HIGH RESOLUTION GENETIC CHARACTERISATION OF SELECTED INDIGENOUS CATTLE BREEDS FROM NIGERIA AND UGANDA

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FACULTY OF AGRICULTURE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

JUNE, 2014

DECLARATION

I declare that the work in this Dissertation entitled "**HIGH RESOLUTION GENETIC CHARACTERISATION OF SELECTED INDIGENOUS BREEDS OF CATTLE FROM NIGERIA AND UGANDA**" has been carried out by me in the Department of Animal Science, Ahmadu Bello University, Zaria - Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Christopher MUKASA	.....	.....
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled **"HIGH RESOLUTION GENETIC CHARACTERISATION OF SELECTED INDIGENOUS BREEDS OF CATTLE FROM NIGERIA AND UGANDA",** by **Christopher MUKASA** meets the regulation governing the award of a Doctor of Philosophy Degree in Animal Science of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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Dean, School of Postgraduate Studies — Date: _____

DEDICATION

This dissertation is dedicated to Almighty God for his infinite mercies, guidance and favour, to Jesus Christ my only saviour and the Holy Spirit my comforter. I also dedicate this great piece of work to my darling wife Margret for whom her constant understanding, support and encouragement has made it a reality

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ABSTRACT

A total of 272 individuals from 12 indigenous Nigerian cattle breeds – Sokoto Gudali (21), Red Bororo (22), Adamawa Gudali (25), Kuri (2), Wadara (3), Bunaji (23), Friesian × Bunaji (24), N'Dama (23), Azawak (2), Keteku (13), Yakanaji (12) and Muturu (12) with five indigenous Ugandan cattle breeds – Karamonjong (16), Sereres (13), Sahiwal Zebu crosses (13), Nganda (23) and Ankole (25) were genotyped for 777,962 single nucleotide polymorphism (SNP) markers to assess genetic diversity, population structure, admixture levels, and relationships. Among the Nigerian cattle populations, the observed heterozygosity varied from 0.582 in Friesian × Bunaji to 0.773 in Muturu with a mean of 0.676 and varied from 0.648 in Karamonjong to 0.622 in Nganda with a mean of 0.637 across all Ugandan cattle populations. The expected heterozygosity in the Nigerian cattle breeds ranged from 0.642 in Muturu to 0.638 in Red Bororo with a mean of 0.641 and ranged from 0.635 in Karamonjong to 0.632 in Serele/Teso Zebu with a mean of 0.633 across all Ugandan cattle populations. The gene flow (*N(nm)*) value combining all 12 Nigerian breeds, amounted to 0.979 and that of Ugandan cattle breeds amounted to 0.953. In turn, when gene flow was examined within breed and country, gene migration in the Nigerian cattle population ranged from 0.981 in Muturu to 0.975 in Red Bororo with the greatest gene migration of 0.955 observed in Karamonjong and the lowest was observed in the Serere (0.950) in the Uganda cattle population. The inbreeding coefficient estimate (F_{IS}) in Nigerian cattle revealed that within population variation accounted for approximately 10.5% of the total genetic variation and 1.6% of the total genetic variation in Ugandan cattle. In the Nigerian cattle populations, only 3056 (0.46%) of 4235 SNPs markers significantly deviated from HWE ($p < 0.05$, $p < 0.01$), as where in Ugandan cattle

populations, 98 (0.02%) of the markers appeared to be in disequilibrium. The first and second principal components explained approximately 15.54 % and 3.66% of the total variation in Nigerian and Ugandan cattle populations respectively and supported the clustering of the populations according to their historical origins and geographical locations. A considerable source of variation among cattle was exhibited at low cross-validation (K = 2, 3, and 4) clustering the populations into African Taurine, European Taurine, African Zebu and detecting the Zebu introgression in African Taurine breeds. Genetic distance, principal component analysis, and population structure analyses, admixture analyses and phylogenic tree, clearly differentiated the cattle population according to their historical origins, and confirmed that Nigerian cattle populations were genetically distinct from the Ugandan cattle populations. This study indicated the necessity for a balance between improving livestock productivity and the conservation of cattle breeds at risk of extinction in Nigeria and the Nganda in Uganda. Hitherto, these populations have represented a unique genetic resource and unexploited opportunity that warrants initiatives for their sustainable conservation and utilization.

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CHAPTER ONE

1.0. INTRODUCTION

Domesticated animals, especially cattle, are an important source of protein in African countries including Nigeria and Uganda. Increasing this protein resource requires the conservation of diversity among indigenous cattle breeds. In order to cope with an unpredictable future, genetic reserves that are capable of readily responding to directional forces imposed by a broad spectrum of environments must be maintained (Hannotte and Jianlin, 2005).

Maintaining genetic diversity is an insurance against future adverse conditions. In Africa, diversity among environments and nutritional standards as well as challenges from multiple infectious agents require diverse breeds and populations. The breeds therefore act as storehouses of genetic variation, which form the basis for selection and may be drawn upon in times of biological stress such as famine, drought or epidemics. The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity (Hanotte and Jianlin, 2005).

It has been estimated that since domestication, over 6,379 documented breed populations from 30 species of livestock have been developed globally in the last 12 thousand years (FAO, 2000). It is generally accepted that the highest amount of genetic diversity in these populations of livestock is found in the developing world, where record- keeping is poor and the risk of extinction is high and is increasing. Recently, loss of genetic diversity within indigenous livestock breeds has been a major concern. The number of mammalian breeds at risk of extinction has increased from 8 to 19% since 1995 (FAO, 2000). The situation with bird breeds is even more serious with the total percentage of breeds at risk of being lost

increasing from 20% in 1995 to 34% in 1999" (FAO, 2000). More particularly, it is estimated that 22% of known livestock breeds have become extinct in the last 100 years and another 27% are at varying degrees of risk (Rege and Tawah, 1999). The losses, as well as the risk to existing cattle breeds, have been partially blamed on indiscriminate crossbreeding between exotic breeds and indigenous animals (Rege and Tawah, 1999).

Globally, this realization has led to efforts to study genetic diversity in livestock species in order to provide a foundation for conserving these potentially-useful germplasms. In addition to loss of diversity, a large proportion of domestic animal breeds in the world is believed to be in danger of extinction (Cardellino, 2004). According to Hanotte and Jianlin (2005), though it may be too late for many livestock and poultry breeds in Europe, optimism in the developing world about slowing down the loss of both diversity and indigenous animals is high. Conservation of indigenous animal resources has been proposed as a method for slowing down the loss in diversity in livestock breeds through extinction.

Apart from preventing extinction, conservation of indigenous cattle breeds is also important for the future health of the animal industry globally as they could be a resource for novel genes that can permit sustained genetic improvement as well as enable adaptation to changing breeding objectives and environments (Notter, 1999). In order to ensure proper conservation and utilization of indigenous cattle breeds, it is necessary to evaluate genetic variations that exist within and among breeds. A large proportion of indigenous livestock populations in the developing world have yet to be characterised or evaluated at phenotypic and genetic levels (Hanotte and Jianlin, 2005).

In the case of African cattle, genetic classification of cattle breeds is still needed. Most existing classifications of cattle breeds in Africa including Nigeria and Uganda are based on historical, anthropological and morphological evidence, which are most often not enough for the purpose of conservation (Tawah and Rege, 1996; Rege and Tawah, 1999; Rege, 1999; Mwacharo *et al.,* 2006). Relative genetic diversity can be determined using phenotypic characteristics and/or molecular markers. Phenotypic characteristics of cattle breeds as well as their adaptive characteristics are important in identifying breed attributes for immediate use by farming communities. In one of the few studies reported, Mwacharo *et al.* (2006) phenotypically characterised two breeds of Zebu in Kenya. The authors identified 12 morphometric measurements to differentiate the Masaai Zebu from the Kamba Zebu in Kenya. The apparent wide within-breed and between-breed variations in linear body dimensions that were observed in the study were indicative of the large genetic diversity inherent in the small East African Zebu cattle (Mwacharo *et al.,* 2006).

The advent of molecular techniques has led to an increase in studies that focus on the genetic characterisation of domestic breeds using genetic markers (Giovambattista *et al.,* 2001). As a tool used in evaluating genetic variation, markers can provide useful information at different levels and purposes such as structure of animal populations, levels of gene flow, phylogenetic relationships, patterns of historical biogeography, and parentage (Feral, 2006). In addition, genetic assessment is also of interest for the design of genetic improvement programmes including appropriate choice of breeds for crossbreeding (Mwacharo *et al.,* 2006).

1.1 Problem Statement

The indigenous cattle populations of Western and Eastern Africa are vital to the subsistence and economic development of these regions, providing essential food products, sustaining the employment and income of millions of West and East Africans living in the rural areas, and providing manure as well as draught power used in ploughing and transport. In comparison to their exotic counterparts, these cattle are better adapted to survive and reproduce under the region's harsh environments. Many of the cattle breeds are named after the community, which keeps the population, or according to geographical localities they inhabit, and the true genetic relationship among the major populations is not yet well known or documented. They are currently at risk of extinction due to indiscriminate crossbreeding and replacement with exotic breeds, uncontrolled introgression and interbreeding, absence of breed development programmes and political instability, and immediate steps must be taken to conserve them (Rege and Gibson, 2003; Hanotte *et al.*, 2010).

The erosion of locally-adapted genetic resources will significantly limit the option and capacity to cope with changes to production environments and breeding goals. Understanding of farm animal genetic diversity is therefore required to contribute to meeting current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing environments and breeding objectives (Notter, 1999; Köhler-Rollefson *et al.*, 2009; Hanotte *et al.*, 2010).

1.2 Justification of the study

Despite their importance, these cattle have not been well-defined, classified or studied adequately in the past. Their current classification based on historical and anthropological evidence, and phenotypic data is inaccurate, making implementation of rational conservation and utilisation strategies difficult. The high levels of Zebu genes reported in some African Taurine breeds by Hanotte *et al.* (2002) is particularly alarming and the African Taurines stand to lose their identity should this trend continue. It is therefore necessary to assess the levels of African Zebu genes, African Taurine and the European Taurine genes in African cattle breeds, particularly the Taurines to enable their effective management.

Previous studies focused on genetic diversity and structures of Nigerian cattle populations (Adebambo *et al.,* 1998) and Ugandan cattle populations (Okomo, 2002; Kugonza *et al.,* 2011) have used low density microsatellite, mitochondrial, or Y-chromosome markers. However, in recent years, analyses of single nucleotide polymorphism (SNP) markers are becoming the standard approach for diversity analysis and genome-wide studies. They represent one of the more interesting approaches for genotypization because they are abundant in the genome, genetically stable, and amenable to high throughput automated analysis (Vignal *et al.,* 2002).

The usefulness of SNPs in analyses of population diversity and structure has been demonstrated in several studies (McKay *et al.,* 2008; Lin *et al*., 2010). The identification of genomic SNPs will provide an opportunity to apply genome based association studies in the future (Matukumalli *et al.,* 2009; The Bovine HapMap Consortium, 2009). Despite a large number of SNPs identified in the bovine genome sequencing project, few have been

validated in tropical cattle populations. Breed characterisation requires basic knowledge of genetic variations that can be effectively measured within and between populations.

1.3 Research questions

Africa is rich in cattle genetic resources being the repository for a number of native breeds adapted or selected for production in tropical, sub-tropical, or marginal production environments. This gives rise to the following research questions for this study:

1. To what extent are the native cattle breeds from Nigeria and Uganda genetically diversified?

2. What is the genetic population structure and admixture levels of the native cattle breeds from Nigeria and Uganda?

3. What is the genetic relationship between the indigenous cattle populations from Nigeria and Uganda?

1.4. Aim and objectives of the study

The broad objective of this study is to apply genomic tools to aid the characterisation of the structure of bovine genomes of selected Nigerian and Ugandan native breeds of cattle and then analyze this data to better understand the relationships among African breeds and also between native and popular European breeds. To be able to distinguish between breeds for conservation and utilization purposes, the determination of the genetic variability, population structure and phylogenetic relationships using genomic tools led to the formulation of the following specific objectives for this study.

1. To quantify the extent of genetic diversity within the indigenous cattle breeds in Nigeria and Uganda based on 777,962 SNP marker genotypes.

2. To determine the population structure and admixture levels of the indigenous cattle breeds in Nigeria and Uganda.
3. To determine the phylogenetic relationships among the indigenous cattle breeds within Nigeria and Uganda.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Classification of cattle

Domesticated cattle belong to the *Bovidae* family within the order *Artiodactyla* or even-toed ungulates, sub-order Ruminantia, family Bovidae and tribe bovinae. The tribe *bovinae* includes different members: beyond cattle like Yaks and Buffalos. The Bovidae first appeared in the Miocene (*circa* 20 Mya) and underwent a rapid radiation, which is considered to have been unparalleled in other large mammals (Gatesy *et al*., 1992). This may have been caused by the first appearance of large grassland ecological niches during the Miocene epoch.

The systematic classification of bovids has not been satisfactorily resolved due to the rapid cladogenesis of the group, convergent evolution and also debatable monophyly for the family as a whole. The most useful diagnostic feature of bovids are the presence of non-deciduous horn cores and horn sheaths which, it is argued, naturally distinguish them as a separate monophyletic clade. Molecular evidence however, would suggest that the Bovidae may actually be paraphyletic in origin (Miyamoto and Goodman, 1986; Kraus and Miyamoto, 1991, Gatesy *et al*., 1992).

Domesticated cattle are members of the *Bovini* tribe within the Bovidae family. Other members of the Bovini tribe include the Bison (*Bison bison*), the European Bison or Wisent (*Bison bonasus*), the Banteng (*Bos* [*Bibos*] *banteng*), the Gaur (*Bos* [*Bibos*] *gaurus*), the Yak (*Pöephagus mutus*) and the Kouprey (*Bos* [*Bibos*] *sauveli)*. There are two recognised forms of domesticated cattle, the humpless *Taurine* cattle of Europe, West Africa and northern Asia, (*Bos taurus*) and the humped *Zebu* cattle of southern Asia and Africa (*Bos*

indicus). Attempts to classify the Bovini tribe have also been beset by the problems of rapid radiation and parallel evolution. *Bison* and *Bos* have been considered separate genera on the basis of craniological evidence and other morphological characteristics (Epstein, 1971). Recent evidence from allozyme studies and mitochondrial DNA sequence analysis would place *Bison* and *Pöephagus* as sub-genera of *Bos* along with the *Bibos* species (Hartl *et al*., 1988; Miyamoto and Boyle, 1989; Miyamoto *et al*., 1989). The fact that all the members of the *Bovini* are interfertile to varying degrees would also argue for congeneric status (Baker and Manwell, 1991).

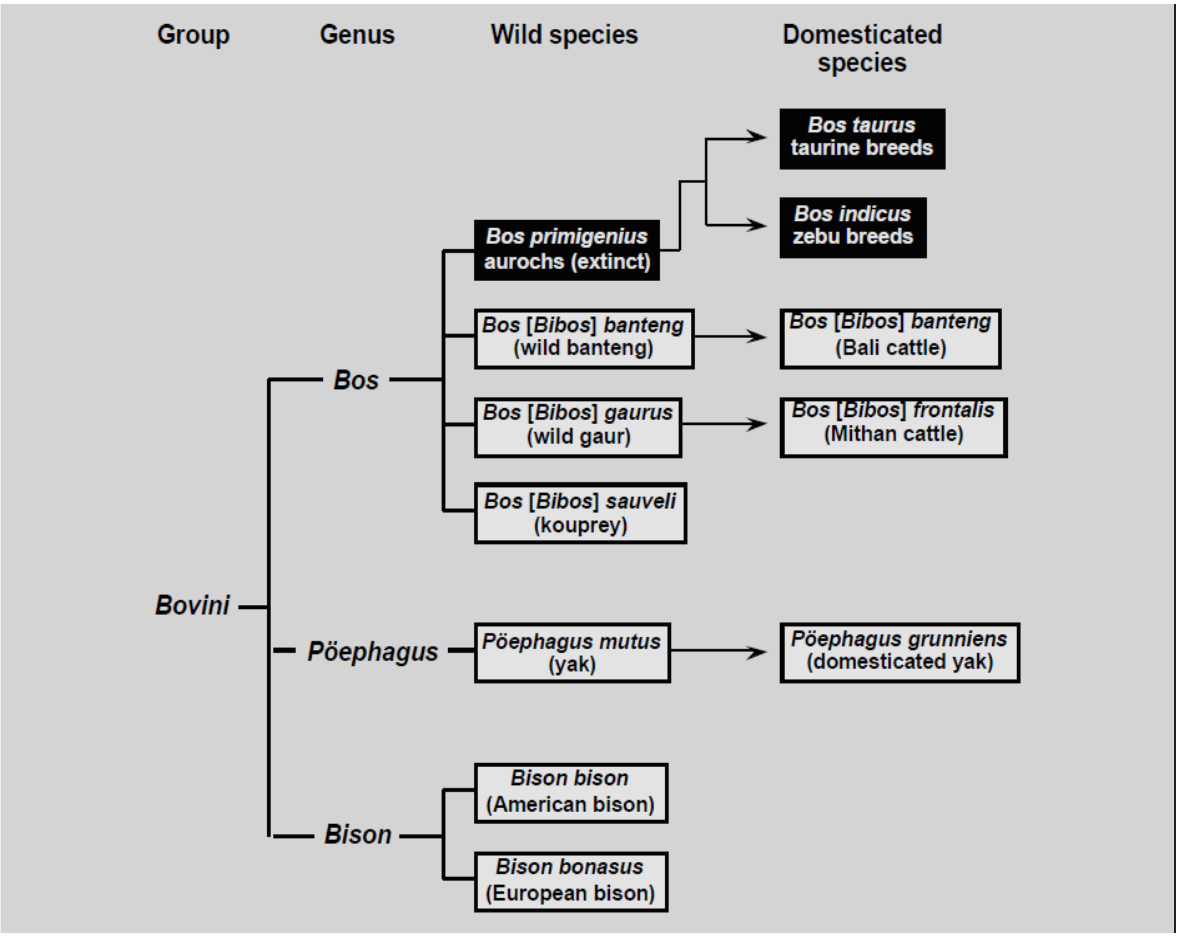


Figure 2.1 Classification scheme for the various members of the wild and domesticated species within the *Bovini* group (Epstein and Mason, 1984).

2.2. The origin of the domesticated cattle

The first authenticated domesticated cattle in Africa appeared in the early civilizations of the Nile Valley about 6,500 BC (Epstein and Mason, 1984). These longhorn cattle dispersed with Hamitic peoples south wards through present-day Sudan, west wards along the northern coastal region, south-west wards into West Africa and also centrally through a much-reduced Saharan region. The cattle which entered West Africa evolved an inherited tolerance or resistance to trypanosomiasis and were able to thrive in the southern forested regions. These longhorn cattle eventually gave rise to the modern N'Dama breed.

Domesticated Taurine cattle are thought to have entered Africa in successive waves from southwest Asia, in a similar fashion and at a similar time to the early cattle of Europe. Populations of Zebu cattle migrated into Africa at a later date from Arabia and the Indian sub-continent. Although there was an indigenous African aurochs, *Bos primigeniuso pisthonomous*, it is widely accepted that this sub-species was not domesticated independently (Epstein, 1971; Epstein and Mason, 1984; Payne, 1991). There has been some speculation in the literature however, that this native African aurochs actually formed or contributed to the early domesticated populations on the continent (Grigson, 1991; Wendorf and Schild, 1994).

In North Africa, shorthorn cattle began to replace the original longhorn populations at approximately the same time as in southern and central Europe. They entered Egypt by way of the Isthmus of Suez towards the middle of the 5th millennium BC. For a considerable period, longhorn cattle prevailed over the shorthorn type. Gradually however, the shorthorn populations increased in numbers as military successes of the Pharaohs in southwest Asia brought new shorthorn herds back to the Nile Valley. Accordingly, during the *Hyksos*

period (*circa* 1700-1,580 BC), shorthorn cattle became the predominant type in North Africa (Epstein, 1971). Shorthorn cattle eventually dispersed throughout north, east and West Africa. In West Africa, they also developed a tolerance to trypanosomiasis and were capable of inhabiting forested regions with a high tsetse challenge (Payne, 1970).

The first Zebu cattle to enter Africa are thought to have been cervico-thoracic humped cattle which entered across the Isthmus of Suez and from Arabia into Somalia and Ethiopia. These introductions are thought to have taken place during the 4th millennium BC (Payne, 1970). These initial introductions did not have a significant impact on the native Taurine populations until local pastoralists realised the Zebu's innate physiological adaptations to arid conditions. Thereafter, Zebu migrants and Taurine cattle were crossed to form populations of *Sanga* cattle. Internal African migrations such as those of the Bantu peoples (*circa* 700 AD) and the Hottentots (*circa* 1,500 AD) were responsible for dispersals of Sanga cattle into southern and central African regions (Epstein, 1971).

Thoracic-humped Zebu cattle did not enter Africa in any great numbers until the Islamic Arab conquests of 669 AD (Hourani, 1991). Again, like their cervico-thoracic predecessors, they were mainly imported through the Horn of Africa into Somalia and Ethiopia. The newly imported Zebu cattle began to displace Sanga cattle due to their arid-adapted physiology and also because they gave higher milk yields (Payne, 1991). Zebu cattle continued to be introduced into Africa as long as Arab power was maintained in north and east Africa. They were brought to West Africa along the Sahelian *corridor* between the Sahara desert to the north and tsetse-infested forests to the south. They were crossed with local Taurine animals to form local intermediate populations such as the White Fulani of northern Nigeria. They were unable to impinge on the Taurine cattle deep within the West

African equatorial regions because they lacked the ability to withstand the effects of trypanosomiasis, unlike the indigenous Taurine cattle.

The spread of Zebu cattle in East and Southern Africa was facilitated by the occurrence of periodic rinderpest epizootics in these regions. Taurine animals are much more susceptible to this viral disease than Zebu cattle and usually die when infected. The last major rinderpest epizootic took place during the 1890's and is thought to have wiped out as much as 80% of the cattle populations of East Africa (Denbow and Wilmsen, 1986). However, indiscriminate crossbreeding of the Zebu with Taurine breeds has been a major contributor to the genetic dilution of this resource in most African counties. This has been accelerated by advances in new reproductive techniques, particularly artificial insemination and increased exchange of breeding stocks between regions (Moazami-Goudarzi *et al.*, 1997).

2.3. African cattle genetic resources

Almost 70% of the populations of sub-Saharan Africa are dependent on agriculture as a source of income and domesticated cattle are tightly-interwoven in the cultural and economic fabric of African life. In many regions of Africa, they provide, in the form of milk and meat, the bulk of the dietary intake for pastoral societies. They are also used for traction while hides, blood, horns and dung are useful by-products (Payne, 1970). The most convenient criteria generally used to distinguish African cattle breeds is their associations with particular tribes of cattle-herding peoples. There have been very few systematic surveys seeking to disentangle and classify the origins and current status of the approximately 130 cattle breeds known to exist on the African continent (Schierf, 1995).

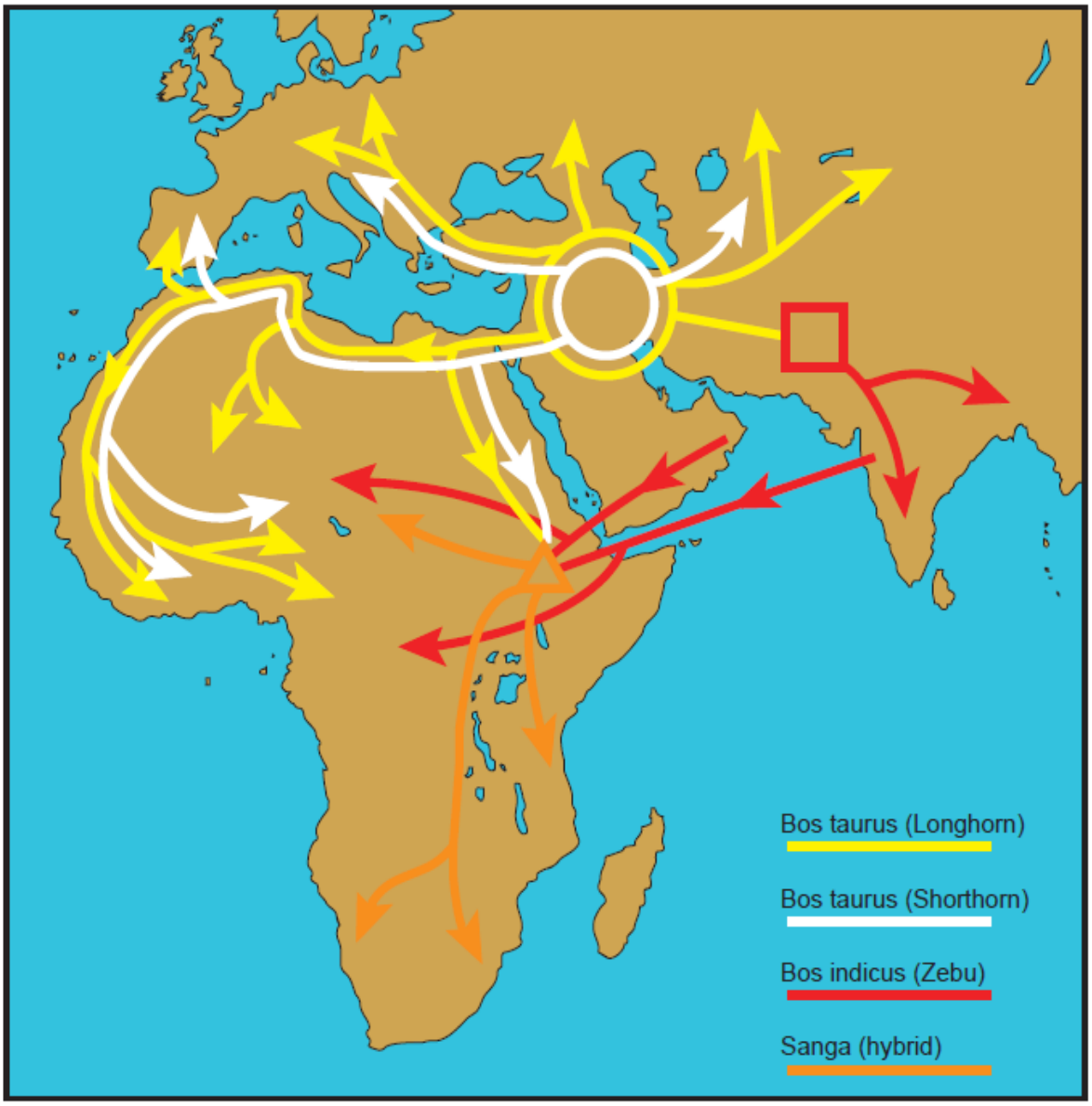


Figure 2.2 Map of the Epstein -Mason model for the origin and subsequent dispersion of domesticated cattle from Southwest Asia in Asia to Africa and Europe (Epstein and Mason, 1984).

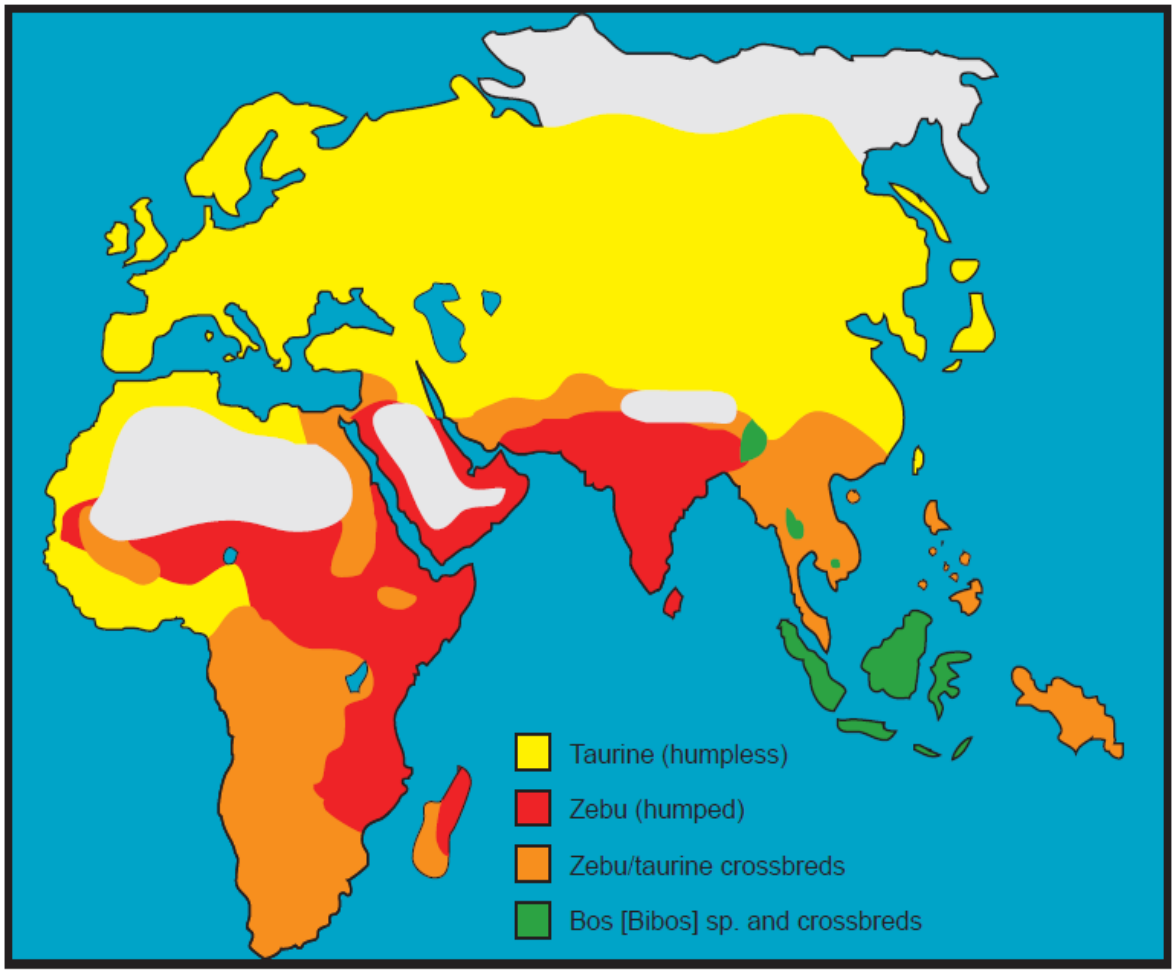


Figure 2.3 Map illustrating the approximate current distributions for the various types of cattle and their hybrids in Asia, Africa and Europe (Epstein and Mason, 1984).

However, the works of Epstein (1971), Payne (1970) and Maule (1990) give some insight into the breed histories and biogeography of present-day cattle populations. The classification of African cattle breeds has been complicated by historical crossbreeding that has taken place on a massive scale between the original, domesticated taurine cattle of Africa and invading herds of Zebu cattle from Asia. Hybridisation between the two sub-species has taken place to varying degrees over most of Africa and today, the cattle population of the continent represents a biological mosaic of the two morphologies (Epstein, 1971).

The distribution of African cattle populations has also been profoundly influenced by the epidemiology of certain livestock diseases. During the 1890's, rinderpest, a disease native to southern Asia decimated domesticated cattle and related wildlife species in East Africa which permanently altered the demography of the East African cattle population (Epstein, 1971). In addition, the protozoan disease, trypanosomiasis, spread by insects of the genus *Glossina*, (tsetse flies) is rife over large swathes of sub-Saharan Africa and severely restricts cattle husbandry in these regions. However, certain Taurine cattle populations, particularly in West Africa have evolved a resistance or tolerance of the disease, presumably due to strong natural selection over several millennia. These cattle which represent the last remnants of the original domesticated cattle of Africa are currently the focus of a concerted international effort to characterise and conserve them (Epstein, 1971).

Indigenous breeds constitute well over 95 percent of the cattle populations in Africa (Rege, 1992). These are well-adapted to the environment and ravages of various kinds such as drought, famine and civil wars that continually plague the continent. Their adaptive features enable them to effectively cope with the stressful nature of marginal lands (Chenyambuga,

2002). Maintaining genetic diversity is an insurance against future adverse conditions. In Africa, diversity among environments and nutritional standards as well as challenges from multiple infectious agents requires diverse breeds and populations which act as store-houses of genetic variation and form the basis for selection to be drawn upon in times of biological stress such as famine, drought or epidemics. The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity.

2.3.1. Cattle sub-species and breeds in Nigeria

Individual breeds and strains of cattle in Nigeria have been characterised, but overviews of the country as a whole are rare. The most important of these is Gates (1952). Standard references such as Epstein (1971) and Mason (1988) constitute useful reviews of the general literature on West Africa but do not cover the specific situation in Nigeria. A specific study of the breeds of cattle recognized by indigenous producers was undertaken as part of the Nigerian National Livestock Resource Survey (NNLRS) in 1990 (Blench, 1999).

It is generally believed that the size of Nigeria's present cattle population is in the range of 10 to 15 million, the majority of which are humped Zebu owned by the Fulani people of which 13.5 million were kept in pastoral systems and 1.5 million in villages, but included Taurine animals such as the 115,000 Muturu, some Keteku, N'Dama and Kuri. Country-wide, the mean density of cattle is approximately 15/km2, or 6.6 hectare/head (Meghen *et al.,* 2000).

2.3.1.1. Indicine Types

Bunaji

Bunaji or White Fulani cattle is a white, black-eared and medium-horned breed, and is the most numerous and widespread of all Nigerian cattle breeds. The NNLRS estimated that they represent some 37% of the national herd. They are found from Lagos to Sokoto, Katsina and Kano States and spread across the Nigerian Middle Belt. The only areas from which they are significantly absent are Borno, where Rahaji and Wadara predominate, and in the south-east, where there are no resident Zebu. The movement into the derived savannah and to the edge of the humid zone has largely been of Bunaji and pastoralists generally agree that they are superior to all other breeds of Zebu in resisting disease (Blench, 1993).

Their estimated population size is 5,118,547 or about 51 % of the national herd (RIM, 1989). They are characterised by White black-eared and medium -horned breed (Plate.I). Most herds have a few black coloured patches on the coat or with blue flecking or red and white with a mortality rate of 15.6%, calving interval is between 388 and 810 days and age at first calving is 40.00–60.00 months. They are more resistant and tolerant to diseases than other Zebu races with an average milk yield of 627 – 1034kg over lactation periods of 194 – 245 days, a weight gain of 880g over 76 days fattening period and conception typically peaks in April-May. The skin is loose and pigmented and the hair is soft with erect ears. Horns are medium to long lyre-shaped and curve outwards and inwards, they have outward tips of the horns in some of these animals with large and well-developed dewlap of many folds. Sheath and navel flap are not large and female udders are developed with medium-sized teats (Buvanendran *et al.*, 1983; Ngere, 1990; Hall, 1991).



Plate.I. Bunaji bull

Yakanaji

Yakanaji is also known as the Bunaji or the White Fulani cattle breeds (Blench, 1999), which original habitat of the northern Nigeria, southern Niger and north-eastern Cameroon with both the Fulani and Hausa tribes; gradually they have spread into southern part of Nigeria especially Borgu and Kwara axis (where they are referred to as Yakanaji). Their characterised by long (80-105cm) and lyre-shaped horns; coat colour is white with patches of black, sometimes with red marks on the ears, feet and sides. It is known to have excellent potential as dual-purpose (milk/beef) cattle (Felius, 1995). These medium-sized animals have fine, short limbs. The average height of the animals is 115 to 130 cm. The face profile is long and straight with the forehead sometimes tapering, resulting in a slightly convex appearance. The poli and orbital arches are slightly prominent. Loose horns occur, but are rare. Polled animals with very peaked polls also occur (Felius, 1995).

In areas where the tsetse challenge is not too severe, Zebu characteristics are perceived as desirable by farmers and crossing humpless females to Zebu bulls has become a common practice (Rege, 1999).

The White Fulani are generally taller and narrower bodied cattle, the hump is of good length but has a marked sloped from hook to pin bones. The udder is well-developed and of a good shape and strong attachment. Teats are well positioned and are of medium to reasonably large size. They have leggy appearance. Their more resistant to dermatophilosis, resistant to helminth parasites and has low mortality rate. They are good beef animals which fatten well in feedlots and on pastures (Rege, 1999).



Plate II. Yakanaji cattle

Sokoto Gudali

There are two quite distinct types of Gudali in Nigeria – the Sokoto Gudali (or Bokoloji) and the Adamawa Gudali. The Sokoto Gudali is a uniform cream, light grey or dun, the dewlap and skin folds are highly developed and the horns almost absent (Plate.III). Although the Sokoto Gudali stereotypically occurs mainly in the northwest of Nigeria, in reality it is now distributed widely throughout the country. The breed is a native to Sokoto, Kebbi and Zamfara States where climate is semi-arid and tropical but widely distributed throughout Nigeria and neighbouring Sahelian countries. The Nigerian National Livestock Resource Survey estimated that they represent some 32% of the national herd with a population of about 4,351,528 (Rege and Tawah, 1999).

They are characterised by being short-horned, short-legged cattle with very short or effectively absent horns. Some have darkened patches around the front half of the body with well developed dewlap and skin folds. This breed is considered good as fattening stock (Nuru *et al*., 1981), however, some consider them to be lacking in stamina. Weight gain over 76 days fattening was 920g with mean milk yield 1,144kg over 266 days to 2,633kg over 322 days (Adebambo *et al*., 1998). The age at first calving was 38.6 – 46.0 months with a 467 days calving interval (Hall, 1991). The national herd structure is 69.6% females and 30.4% males. No marked trend with respect to seasonality of conception but higher conception rates are typically in March and April (Johnson *et al.*, 1984; Adebambo *et al*., 1998).



Plate.III. Sokoto Gudali bull

Adamawa Gudali

Restricted to Adamawa and southern Borno States, the Adamawa Gudali, is owned by both the Kanuri and Fulani pastoralists. It is rare for them to have complete herds of Adamawa Gudali, and often they are mixed with Wadara, Bunaji or Rahaji. Adamawa Gudali is regarded by many farmers as the indigenous race, of the region and they are common in villages. The animals are favoured for ploughing, but when they become too large to pull a plough effectively they are further fattened in the compound and sent to market (Rege and Tawah, 1999).

The Adamawa Gudali resembles the Bunaji in conformation with a medium to large size, medium-length horns, and usually pied, or a white, black, red or brown coat. It has thick, crescent-shaped horns, a pendulous hump, and a short head and muzzle (Plate.IV). The pendulous hump is the feature that most reliably distinguishes it from the Bunaji (Tawah and Rege, 1996). The NNLRS estimated that Adamawa Gudali represents about 2% of the national herd with a population size of 263,019. At least two local types were originally recognized in Nigeria: the Banyo, with Rahaji blood and rather large horns, often with a white face and red eye patches, and the Yola, which had an admixture of Muturu (Gates, 1992; Blench, 1993).

At least two major types were recognized in Nigeria: the Banyo, with Rahaji blood and rather large horns, often with a white face and red eye patches and the Yola which has and admixture of Muturu, indigenous to the region, larger than Bunaji. They are better traction animals than Bunaji and have good fattening abilities. In their herd structure, 71.4% are females and 28.6% males with a conception pattern curve which is bimodal with peaks in June - July and December – January (Buvenendran *et al.*, 1983; Ngere, 1990).



Plate.IV. Adamawa Gudali bull

Red Bororo (Rahaji)

The Rahaji is one of the largest Zebu breeds and is distinguished by its deep burgundy-coloured coat, pendulous ears and long, thick horns. It is the third most numerous breed of cattle in Nigeria with a population size estimated to be 3,029,541, about 22% of the national herd. The Rahaji (Plate.V), is widely distributed in Kano, Daura, Borno and Adamawa areas. (Blench, 1993).

The Rahaji is adapted to arid and semi-arid regions and rarely goes further south than Kaduna in the wet season, except for the isolated population on the Mambila Plateau in the north-east (Blench, 1993). Fulbe pastoralists consider the Rahaji an extremely prestigeous breed and many herds of 'white' cattle include a few Rahaji for crossbreeding.Nonetheless, it tolerates neither humidity-related diseases nor poor nutrition. Strikingly, a Fulbe clan, the Rahaji, named for the breed they traditionally herded, has been obliged to exchange their stock for Bunaji as they have moved south into the Middle Belt because of high mortality among the 'red' animals (Blench, 1993).

The mortality rate is lower than 15.6% for Bunaji with a 7.3% slaughter figures higher than the Bunaji. Their calving interval is 537 – 585 days with the age at first calving ranging from 47.9 to 53.0 months and calving occurs all-year- round but peaks in April (Ngere, 1990; Adebambo *et al*., 1998).



Plate.V. Red Bororo bull

Wadara

Wadara cattle are the 'indigenous' cattle of Borno i.e located in the north-eastern part of the State and are referred to by the Koyam and related pastoralists as 'our' cattle. They are frequently called 'Shuwa' in the literature, after the Shuwa Arabs who also herded them. A related breed with a white coat, the Ambala, is often traded into Nigeria from Chad. Wadara cattle are medium-sized, lightly- built cattle, and are usually dark red, black, pied or brown (Blench, 1993).

These medium-sized, lightly built cattle are usually dark red, black, pied or brown. They are short-horned and have a small erect hump (Plate.VI), representing about 6.6% of the national herd with a population size of 904,731. Their good dairy animals with average milk yield of 1,212kg over 259 days. The age at first calving is 45.4 - 52.4 months and they have a annual pattern of conception, which peaks in July with a herd structure of 60.4% females and 39.4% males (Olutogun, 1976; Ngere, 1990; Hall, 1991).

Azawak

The Azawak is said to be native to the Azawak Valley north-east of Nigeria and is distributed along its north-western border. They are generally, found on the Nigerian border in western Sokoto State and also occur in north-western Kwara State and dotted along the northern baorder of Nigeria from Sokoto to eastern Kano (Blench, 1993).



Plate.VI. Wadara bull

The NNLRS estimated that they represent just 0.7% of the national herd with a population size of 103,280. A small population of Azawak cattle exists in Nigeria throughout the year, but the majority of the herds are seasonal transhumants. Azawak are generally only found on the border north and west of Sokoto but there were also some in the north-west of Borgu and dotted along the frontier from Sokoto to Katsina. It is lightly-built with medium-length horns. Although Azawak in Niger are commonly described as red, the Azawak that enter Nigeria are usually a light fawn colour, though they can also be white, brown, pied and black, but at times, they are white, brown, pied and black (Plate.VII). They calve annually, are more resistant to diseases, eat less, and on a wider variety of vegetation than the Rahaji (Rege and Tawah, 1999).

Azawak cannot trek long distances as Rahaji. They have placid temperament and can be herded away by thieves at night. They are good fattening animals, docile, and can be trained for ploughing and carting. They are very productive, have shorter calving interval and earlier first calving. Their calving interval is 426 – 507 days with age at first calving of 37 – 52.2 months and their conception pattern has a single peak, from January to October. The herd structure is 78.2% females and 21.5% males (Ngere, 1990; Hall, 1991).

2.3.1.2. Taurine Types

This section covers animals usually described as ‘trypanotolerant’, the West African dwarf shorthorn or Muturu and the various types of Keteku, Zebu x Muturu and Zebu x N’Dama crosses (ILCA, 1979). No judgement is offered on their powers to resist disease.



Plate.VII. Azawak bull

West African Dwarf Shorthorn (Muturu)

The West African dwarf shorthorn or Muturu is small-bodied, and blocky in conformation with short, fine-boned limbs (Plate.VIII). It has a compact body, no hump, a straight back, and a broad head. The face is slightly-dished, and the horns are very short. In south-central Nigeria, the Muturu is generally black, or black and white. Animals on the Jos Plateau are usually black and white but are distinctly larger than lowland animals. There are more variations in the northern populations; brown, red or tawny animals were recorded (Blench, 1993).

Within Nigeria, Muturu cattle have a very disjunct distribution, suggesting the gradual retreat of a once widespread population. The history, distribution, management and productivity of Muturu have recently been reviewed in Blench *et al.* (1998). Muturu are widely-dispersed and are often stall-fed, and so are less visible than Zebu. As a result, published population figures are little more than informed guesses though the NNLRS reported a population size of 115,172. Moreover, since northern Muturu are barely known and their trypanotolerance is unmeasured, they have usually been excluded from estimates of 'trypanotolerant' cattle (Rege and Tawah, 1999). The isolated populations of Northern Muturu along the Republic of Cameroon frontier range up as far as south-eastern Borno, adjoining the Michika-Mubi area of Adamawa. Small clusters exist in the Atlantika mountains, south-east of Yola and near Cham, east of Bauchi. Muturu are still relatively common south-east of the Jos Plateau in the dry savannah. There is another nucleus of Muturu north of Tegina in the north-west, with diverse coat-colours suggesting a link with the north-eastern populations (Akinwumi and Ikpi, 1985).

Muturu were probably once kept throughout the whole of southern Nigeria and their disappearance from many areas is relatively recent. West of the river Niger, Muturu were once widespread but are now uncommon. They have either been replaced by Keteku and Zebu, or communities have ceased keeping them. At present, the major concentrations of Muturu are in the south-east, in the Cross River area and among the Tiv people in and around Makurdi. Muturu are kept throughout the Igbo areas but in very low densities. The division of Muturu into different populations is somewhat controversial. The research defined four sub-sets; Northern Savannah/Montane, plateau, derived savannah and forest. Only the first group is distinctive in physical appearance. Muturu in the derived savannah and forest are similar in colour, conformation and management and contrast with those in the northern savannah/montane environments (Adebambo *et al*., 1998).

The humpless and short-horned Muturu is generally black in colour or black and white for those found south of Niger-Benue Rivers and Plateau State. The forest Muturu have a white patch around the muzzle and a tawny line above the vertebral column. In the north, brown, red and tawny-coloured occur. Milk yield was 421kg over 216 days with 28.3 – 41.8 months mean of age at first calving while their calving intervals were between 13.9 and 26.8 months with their conception pattern peaking in July with a series of high values in the dry season (February – April and December – January). They are trypano-tolerant whose herd structure is 70.5 – 73.2% females and 27.0 – 29.7% males. The progeny mortality rate is 4.7 – 15.6% and lowest with 4.7% for southern savanna (Ngere, 1990; Hall, 1991).



Plate.VIII. Muturu bull

Keteku (Taurine × Zebu crosses)

Apart from the Muturu, there are essentially three cattle types that fall under the broad rubric of trypanotolerant:

(a). Crosses of the West African Dwarf Shorthorn (Muturu) with Zebu, (b) with N'Dama

(c). Pure-bred N'Dama.

The first two are known as Keteku within Nigeria, although they are very different in conformation.

The Muturu × Zebu cross is called the Borgu Keteku and the Muturu × N'Dama the Lagos Keteku. The N'Dama, unlike the Zebu and Muturu, are not indigenous to Nigeria but were imported from the Senegambia during the twentieth century. The distribution and productivity of Keteku have been studied in more detail in Blench *et al*. (1998). The definition of Keteku has become more problematic in recent years with an increasing proportion of Zebu blood in 'Keteku' herds. As Fulani pastoral herds push ever further south and increasingly inhabit regions previously restricted to trypanotolerant stock, more Zebu are bought in for village herds. For example, the 'Biu', a Zebu × Savannah Muturu cross found near Biu in southern Borno and described in the literature (Gates, 1992), has effectively become submerged in the local Zebu gene pool.

Borgu Keteku

The Borgu Keteku also called Kataku, Ketari, Borgu, Borgawa and Kaiama, is a trypanotolerant, stabilized Muturu × Zebu cross (Gates, 1992). It combines Muturu and Bunaji features with white, grey and black types predominating, and more occasionally, red and brown. The horns are long, compared with a Muturu, but the hump is smaller, and the legs shorter than a Bunaji. In Nigeria, Keteku in herds are restricted to a narrow band along

the Benin Republic border in the region usually known as 'Borgu'. Further to the east, Keteku are occasionally kept adjacent to villages in northern Yorubaland. West African dwarf short-horns were once common through out this region and the Keteku fills the same niche. Keteku are sometimes bought as investment stock in the Ondo area by farmers who value their combination of size and trypanotolerance. Keteku were formerly distributed from breeding farms as part of livestock extension programmes and the Government Livestock Centre in Ado-Ekiti still keeps a stock of Keteku (Blench, 1999).

In contrast to other West African countries, there has been very little 'new' crossing of Zebu and Muturu in southern Nigeria. In some ways, it is surprising that the crossbreeding of Zebu and Muturu did not take place all along the line where the two types came into contact. Further to the east, among the Igbos, farmers tend to assume that the two breeds are incompatible; attempts at crossbreeding would conflict with religious structures (Adebambo *et al.,* 1998). The trypano-tolerant Muturu is predominatly white, grey and black in colour. Pied and brown colours occur. Horns are longer than Muturu's but the hump is smaller and the legs shorter than those of Bunaji (Plate.IX). The mean age at first calving is 44.6 months with a calving interval of 20.6 months and an even all-year-round conception and 24 – 72 months age at first calving (Ngere, 1990).



Plate.IX. Keteku bull

N'Dama

N'Dama cattle are native to Senegambia and adjacent parts in the west of West Africa (Blench *et al.* 1998). They were first brought into Nigeria from Guinea in 1939 on an experimental basis, because they were trypanotolerant and yet were larger than Muturu. Although those imported into Nigeria are generally light brown (Plate.X), there are black and pied animals in Guinea. N'Dama cattle have been sold to farmers and pastoralists with a view to improving the resistance of local herds to trypanosomiasis. In most cases, herders cross them with Zebu and there are now few pure N'Dama, although some were recorded in northern Yorubaland (Blench, 1999).

The N'Dama has a medium-sized, compact body with lyre-shaped, black-tipped horns, short legs, broad, straight back and no hump. There is a small dewlap in the male, but a fairly large head. Those in Nigeria are light brown and in Guinea, black and pied colours are found. Females have small dew-lap than males which have fairly large head. They have an average weight of 299kg and bulls can weigh 550kg. They are exceptionally fertile, early-maturing, poor milkers and are characterised by nervous tendencies, stubbornness and easily-provoked into action but above all they are trypano-tolerant (Ngere, 1990).

Kuri

In Nigeria, Kuri are found not only on the Lake Chad but on its shores and along the Yobe valley, as far west as Gashagar. The breeds along the Komadugu Yobe are crossed with Zebu and are generally referred to as Jetkoram in the literature (Blench, 1993). Kuri are noted for their extremely-variable colours and their ability to thrive in semia-quatic conditions.



Plate.X. N’Dama cattle

The nucleus of the Kuri cattle population is within the region of the former Lake Chad, and along its eastern shores (Meghen *et al.,* 2000). The Kuri are large-bodied, humpless, long-horned cattle whose exact historical origin is unknown (Blench, 1993; Meghen *et al.,* 1999).

The Kuri has distinctive, inflated, spongy horns (Plate.XI), useful in swimming, unknown in any other breed and with a mean height of 1.5 m, and weight of some 550 kg, is one of the largest breeds of African cattle. Conception peaks around April and August and calving peaks in January and May. Average age of breeding for females is 86 months; average age at first calving is 49.9 months and their highly adapted to they are natural new environment. Their milk yield is 3-6 litres per day and lactation period is 6-10 months although record of 1,350 litres per lactation exists and the calving interval is 18.7 months, though it can be less (15.8 months). For the herd structure, 63% are females and 37% are males with an overall mortality rate of 27% which is the highest for any Nigerian breed (Ngere, 1990)

Friesian × Bunaji crosses

In a bid to exploit this genetical advantage, Nigeria, many decades ago imported several Holstein-Friesian (HF) bulls for crossbreeding with the local dairy breeds, especially Bunaji (BJ) heifers. This resulted in considerable improvement in milk yield and reproductive potentials (Mbap and Ngere, 1995). Crossbreeding has therefore been exploited as an efficient tool of blending the adaptability of tropical cattle with the high milking potentials of exotic breeds for increased milk production (Oni *et al.,* 2001). Thus results of studies conducted at the National Animal Production Research Institute (NAPRI), Shika, Nigeria on the performance of cross breeds of Friesian with Bunaji, have indicated

an improvement of over 60% in milk yield by the first cross, and a further increase in the level of Friesian blood resulted in an additional gain in milk yield but with decreasing magnitude. There was also a marked reduction in the calving interval and age at first calving in the crosses (Oni *et al*., 2001).

Also research conducted at Vom and Agege in Nigeria on crossbreeds of White Fulani cows and Friesian bulls showed considerable improvement in milk production in the first filial (F_1) generation (Ojedapo *et al*., 2004). This brought the idea of setting up West African Milk Company (WAMCO's) Integrated Dairy Farm (IDF), Vom, Nigeria. Efforts at increasing the productivity of the Nigerian breed (*Bos indicus*) by cross breeding with European Friesian stock of improved genotype (*Bos taurus*) has been a major pre occupation of WAMCO via its IDF for many years.

In Nigeria, Friesian sires are the most predominant dairy breed of cattle used in cross breeding the Bunaji cows (Alphonsus *et al.,* 2010) and Holstein Friesian x Bunaji cross-combination are the most compatible dam and sire breed combination with the highest percentage of normal calving (83%) and without a single mortality recorded (Akpa *et al.,* 2011). Friesian x Bunaji had a high to low pattern for percentage incidence of Hb AA and AB respectively, with HbBB conspicuously absent (Essien *et al.* 2011). The gene and genotypic frequencies of haemoglobin variants revealed very high frequency of Hb AA in the Freisian x Bunaji crosses. The absence of Hb BB genotype in the crosses suggested some adaptive superiority of the Hb AA and AB over the Hb BB (Essien *et al.* 2011). The Hb type AA in the Nigerian Friesian x Bunaji crosses of cattle of Zaria environment was favoured by natural selection, possibly for adaptation against the hot tropical weather and

tolerance of tick and helminthic infections which was not achievable with exotic cattle breed (Essien *et al.* 2011).

Akpa *et al.* (2011) reported the lowest percentage of calving difficulty (17%) with zero mortality in Friesian x Bunaji crosses, indicating the high compatibility of these two breed origins. Mukasa *et al.* (2011) reported an average total milk yield of 3687.096 ± 104.673kg in the Friesian × Bunaji, which was lower than 4457.72 ± 330.41kg reported by Ojedapo *et al.* (2004). Other authors reported an average total milk yield of 1988 ± 118.1kg Friesian × Bunaji at Shika (Malau-Aduli and Abubakar, 1992) and 1189.00 ± 130.86 for the Friesian × Bunaji in WAMCO (Ojedapo *et al.,* 2004). The beneficial effect of cross breeding has no doubt been reflected in the total Milk yield of the Friesian × Bunaji crosses compared to the Pure Bunaji which explains the high coefficient of variation. The overall lactation length averaged 250 ± 4.8 days for Friesian × Bunagi crosses at Shika (Malau-Aduli *et al.,* 1996). Age at first calving was 30.250 ± 0.194 months (Mukasa *et al.,* 2011), which was lower than 3.5 ± 2.3 years reported by Malau-Aduli *et al*. (1996) for Friesian x Bunaji cross, and in agreement with that of 30.9 months at Vom (Ojedapo *et al*., 2004).

It was however much shorter than the 60 months reported in pure Bunaji under traditional management in Northern Nigeria (Oni *et al.,* 2001). Also the overall mean AFC of 30.250 ± 0.194 months was lower than the average age at first calving in *Bos indicus* cattle of about 44 months compared with about 34 months in *Bos taurus* and *Bos indicus* × Bos *taurus* crosses in the tropics (Roche, 2000). Friesian × Bunaji crosses are big in size with a skin is loose and pigmented with white and black parches, white and black spots, black and the hair is soft with big and slightly erect ears. Horns are medium to short with a small dewlap and have a well developed udder (Plate XII).



Plate.XI. Kuri bull



Plate XII. Friesian × Bunaji crossbred cow

2.3.2. Cattle sub-species and breeds in Uganda

2.3.2.1. East African Zebu

Rege and Tawah (1999) coined the term 'East African Zebu', to embrace the substantial variation in the morphology of the shorthorn Zebu of Eastern and Southern Africa. The term 'East African Zebu' is used to describe all the 'Shorthorn Zebu' of Eastern and Southern Africa (Rege and Tawah, 1999). Based on their body sizes, the East African Zebu breeds can be divided into two sub-groups, the 'Small' and the 'Large'.

The term 'Small East African Zebu' was suggested to portray the small frame of these animals and to avoid confusion with other types of East African cattle (Rege, 1999). The Small East African Zebu breeds are the majority, being represented by 49 breeds. The Large East African Zebu is represented by 13 breeds, which are restricted to the relatively-drier parts of Sudan, Eritrea, Ethiopia, Somalia, Kenya, Tanzania and Uganda. The isolations imposed by tribal boundaries, whether physical and/or cultural, and those due to ecological restrictions are partially-responsible for the genetic differentiation leading to the existence of different breeds and strains (Rege and Tawah, 1999). However, variations in nomenclature associated with different tribes and ecologies do not, in themselves, imply genetic differences. For this reason, breeds or strains that have a common ancestry can be classified further, according to whether or not they occupy the same geographical area (for example, a country) and/or a defined ecological zone within one or more countries. Rege and Tawah (1999) referred to these two classifications as clusters and groups, respectively.

Large East African Zebu

Karamanjong Zebu

This breed inhabits north-eastern Uganda (Jie and Karamanjong), south-eastern Sudan (*Toposa* strain) and north-western Kenya (*Turkana* strain). The Karamanjong Zebus are morphologically quite similar to the Boran, being large and heavy-boned. Major coat colours are red, roan, grey and white; while horns vary in length and shape. The hump is large and dome-shaped while the dewlap, umbilical fold and sheath are larger in Karamanjong type (Plate.XIII) than the Toposa. Usually, the udder is moderately well-developed (Rege, 1999).

Small East African Zebu (Teso group)

This group comprises three strains inhabiting the Teso district of Central Uganda (the *Kyoga* and *Usuk* strains) and in the Serere Peninsula of Lake Kyoga (the *Serere* strain). These strains are at risk of loss due to continued interbreeding with the Nganda and Ankole cattle, especially in recent times (Rege, 1999).

Nkedi

The Nkedi of north-eastern Uganda are thought to have come to their present breeding area from southern Sudan. Earlier the Nkedi cattle were classified with the Mongalla and the Abyssinian Shorthorned Zebu (Rege and Tawah, 1999). They are maintained by Hamitic, Nilotic and Bantu tribes, and hence various names are used. They are found mainly in north-eastern Uganda in the Pallisa, Iganga, Kamuli and Tororo areas; recently also in Soroti and Serere districts after recent cattle raids of the Teso district, where the Nkedi used to represent the dominant cattle. They are small and fine-boned, with varying hump sizes

but usually large and hanging backwards in the bull. The predominant colours are black, black-and-white, brown and brown-and-white (Plate.XIV), but grey, roan and brindle also exist (Rege and Tawah, 1999).

Serere

The Serere of south Teso around the shores of Lake Kyoga are quite similar to the Kyoga cattle. There are also reports that the Serere have evolved from interbreeding of the Nkedi with Ankole. This is supported by their larger body size and smaller hump of the Nkedi, reflecting Sanga attributes (Ankole) (Felius, 1995). Although they have some conformational similarities with the Nkedi, they are slightly larger, but the hump of the Serere is smaller, indeed inconspicuous, in cows (Plate.XV). Horns are typically similar to those of the Nkedi (short and thick at the base) but animals with long and thin horns similar to those of the Bahima cattle are also quite common (Rege and Tawah, 1999). The Zebus are good dairy animals with average milk yield of 693 kg over 239 days and 239 days of dry period (Galukande *et al*., 1992). Maturity was attained at 298.5 Kg and their age at first calving is 43 months with a calving interval of 420 days. Their weight at birth was 20.9 with a weight of 94.8 Kg at 6 month and a yearling weight of 195 (Kisamba, 1991).

2.3.2.2. Zenga

After the initial cattle plague (rinderpest) epidemic and decimation of the dominant Sanga populations, Zebu cattle were introduced from Asia into Africa at various points on the east coast of the continent, and interbreeding with Sanga remnants resulted in several Zebu-Sanga and Sanga-Zebu admixture populations. The breeds that emerged from these crosses have been classified in a separate group of "Zenga" cattle. Naturally, the Zenga are localized in eastern Africa, the natural point of contact of the Zebu and Sanga populations.

No breed improvement programmes exist for any of the Zenga. However, there is an on-going on-station characterisation of the Horro in Ethiopia (Rege and Tawah, 1999).

Nganda

The Nganda was developed out of interbreeding of Small East African Zebu in Uganda (mainly the Nkedi) and the Ankole Sanga (mainly the Bahima, but also the Watusi in recent times). The Ankole cattle have been mixed with the adjacent Bukedi Zebu since the 17th century (Rege and Tawah, 1999). The Nganda have white, long, round and thick based horns, while the hump is small and cervico-thoracic in location. The coat colour varies but red, black and brown are more common with a moderate dewlap (Plate. XVI). Their used for milk, work and meat (Rege and Tawah, 1999). They are very productive, with a calving interval of 362 days and a first calving at 52 months (Mbuza, 1995). Their lactation length was 267 days with a birth weight of 19.1 Kg and matured at 453 Kg with a yearling weight of 217.3 Kg (Mahadevan and Marples, 1991).

2.3.2.3. Sanga

The area of distribution of Sanga cattle extends from Eritrea, through Ethiopia, southern Sudan and the Great Lakes region of East Africa to southern Africa where they are the traditional cattle in all countries south of the Zambezi. It is also considered that before short-horned Zebu became dominant in eastern Africa, the Sanga was ubiquitous to this area. Ethiopia and environs are considered to be the centre of dispersal of the Sanga (Rege and Tawah, 1999).



Plate.XIII. Karamonjong cattle



Plate.XIV. Nkedi cow



Plate.XV. Serere cows

Sanga cattle vary in size from very small to large. The head is usually long, moderate in width with a convex or straight profile. The horns vary greatly in length, base circumference and orientation. Polled Sanga are rare. On the basis of horn size and form, there are two types: Those with long, relatively slender horns; and those with gigantic horns of great basal circumference.

The Sanga humps range from small, almost inconspicuous, to medium. Like in the Zebu, the hump is more prominent in bulls than in cows and is better developed in some breeds, than others. The hump is more thoracic (and larger) in areas with large Zebu influence. The Sanga hump is typically muscular in structure. While there are hardly any breed improvement programmes for the Sanga of Eastern Africa, the majority in Southern Africa have well-organised programmes and most have Breed Societies (Rege, 1999; Rege and Tawah, 1999).

Ankole

The original Ankole stock was supposedly brought to northern Uganda by the Bahima pastoral Nilotes from the north, from the epicentre of Sanga dispersal in the Ethiopian highlands, sometime between the 13th and the 15th centuries. Subsequently, the distribution of Ankole cattle shifted southwards and westwards due to natural selection pressure and human migration to avoid the disease-carrying tsetse flies. The Bahima settled in western Uganda, but some clans proceeded farther south, west of Lake Victoria, down the Eastern border of the then Tanganyika (Rege and Tawah, 1999).



Plate.XVI Nganda cattle

The Ankole of the Bahima are among the most typical Sanga and, until the recent conflicts in the Great Lakes region (civil strifes in Uganda in 1970s to 1980s and the conflicts in Rwanda and Burundi starting in the early 1990s), had been free from the Zebu influence seen in the Sanga of Ethiopia and Sudan. These cattle are large to medium in size, tall at the withers, but not particularly heavy. The horns are the most conspicuous and highly regarded feature of the Ankole cattle. The horns are very large, projecting from a pedestal-like base outwards and upwards, from where they may curve to form a circle or crescent or they may spread out. The hump is small and cervico-thoracic and the dewlap is moderately developed. Coat colour is mainly dark-red, but black, brown, white, grey and dun, as well as combinations may also be found (Plate.XVII). Almost all Bahima cows are milked, despite the low yields (Rege and Tawah, 1999).

Most of the Ankole heifers conceive for the first time between the ages of 2.5 and 4 years. However, some well-suckled heifers conceive for the first time between the ages of 1 and 2 years. The cows with short calving intervals commonly have little milk and those with long calving intervals can produce 7 – 10 litres. The well-fed cows will give milk for up to a year but towards the end of this period, the amount will decrease. Most Ankole cows normally produce one calf per calving but a few have twins and seldom do they produce three (Rege, 1999).

2.4 Conservation of domestic animal diversity

2.4.1 Meaning and importance of domestic animal diversity

Domestic animal diversity has been defined by Hammond (1993) as the spectrum of genetic variation existing among species, breeds and individuals of all animal species, which have been domesticated, and their immediate wild relatives. This genetic variation

has been developed during the millions of years of evolution to form and stabilize each species. Globally, there are about 40 species of domestic livestock (FAO, 1995), which have been domesticated by humans over the last 12,000 years. In the process of domestication, however, separate and genetically-unique breeds and strains have developed within each species as a result of human development and occupation of new areas over the planet.

According to FAO (1995), there are about 4,000 to 5,000 breeds and strains of domestic animals in the world, of which 250 are breeds of cattle (FAO, 2004). These breeds and strains are referred to as the global animal genetic resources and the genetic variation both between and within the breeds are described as the diversity within the species of domestic animals.

The selection process, both environmental- and human-directed, has resulted in much of the diversity existing between the breeds. Differences among the breeds have been created by reproductive isolation, often imposed by human-directed selection through pursuit of different breeding objectives and physical separation for various length of time. As a consequence of physical separation, each breed/strain has been adapted to particular ecological condition to suit the local climate and the requirements of the community. Thus, in the conservation of domesticated species, it is the diversity between breeds, rather than the variation between species, which is of crucial importance (Hammond, 1993; Barker, 1999).



Plate.XVII. Ankole cattle

Intra-specific diversity has got both within-population and between-population genetic components. Genetic variation at the population level consists of the differences in the types of alleles present and their frequencies across all members of a population considered together. Genetic variation within population is caused by change of allele frequencies over time due to selection, random genetic drift and gene flow (immigration from or emigration to other populations). Genetic variation within population is important as it is related to heterozygosity, which is known to enhance fitness-related characteristics (Allendorf and Leary, 1986).

Variability between populations arises both from random processes (founder effects, demographic bottlenecks, genetic drift and mutations) and from local selection imposed by environment and humans (Hartl and Clark, 1997). Variability between populations is also the result of adaptation of populations to their local conditions and it is important as locally-adapted populations may have particular genes or gene combinations critical for viability in their local environments. For conservation of animal diversity, it is important that both within-and between-population variation be maintained. This will enable better utilization of animals for production of food and agriculture to meet the current need of humankind while maintaining the potential to meet the range of future possible changes and needs (Hammond, 1993; FAO, 1995). This is because genetic diversity is the basis for a species' evolutionary flexibility and responsiveness to environmental changes.

The main reason to advocate conservation of genetic diversity is that the improvement of domestic animals in order to meet human needs, is dependent on genetic variation both within- and between-breeds/strains. Loss of genetic diversity will likely decrease the ability

of animals to respond to environmental changes and will result in loss of genetic information potentially useful for breeding improvement (Delgado *et al.,* 1999). The earth's climate varies widely and is continuously changing. It is the diversity of species and breeds that will allow the environment to be utilized efficiently throughout the years both in temperate and tropical areas. The urge of conservation, particularly in developing countries, is strengthened by the predictions of the scale of human population growth. It has been predicted that, human needs for food will double over the next two generations with the demand for animal products increasing more rapidly than that of plants (Hammond, 1993; Hammond, 1994; Delgado *et al.,* 1999). With increasing population pressure, the quantity of food and other products must increase. In developed countries, consumer emphasis on product quality is increasing. Changing the production level and product quality requires different types and varieties. In addition, changes in food and agricultural production will require new combinations of plant and animal species and lines (for example, breeds and varieties) and changes of management and production strategies. Sustainability in this new production system will require different genetic types (FAO, 1995).

The second reason to advocate conservation of domestic animal diversity is that, we can reasonably expect that large differences observed today between geographic regions in human needs for food and agriculture and in production capability will persist into the future. According to Hammond (1994) almost three quarters of the world agriculture will remain at the low- to medium-input levels where animal production environments incorporate combinations of stress such as feed shortage, drought and heat. Breeds in these environments are reservoirs of genetic information responsible for the adaptations necessary for production under adverse environment. Although they have been overlooked,

the needs for those, which are able to produce in hard conditions, is making and will make future important contribution to feeding humans (Simm, 1998).

A third reason for conservation of domestic animal diversity is that sustainable crossbreeding schemes require at least two viable purebred populations and sometimes more. Trends towards fewer breeds will reduce the opportunities for improvement of productivity through crossbreeding. The fourth reason is that the wide variety of livestock breeds and strains available today is part of our cultural heritage and it deserves protection. At the breed level, unique genetic differences exist among the breeds of each domestic species; thus, genetic diversity at the level of species can be exploited to satisfy human needs for varieties (Simm, 1998).

2.4.2 Loss of genetic diversity in domestic animals

Reduction in genetic diversity has been expressed primarily in terms of loss of breeds and strains. It is postulated that the current rate of extinction of species, breeds and strains is greater now than at any time in the past (Hammond, 1993). It is estimated that at least 30% to 40% of all animal genetic resources are currently at high risk of extinction (Hammond, 1994). However, adequate records do not exist to enable reliable estimates of either loss rates of the breeds or of domestic animal diversity itself. The existing data on the number of endangered breeds are likely to be underestimates of the magnitude of the problem. The loss of genetic diversity is occurring both within populations and among populations.

2.4.3 Loss of diversity within population

The factors that diminish genetic diversity within populations are genetic bottlenecks, random genetic drift, inbreeding and human activities. All these four factors are functions of population size (Lacy, 1987; Lande and Barrowclaugh, 1987), which are described in the following section.

2.4.3.1 Genetic bottlenecks

A bottleneck occurs when a large population experiences a severe, temporary reduction in size due to environmental or demographic events. For example, natural catastrophes, which occur at unpredictable intervals including event, like drought, disease outbreak and war. These events may kill a certain percentage of a population and therefore reduce the effective population size. The result is that the genetic variability of all subsequent generations is contained in the few individuals that survive the bottleneck and reproduce. Hence, some genetic diversity is lost in the process. The magnitude of the loss in diversity depends on the size of the bottleneck and the growth rate of the population afterward (Hunter, 1996). Another demographic event which may lead to bottleneck effect is the founder event. A founder event occurs when a few individuals of a population establish a new population. The genetic constitution of the new population depends on the genetics of the founder animals.

The genetic diversity of the original larger populations is reduced because the sample of genes in the few founder animals is not likely to be representative of the original gene pool. Generally, passing through a genetic bottleneck can create two problems (Carson, 1983): a loss of certain alleles, especially rare alleles, if no individuals possessing those alleles survive, and a reduction in the amount of variation in genetically-determined characteristics

due to the presence of fewer alleles and decline in heterozygosity. Baker and Moeed (1987) have shown that the founder populations of several species of introduced birds have fewer alleles and lower heterozygosity than the source populations. Bottlenecks have been shown to reduce allozyme heterozygosity. Leberg (1992) suggested that extremely-low levels of allozyme heterozygosity in broad geographical surveys imply the occurrence of one or more recent severe bottlenecks. The overall effect of bottlenecks is the decline in fitness of the individuals in the population.

2.4.3.2 Random genetic drift

Genetic drift is a random change in gene frequencies in small populations, attributable to sampling error. In small populations, each generation retains just a proportion of the gene pool of the previous generation. Subsequently, after many generations of random genetic drift, small populations will usually result in loss of variability as a consequence of fixation of some alleles on loci and loss of other alleles (Charlesworth *et al.,* 1994).

2.4.3.3 Inbreeding depression

Inbreeding results from the mating of two closely- related individuals. However, the degree of relationship may vary. “Close breeding” refers to the mating of very close relatives such as sibling to sibling or parent to offspring. Its probability of occurrence increases in small populations if mating occurs at random. Inbreeding allows the rare, harmful recessive alleles to become expressed in the homozygous form, with resulting harmful effects on the offspring (Charlesworth *et al.,* 1994; Lomker and Simon, 1994) such as reduction in fertility, fecundity, offspring size, growth and survival, and physical deformities. Since inbreeding depresses reproductive fitness, it is assumed to increase the risk of extinction. This presumption is supported by correlation between extinction and inbreeding on

laboratory and domestic animals. However, gene flow from outside populations is beneficial in avoiding inbreeding and the erosion of genetic diversity (Miller and Waits, 2003).

2.4.3.4 Human activities

Extinction of species, breeds and strains/varieties through human activities represents the greatest threat to genetic diversity. The major threats to genetic diversity that result from human activity are habitat destruction and degradation, pollution, introduction of exotic species, and over-exploitation (Frankham, 1994). These threats are all caused by an ever-increasing use of the world's natural resources due to expanding human population and development of market economy. The growth of towns, factories and mines in developing countries creates a cash market for livestock products. Consequently, the traditional farmers who formerly kept animals for their own needs begin to supply the cash market. Genetic variation is being lost as farmers in developing countries abandon their local breeds in favour of high-yielding breeds for commercial production. Even if human activities do not directly eliminate a breed or strain, loss of genetic variation is taking place as the number of individuals in these populations is reduced. In the long run, the population size of a breed or strain may become so small that the breed/strain is no longer viable and may eventually go extinct.

2.4.4. Loss of diversity among populations

Loss of genetic diversity among-populations occurs when historically divergent and isolated populations experience an artificially high rate of gene flow from other populations. In domestic animals, this is typically happening, as traditional farmers mix and interbreed their populations by moving from one place to another when they set up new

settlements. It also occurs through crossbreeding by increasingly promoting universal use of very few superior breeds per species to upgrade local breeds. The result is that the uniqueness of formerly-distinct breeds/strains is diminished or lost. Moreover, there are concerns that, if there exist co-adapted groups of genes within each sub-population, merging may result in the break-up of these and the loss of the particular adaptability of each sub-population to its environment (Frankham, 1994). Lacy (1987) has shown that the effect of gene exchange between sub-populations was to increase the variance within groups, decrease the variance between groups and decrease the total variance. According to Hammond (1994), about 50 % of the total variance for several quantitative traits in cattle, sheep and pig breeds is at the between-breed level, the remainder being common to all breeds. Hence, a move to one breed would eliminate half of the total variation. Loss of genotypic variance is irreversible as is the loss of alleles from a population. This is because it is impossible to sort out the alleles into groups of genotypes resembling the original sub-populations (Lacy, 1987) without prior knowledge at the molecular level of the genomes being lost.

2.4.5 Approach to conservation of animal genetic diversity

Conservation of genetic resources has become a major issue of public interest in the last decade or so. This is due to increase in awareness that genetic diversity is the basis for a species' evolutionary flexibility and responsiveness to environmental changes and that loss of genetic diversity will restrict the opportunities available to mould domestic animals to meet unpredictable future changes and requirements. Hence, genetic diversity needs to be preserved. The loss of variation within breeds is continually balanced by the introduction of new variation through new mutations; however, it is unwise to rely on new mutations as

they are rare events and generally have deleterious effects on reproductive fitness (Frankham, 1994). Moreover, the genetic variation present as differences among breeds cannot be regenerated. Each breed or strain is a result of mutation, genetic drift as well as separate adaptation and evolution, often over many generations following different selection pressures imposed by climate, endemic parasites and diseases, nutrition and human selection. Each breed thus comprises a unique set of genes, which must be conserved.

Conservation of domestic animal diversity has been defined as the sum total of all operations involved in the management of animal genetic resources so that the pool of genetic diversity is maintained over time (Hammond, 1993). It encompasses management of human activities in such a way that animal genetic resources are best utilized and developed to meet immediate and short-term human needs for future generations. Most attention in the conservation of animal diversity has been directed toward rare breeds. However, in the management of animal genetic resources, the fundamental problem is not the distinction between the breeds that are endangered and those that are not, but between those that are perceived to have little or no current utility and those which do have current utility or seem likely to have an immediate future use (Barker, 1994).

The critical issues in the conservation of domestic animal diversity according to Notter *et al.* (1994) include: (i) defining utilization strategies for available breeds to optimize production efficiency and sustainable utilization throughout the global range of environments and production systems; (ii) livestock improvement strategies that make appropriate use of global domestic animal diversity; (iii) development of optimum strategies for assessment and preservation of the genetic diversity, which exists in breeds

that have currently little or no value in commercial production. FAO (1995) has recommended the following strategies for effective management of domestic animal diversity at global level and for each species:

- Identifying and listing all breeds.
- Describing and characterizing breeds in order to understand their unique qualities and potential contributions and to understand which breeds have the potential to make the greatest variety of future contributions.
- Monitoring the population statistics for each breed and regularly reporting to the world the populations currently at risk of extinction.
- Sorting adequate samples of as many breeds as possible, generally in the form of frozen semen, ova, and embryos, to enable the future regeneration of lost populations of animals.

An overview of the historical background of animal genetic resources and the reasons for concern about their future, both in the developing and developed world, has been presented by Barker (2002). He concluded that the primary focus in the conservation of domestic animal diversity was on the conservation of breeds. In general, two methods are used for the conservation of genetic resources to support future livestock improvement: *in situ* conservation and *ex situ* conservation.

2.4.5.1 In situ conservation

This involves maintaining breeds in their farm environments. *In situ* conservation is primarily the active breeding of animal populations for food and agriculture, such that diversity is best utilized in the short-term and maintained for the longer term (Hammond, 1994). Operations pertaining to *In situ* conservation include performance recording and development of breeding programmes. It also includes ecosystem management for

sustainable production of food and agriculture. It has been suggested that the number of animals required in maintaining a breed in western countries ranges from 150 to 1500 breeding females, depending on the species and reproductive rate. However, in developing countries, the number should not decline below 5000 breeding females (Hodges, 1990). The advantages of this approach are that the animals are still being utilized, the performance characteristics can be properly recorded and evaluated, and the breeds have the opportunity to evolve. The disadvantages are that selection and genetic drift may result in unfavourable genetic changes if the population is small. There is a risk of increasing inbreeding and hence homozygosity, which is associated with reduced fitness. The animals are at risk from disease and other natural disasters. Also, they are likely to be less productive and so, more costly to maintain.

2.4.5.2 Ex situ conservation

In the context of conservation of domestic animal diversity, *ex situ* conservation means storage. Generally, three approaches are used for *ex situ* conservation. Maintaining breeds in farm parks or other animal collections**:** It involves the breeding of animals of a sample of a breed outside its normal production environment or habitat. Many of the pros and cons of this approach are similar to the *in situ* conservation method. However, there is potentially more control over management of the population. It has been suggested that 50 individuals should be the minimum number necessary to maintain genetic variability (Franklin, 1980). With such population, only 1 % of the genetic variability will be lost per generation. This figure is on the safe side. Indeed, it has been suggested that animal stocks can be maintained with a loss of 2–3% of the variability per generation. According to Notter *et al.* (1994) populations can be maintained at an inbreeding rate of 1% per generation

corresponding to an effective population size (*N*e) of 50%, which can be achieved with 14 males and 100 females under random mating.

Creating a conservation herd (gene pool): This involves crossing several rare breeds together, then breeding them to maintain genetic variability. It is an effective way of conserving genetic variation for two or three breeds. Maintenance of genetic diversity is almost better served by pooling five breeds in a conservation herd with *Ne* of 250 (Notter *et al.,* 1994). However, there is a greater risk of losing useful genes when more populations are combined. The disadvantage of this approach is that, although useful genes may be conserved, the identity of individual breeds is lost.

Cryopreservation: This involves frozen storage of rare breeds in the form of living semen, ova, embryos or tissues, which can be used to regenerate animals. Cryopreservation of semen and embryos is a powerful tool for the preservation of genetic diversity (Moore, 1985). In the situation of a critical threat with high probability that a breed will become extinct, preservation of genetic material of individual animals in the form of germ cells and embryos is necessary to ensure that an adequate genetic pool is retained for future improvement programme. Breeding technologies such as artificial insemination (AI) and embryo transfer (ET) may provide support for this approach. The use of frozen semen in conservation programme is particularly feasible when a tradition of use of AI is already strong. Collection of semen of endangered local breeds should take place as part of the AI programme. According to Smith (1984), semen from 25 unrelated sires would provide an exceptionally good sample of the genetic diversity of a breed.

Also Notter *et al.* (1994) recommended cryopreservation of semen from five breeds with ten sires from each breed and argued that this resource format provided greater preserved diversity than frozen semen of 25 sires of each of two breeds. For loci with multiple alleles, the probability of retaining all alleles from the rare breed is higher for the conservation herd supported with frozen semen than for a conservation herd maintained by random mating alone (Lomker and Simon, 1994). However, the ability to rapidly recreate living purebreds is a key issue in germplasm utilization. If frozen semen is used as the only method of conservation, then several generations of backcrossing are needed to reinstate the breed concerned. In contrast, breeds can be reinstated rapidly from frozen embryos. Embryo preservation is particularly important when the reproductive rate is low or the generation interval is long. Furthermore, maternal cytoplasmic genes (for example, mtDNA) cannot be preserved from semen, but can be effectively conserved by the maintenance of even a modest number of female lineages. However, for all species, the likely cost per haploid genotype maintained or per animal sampled is much less for semen than for embryos (Notter *et al*., 1994). In general, embryo preservation should be attempted only when embryo transfer (ET) is already in use for livestock improvement or when a breed has both clear potential value and a high likelihood of extinction (Notter *et al*., 1994).

Frozen storage of semen and embryos are relatively expensive, but it has the advantage that after the initial investment, semen and embryos provide much greater flexibility for finding ways of enhancing the reproduction of individuals, which are refractory to natural breeding conditions (Moore, 1985). In addition, the breed being conserved is free from unintended genetic changes (Simm, 1998). During storage, frozen genetic material is at less risk from disease and natural disasters than live animals, but obviously at risk from technological

failures. Splitting the material collected from a particular breed or population and storing it at different locations can minimize these risks. The disadvantages are that reproductive technologies are not uniformly successful or presently available for all individuals or all species and the expertise is not always available in the places where it is needed most. Also, the breeds conserved in this way are not able to adapt to changes in the production environment or new disease challenges. Semen or embryos frozen now may as well be unable to meet future requirements for improvement.

2.5 Genetic markers

To understand the history and evolution of populations, it is usually necessary to study a large number of polymorphisms (Cavalli-Sforza, 1998). Through molecular revolution over the last few decades, a lot of techniques have been developed to study population structure using genetic markers. At the first stage of research, almost all markers identified have been protein and blood group polymorphism, and only a few hundred were previously known (Nei and Roychodhury, 1988). These markers are also known as 'classical polymorphisms' to distinguish them from those obtained by direct deoxyribonucleic acid (DNA) analyses.

The use of DNA segments to analyze genetic polymorphisms has resulted in the identification of a great number of markers which is of great benefit as a lot of markers are needed to detect population structure and selection signatures within populations, consequently identifying quantitative trait loci (QTL) affecting traits of economic interest (MacNeil and Grosz 2002; Thaller *et al.,* 2003), as well as qualitative trait loci (Drögemüller *et al.,* 2005; Drögemüller *et al.,* 2009; Fontanesi *et al.,* 2010). Commonly considered DNA markers are Microsatellites and Single Nucleotide Polymorphisms (SNPs or 'snips').

2.5.1 Microsatellites

Microsatellites (MS) are direct tandem repeated sequences of DNA with a repeat size ranging from 1 to 6 base pairs (bp). Hence, a microsatellite is also called a simple sequence repeat (SSR). To identify microsatellite loci suitable for use as genetic markers, different approaches have been developed. One of the prevailing methods to identify microsatellites is the use of primers, which are commonly designed directly from the sequence data. In this context, the primers serve to detect the variation of microsatellites, by flanking the different numbers of repeats provided by Polymerase Chain Reaction (PCR) (Saiki *et al.,* 1988; Newton and Graham, 1997).

Microsatellites are highly polymorphic in many animal and plant species (Morgante and Olivieri, 1993; Kim *et al.,* 2004). The relatively high variability of microsatellites has allowed a large number of population structure studies. Prior to 2004, approximately 90% of all projects used MS markers while Single Nucleotide Polymorphism (SNP) markers were only investigated in about 10% of all projects (Baumung *et al.*, 2004). In contrast to the past decade, recent analyses in human (Hannelius *et al.,* 2008), cattle (The Bovine HapMap Consortium 2009) and sheep (Kijas *et al.,* 2009) show that currently genome wide SNP are the most robust and efficient markers for uncovering comprehensive and unbiased population structures.

2.5.2 Single Nucleotide Polymorphisms (SNP)

A SNP is a small (single base pair) genetic change, or variation, that can occur within an individual's DNA sequence. This property of SNPs allows us to make associations between marker alleles with quantitative trait locus (QTL) affecting important economic traits in livestock. It has been proven that these genome-wide studies are the most powerful method

for identifying genes that are responsible for important traits, for example, Myostatin (MSTN) for double muscling within animals (Hill *et al.,* 2010) as well as for undesirable genes such as bovine leukocyte adhesion deficiency (BLAD), complex vertebral malformation (CVM) and congenital muscular dystony (CMD) (Charlier *et al.*, 2008).

Single Nucleotide Polymorphisms are a good choice of marker for these studies because of their low mutation rate, high incidence throughout the genome and bi-allelic nature making them amenable to automated detection techniques (Dawson, 1999). It has been estimated that a well-designed genome-wide SNP map requires as many as 500,000 SNPs in human (Vega and Kreitman, 2000) and likely up to 300,000 SNPs in cattle due to the breed bottlenecks for the most popular cattle breeds in the world (Eck *et al.*, 2009; Shannon, 2010). Hence, the numbers of available SNP chips are increasing annually for example, within, cattle six different SNP chips have been released in the last three years namely Illumina's BovineHD and LD, Neogen's GCP-LD and GGP_HD 1 and 2, and Affymetrix's Bos1 which complements the *de facto* standard Illumina SNP50 (Michael, 2010).

Through the ongoing innovations in high-throughput sequencing and array technology, the number of validated SNP is rapidly increasing, for example, The Human International HapMap Consortium is currently using a second generation haplotype map spanning up to 3.1 million SNPs (The International HapMap Consortium, 2007). This rapid development in the SNP chip technology also provides new insights into the population structure of cattle breeds since previous studies have been focused on small sets of microsatellite loci although more than 50million SNP have been identified in cattle but no all this data are publicly available.

2.6 Genetic distances in population genetics

The measure of genetic distance allows one to quantify genetic relationships between two individuals. It is used to describe the proportion of genetic elements (alleles, genes, gametes and genotypes) that the two individuals do not share. Depending on the kind of similarities (*S*) between individuals, most genetic distances vary from 0 to 1 for example; there is a genetic distance of 1, when two samples have no genetic element in common. Depending on the similarities of individuals (*S*), genetic distances (*D*) can be calculated in three different ways (The International Plant Genetic Resources Institute, Cornell University, 2003).

1. D = 1 – *S*, known as linear distance, because it assumes that the relationship with *S* is linear (Figure 2.4A).
2. D = $(1-S)$, known as quadratic distances, the similarity relationship follows a quadratic function, so that, to make it linear, the square root must be calculated (Figure 2.4B).
3. D = (2) $1-S$, describes a circular distance (Figure. 2.4C).

2.6.1 Genetic distances between individuals using the proportion of shared alleles

To determine the genome-wide average proportion of shared alleles between two animals the PLINK software is used (Purcell *et al.*, 2007), where the genome-wide average proportion of alleles shared identical by state (IBS) between animals *i* and *j* is generally defined as

$$AS = \frac{1}{L} \sum_{l=1}^{L} a_l$$

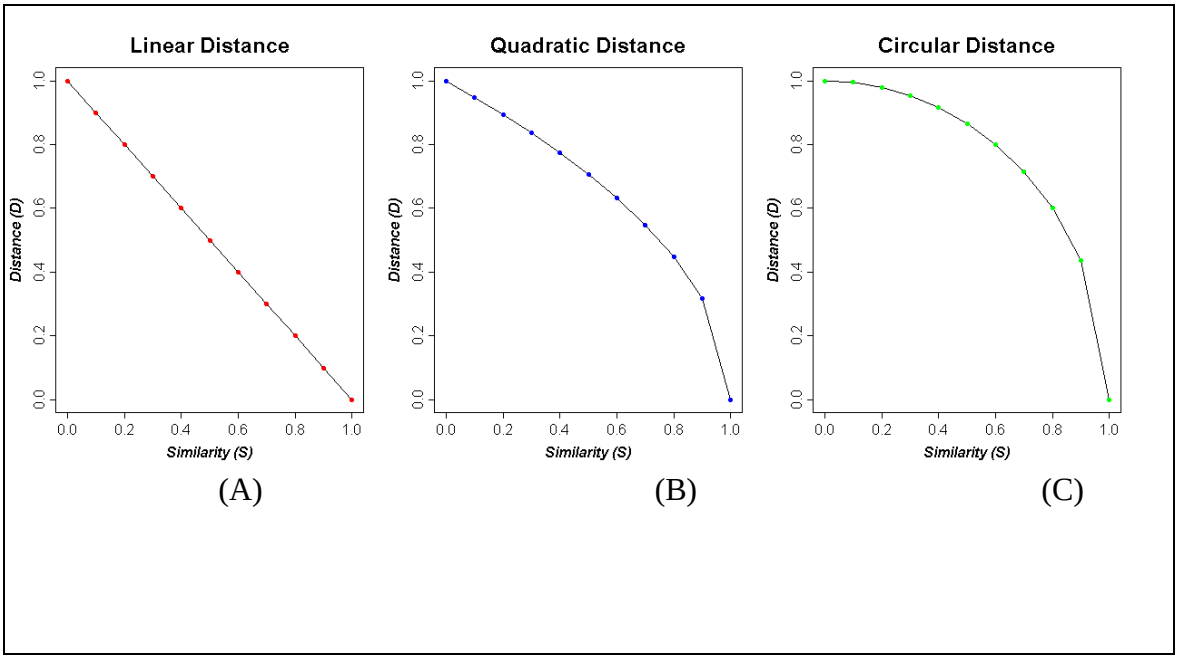


Figure. 2.4. Relationship between Similarity (*S*) and Genetic Distance (*D*). (A) Linear Distance, (B) Quadratic Distance and (C) Circular Distance. Source: (The International Plant Genetic Resources Institute, Cornell University, 2003)

where a*l* = 0, if animal *i* and *j* have no allele in common at the l-th locus,

a*l* = 1, if animal *i* and *j* have only a single allele in common at l-th locus,

a*l* = 2, if animal *i* and *j* have two alleles in common at the l-th locus,

and L is the number of SNP loci used.

The genome- wide proportion of shared alleles generally describes a linear relationship between animals, thus this value can be easily transformed into a so-called allele sharing distance (ASD) by subtracting it from 1. Another widely-used method in cattle breed analyses is the genomic relationship matrix presented by VanRaden (2008), since this approach additional considers allele frequencies within populations besides the alleles shared by IBS. Another possibility to determine genetic distances are based on genetic models considering allele frequencies for example, Wright's F- statistics (Wright, 1931)

and Nei`s standard genetic distance (Nei, 1972). The major difference between ASD and the classical model-based distances is that in the computation of classical ASD allele frequencies are completely ignored (Gao and Starmer, 2007).

2.6.2 Genetic distances between sub-populations

One of the classical methods to measure population differences is the afore-mentioned F statistics. Wright`s F- statistics use genotypes of pre-defined sub-groups to calculate variance in allele frequencies, which may also be used to measure the genetic distance of sub-populations. This concept is based on the idea that those sub-populations that are not inter-mating will have different allele frequencies to those of the whole population. The genetic distance calculated by F statistics is termed FST. The FST values ranges from 0 (no genetic divergence) to 1 (fixation of alternate alleles in different subpopulations), with values approaching 0.25 determining a high degree of genetic differentiation between populations (Mousadik and Petit, 1996; Davies *et al.,* 1997).

The determined FST values between sub-populations are normally presented by distance matrices and phylogenetic networks using common available software packages for example, TREEVIEW (Page, 1996) and SPLITSTREE (Huson and Bryant, 2006). The two additional indexes provided are F_{IS} and F_{IT} which measure the deficiency of average heterozygotes in each population and the excess of average heterozygotes in a group of populations respectively. For a preferable vertical interpretation of the results provided by this analysis, it is necessary to separate the individuals into appropriate sub-groups. At present, this population separation is mostly based upon geographical origin of samples or phenotypes (Liron *et al.*, 2006).

2.7 Methodologies in population structure analysis

To date, many statistical methods have been proposed to examine the relatedness among populations and to assign individuals to their population of origin. The two major methods currently applied are distance-based clustering methods, which utilize dimensionality reduction techniques for example, Principal Component Analysis (PCA) in combination with standard clustering tool for example, k-means (Liu and Zhao, 2006; Paschou *et al.,* 2007; Lee *et al.,* 2009) and model-based methods for example, STRUCTURE (Pritchard *et al.,* 2000; Falush *et al.,* 2003).

2.7.1 Distance based methods

Principal Component Analysis (PCA) is a technique for linear dimension reduction of data complexity, by transforming original vector of correlated variables to the vectors of principal components which are uncorrelated. Through this process, a new coordinate system with inherent statistical properties emerges, with the first principal components explaining the main variance in the data (Menozzi *et al.,* 1978). In this context, PCA is providing an optimal sub-space to investigate data variation in complex population structures and to allocate individuals to their respective population using a common applied clustering algorithm (Patterson *et al.,* 2006; Price *et al.,* 2006). The k-means algorithm is an iterative descent algorithm that minimizes the within-cluster sum of squares (WSS) given the number of cluster K.

$$W_K = \sum_{i=1}^{K} \sum_{j \in C_i} \left\| x_j - \mu_i \right\|^2,$$

where x_j is the feature vector representing sample j,

μ_i is the center of cluster *i*, and C_i is the set of samples in cluster *i*.

Principal component analysis followed by k-means describes a two-stage design that utilizes the proportion of shared alleles between individuals to infer population clusters. The main features of this two-stage design are, that it is computational efficient and assigns individuals to subpopulations without the need of any modeling of data (Liu and Zhao, 2006). Principal component analysis is closely related to multivariate technique called Multi Dimensional Scaling (MDS).

Indeed, PCA is equivalent to applying MDS on the distance matrix representing the data. Hence, this approach is also termed distance-based clustering method (Gao and Starmer, 2007). Both approaches have been recently introduced in population structure analyses (Paschou *et al.,* 2007; Kijas *et al.,* 2009). They are providing an optimal sub-space to investigate genome-wide data sets including thousands of individuals by maximising variance on the first components (Behar *et al.,* 2010). However, the current Bovine HapMap study reported that PCA is becoming impractical and less useful for the analysis of larger data sets (The Bovine HapMap Consortium, 2009).

2.7.2 Model based methods (STRUCTURE/ADMIXTURE)

Model-based methods use standard statistical methods to estimate population parameters, and usually assume Hardy-Weinberg equilibrium for each population. The inference may not be good in the presence of small sample sizes due to the inaccurate estimation of allele frequencies (Gao and Starmer, 2007). Model-based inference also depends heavily on the modeling assumptions. The programme STRUCTURE is a popular model-based programme using Markov chain Monte Carlo (MCMC) within a Bayesian framework (Pritchard *et al.,* 2000). This method has been shown to be an effective and popular

procedure in numerous studies (Lao *et al.*, 2006; Kim *et al.*, 2005). However, the detailed modeling of the data causes intensive computational demands and associated costs (Liu and Zhao, 2006; Patterson *et al.*, 2006; Price *et al.*, 2006), making this algorithm impractical to be applied on high-density genome-wide SNP panels. Furthermore, it has been noted that the identification of the true number of clusters (K) in a sample of individuals is not sufficiently accurate by the algorithm applied (Evanno *et al.*, 2005). Therefore, Evanno *et al.* (2005) introduced an *ad hoc* statistic *_K* for STRUCTURE to identify stable cluster solutions.

However, the final cluster assignments are still very sensitive on the choice of prior distribution parameters of the data used. For example, in the study of McKay *et al.* (2008) the *ad hoc* statistic *K* identified two clusters in a data sub-set containing eight distinct cattle breeds. Recently, alternative model-based algorithms as implemented in the programmes ADMIXTURE (Alexander *et al.*, 2009) and FRAPPE (Tang *et al.*, 2005) have been used for uncovering genome-wide population structures. These algorithms are computationally efficient and can be easily applied on genome-wide data sets to infer individual ancestry. However, these methods are still sensitive to the choice of prior distributions of model parameters and rely on *a priori* ancestry information (for example, ADMIXTURE should only be considered for unrelated individuals).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of the study

This study of indigenous cattle breeds was carried out in the various parts of the countries of Uganda for East Africa and Nigeria for West Africa (Figure.3.1).

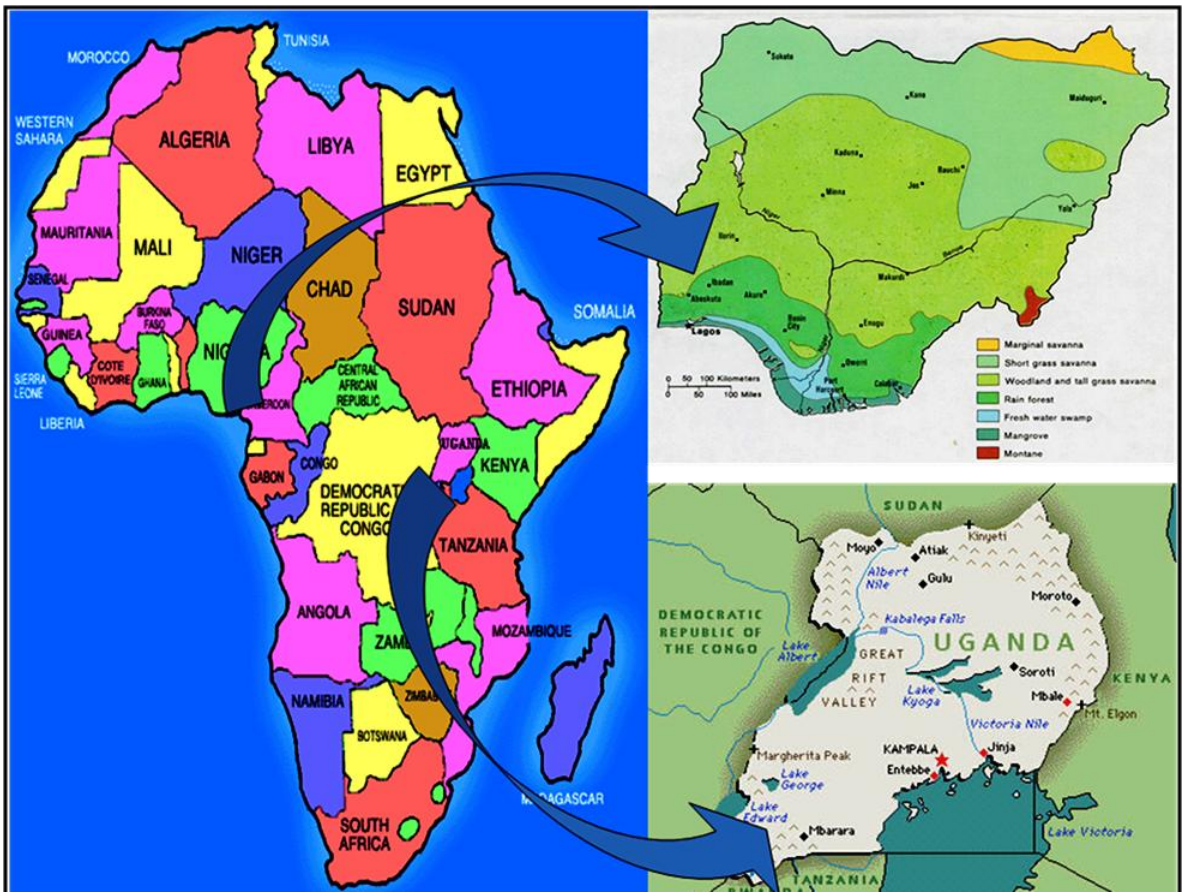


Figure. 3.1. A map of Africa showing the location of Nigeria in West Africa and Uganda in East Africa

3.1.1 Nigeria

Nigeria, the most populous country in Africa, is situated on the Gulf of Guinea in West Africa. Its neighbours are Benin, Niger, Cameroon, and Chad (Figure.3.2). The lower course of the Niger River flows south through the western part of the country into the Gulf of Guinea. Swamps and mangrove forests border the southern coast; inland are hardwood forests. Nigeria weather is highly influenced by the south-western winds and the north-eastern winds. The south-eastern winds are full of moisture and blusters from the Atlantic Ocean whereas the northern easterlies are dry and dirt-laden winds that primarily blow from the deserts of Sahara. The rainy seasons come into existence due to the movement of the Inter-Tropical Convergence Zone winds into the northern region of Nigeria. The south-west winds bring about rainfall in the extreme southern areas of Nigeria. Nigeria's climate is arid to semi arid in the north, tropical in the center, and equatorial in the south. Variations are governed by the interaction of moist south-west monsoon and dry north-east winds. Mean maximum temperatures are 30º C–32º C in the south and 33º C–35º C in the north. High humidity is characteristic from February to November in the south and from June to September in the north. Low humidity coincides with the dry season. Annual rainfall decreases north ward; rainfall ranges from about 2,000 mm in the coastal zone (averaging more than 3,550 mm in the Niger Delta) to 500–750 millimeters in the north (Irmiya and Shadrach, 2010).



Figure 3.2. A map of Nigeria showing the sample areas for the various indigenous cattle populations

Key

- Sokoto Gudali (SO)
- Red Bororo (OR)
- Adamawa Gudali (AG)
- Kuri (KU)
- Wadara (WD)
- Bunaji (BJ)
- Friesian × Bunaji (FB)
- N'Dama (ND)
- Azawak (AZ)
- Keteku (KT)
- Yakanaji (YK)
- Muturu (MT)

3.1.2 Uganda

Uganda is located in Eastern Africa, west of Kenya, south of South Sudan and east of the Democratic Republic of the Congo (Figure.3.3). It is in the heart of the Great Lakes region, and is surrounded by three of them, Lake Edward, Lake Albert, and Lake Victoria. While much of its border is lakeshore, Uganda is land-locked with no access to the sea. The latitude and longitude of Uganda are 1° 0' 0" N and 32° 0' 0" E, respectively. Uganda enjoys a tropical climate with two dry seasons from December to February and from June to August. Other than that it mostly encounters rainy days with Lake Victoria as a major source of atmospheric moisture and thunderstorms. Also Southwesterly wind from Congo brings rain to this country. Uganda weather has got an ideal condition. While the lowlands enjoy a warm weather and the Kigezi highlands enjoy a cool atmosphere most of the time, Uganda encounters bright sunny days with the average temperature of 26°C. It rarely rises above 29°C. The highest temperature can be 35°C while the lowest can be 8°C. Humidity ranges from 70% to 100%, while the hot dry season is from December through February. Intermittent rains start in March with the heavier rainy season being in April and May. The cooler dry season is from June through October. The month of November has short rains including intermittent showers and some flash flooding. Rainfall ranges between 500 mm to 2500 mm. The country is completely landlocked. It is 800 km inland from the Indian Ocean (Bakama and Bakama, 2011).



Figure.3.3. A map of Uganda showing the sample areas of the various indigenous cattle populations.

3.2 Tissue sampling

Tissue blocks were sampled from a total 272 unrelated animals comprising the five (5) indigenous cattle breeds from various parts of Uganda and twelve (12) populations from Nigeria where the cattle breeds are indigenous as shown in Table 3.1. The tissue block samples were obtained from the ears of the animals using Allflex ear punch tissue sample collector and aliquoted into plastic tubes containing the Allflex tissue preservative. To load the Allflex Tissue Applicator, the punches from the box supplied were removed and assembled as one piece, since they came apart, reassembled. The tube retainer at the base of the applicator gun was opened. Twisting the retainer base until the retainer was opened, the punch was inserted into the tissue applicator and the tube retainer was twisted back to lock the punch into the device. Removing the red plastic clip by pushing it downward until it unclips, the applicator was fully loaded and ready to take a sample. The gun was slid over the ear, and the cutter positioned at least 1cm to 2cm from the edge of the ear with care to avoid any obvious veins and ridges. The handles were squeezed together to take a sample and then released to free the ear in one swift, fluid motion. The punch was then removed from the device and checked to see whether the sampling was successful, after which the sample was recorded and put in the punch supply box for further analysis (Genemark, 2013).

Table.3.1. Summary of the indigenous cattle populations sampled from Nigeria and Uganda with their locations and global positioning system (GPS) coordinates.

Breeds	Breed Code	Number of samples	Location	GPS coordinates
Nigeria				8°.00□ N
Sokoto Gudali	SO	21	Sokoto	10°.00□ E
Red Bororo	OR	22	Sokoto	13°.04□ N
Adamawa Gudali	AG	25	Yola, Adamawa	5°.14□ E
Kuri	KU	2	NAPRI, Zaria	13°.04□ N
Wadara	WD	3	NAPRI, Zaria	5°.14□ E
Bunaji	BJ	23	NAPRI, Zaria	9°.13□ N
Friesian × Bunaji	FB	24	NAPRI, Zaria	12°.27□ E
N'Dama	ND	23	NAPRI, Zaria	11°.04□ N
Azawak	AZ	2	NAPRI, Zaria	7°.42□ E
Keteku	KT	13	Ilorin	11°.04□ N
Yakanaji	YK	12	Ilorin	7°.42□ E
Muturu	MT	12	Fashola, Oyo	11°.04□ N
Uganda				7°.42□ E
Karamonjong	KR	16	North-East (Katakwi)	11°.04□ N
Serere	ZS	13	North-East (Serere)	7°.42□ E
Sahiwal × Serere	SW	13	North-East (Serere)	11°.04□ N
Nganda	NG	23	Central (Wakiso)	7°.42□ E
Ankole	AO	25	West (Mbarara)	11°.04□ N
				7°.42□ E
				8°.30□ N
				4°.33□ E
				8°.30□ N
				4°.33□ E
				7°.85□ N
				3°.93□ E
				1°.00□ N
				32°00□ E
				01°.54□ N
				34°.00□ E
				01°.30□ N
				33°.33□ E
				01°.30□ N
				33°.33□ E
				00°.23′ N 2°.28′ E
				00°.36□ N
				34°.00□ E

3.3 Deoxyribonucleic Acid (DNA) extraction

DNA was extracted from the tissue cells using commercial kits, QIAamp DNA Tissue Mini Kit (Qiagen DNeasy kit, 2011) according to the manufacturer's specifications and procedures. AE buffer was placed into 70°C water bath and the Allflex tissue tube containing the sampled tissue was cut to remove the fresh ear tissue block using sterile foreceps and placed onto a sterile cutting surface. The ear tissue was sliced thoroughly using a sterile razor blade to increase the surface area and to allow quicker and more complete lysis of tissue and placed into a 2ml tube. Then 180 µl of Buffer ATL and 20 µl of Proteinase K were added respectively. The mixture was incubated at 56°C until completely lysed and vortexed occasionally until lysis was complete in 2-3 hours. After incubation, the supernatant was briefly centrifuged in the tube to collect condensate. Then 200 µl of Buffer AL was added, mixed by vortexing for 15 seconds, incubated in the tube at 70°C for 10 minutes and briefly centrifuged to collect condensate. Then 230 µl of ethanol (96-100%) was added and mixed by vortexing for 30 seconds to yield a homogeneous solution. Carefully, the sample was applied to a QIAamp Spin Column (in a 2-ml collection

tube) without wetting the rim and then centrifuged at 6000 xg for 1 minute; the spin column was then placed in a clean 2 ml collection tube and the filtrate discarded. Then 500 µl of Buffer AW1 was added without wetting the rim and centrifuged at 6000 xg for 1 minute, spin column, placed in a clean 2-ml collection tube and the filtrate discarded. 500 µl of Buffer AW2 was added without wetting the rim and centrifuged at full speed for 1 minute, spin column, placed in a clean 2-ml collection tube and the filtrate discarded. Then the sample was centrifuged at full speed for 3 minutes; placed spin column in a labeled 1.5 ml-tube and the filtrate discarded to ensure removal of any residual Buffer AW2 which otherwise could hinder downstream applications. 200 µl of the pre-heated (70°C) Buffer AE was added and the tube incubated at 70°C for 5 minutes and then centrifuged at 6000 xg for 1 minute and the filtrate solution (~200µl) was placed back onto the spin column, incubated the tube at 70°C for 5 minutes and then centrifuged at 6000 xg for 1 minute to increase the final concentration and total amount of DNA obtained. The spin column was discarded and then the DNA concentration and quality was determined using a nannodrop and placed the extracted DNA into storage at -20° C for further analysis.

3.4 Single Nucleotide Polymorphisms (SNP) genotypes

The SNP genotypes of all animals were determined on about 777,962 SNP present on the Illumina BovineHD. Approximately 300 ng of genomic DNA from each animal was processed according to the manufacturer's protocol (BovineHD Genotyping Beadchip, 2012). Beadchip analyses were done on an Illumina iScan instrument Image files were analysed in Genome Studio software from Illumina and marker genotypes were output using the PLINK final report function (Purcell *et al.,* 2007). SNP quality threshold criteria were as follows: marker call rates > 95%, Marker Allele Frequency (MAF) < 1e-05,

departure from Hardy-Weinberg Equilibrium (HWE) (Wiggintion *et al.*, 2005) P < 0.01 in at least one population and P < 0.02 in at least two populations using SNP and Variation Suite v7 in Golden Helix (2013). After filtering, 671,317 SNPs were retained for 272 animals and various SNP files were generated according to the country of origin.

3.5. Data Analyses

3.5.1. Determination of the genetic diversity in the indigenous cattle populations from Nigeria and Uganda

Genetic diversity within each population was determined by estimating the expected hetreozygosity (H_E) or gene diversity, Gene flow and Wright's F_{IS} (Wright, 1978)

i. Expected Hetreozygosity: The gene diversity or expected heterozygosity (HE) estimates the probability that two randomly-chosen alleles from the breed are different. The unbiased estimator of HE provided by Weir (1996) at the l-th locus is hereby defined as $H_E = \frac{1-\sum_{i=1}^{n} Pi^2}{1-\frac{1+f}{n}}$

where *Pi* is the frequency of the l-th of *k* alleles and *f* is the inbreeding coefficient across animals (*n*).

ii. Gene flow was determined by the private allele method by Slatkin (1993).

Thus Gene flow (N(nm)) = $\frac{1}{4}\left(\frac{1}{FST} - 1\right)$

where *FST* is the genetic differentiation among the sub-populations

iii. Inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984) were calculated for allozymes and SNPs data using PLINK vs1.07 programme (Purcell, 2007). The significance of F_{IS} estimates was determined by permuting alleles within the total population, genotypes within the total population and alleles within samples, respectively, with 5000 permutations (Goudet, 2002) i.e, $F_{IS} = 1 - \frac{H_I}{H_S}$

Where: H_I = intrapopulation gene diversity or average observed heterozygosity in a group of populations

H_S = average expected heterozygosity estimated from each subpopulation

iv. Deviation from Hardy–Weinberg equilibrium (HWE; heterozygote deficiency) was assessed by performing a chi-square test (Liu and Muse, 2005) for SNPs in the various population.

v. Relatedness is often defined as family-relatedness but identity by descent (IBD) estimation can also detect duplicate samples, duplicate samples from one of a pair of genotyping chips but not the other, or sample contamination. Percentage Identities (PI) was computed from IBD (Identity by Decent) using Golden Helix (2013)

3.5.2 Determination of the population structure and admixture levels in the indigenous cattle populations from Nigeria and Uganda

To explore the possible underlying factor structure of the studied indigenous breeds of cattle from Nigeria and Uganda without imposing any preconceived structure on the outcome, exploratory factor analysis was used to estimate the unique factors using the

PROC FACTOR procedure of SAS, (2004) and means separated according to Victor *et al.*, (2008).

The population structure of 12 cattle breeds for Nigeria and the 5 cattle breeds for Uganda was first analyzed with Principal Component Analysis (PCA) using 671,317 SNPs in an unsupervised clustering approach implemented in the Golden Helix software. Criteria were set to 10,000 iterations on low-dimensional data to separate the individuals to their respective populations (Golden Helix, 2013).

Estimation of genetic admixture proportions of the studied cattle breeds from Nigeria and Uganda were estimated using a coalescent approach (mY) (Bertorelle and Excoffier, 1998) which takes into account allele frequencies and the molecular distances between alleles. Alleles at African Zebu, African Taurine and European Taurine diagnostic loci and the programme Admix 2.0 (Dupanloup and Bertorelle, 2000) were used to estimate mY. The parental populations used were the following: P1 = a pool of genes from the African Zebu breeds, P2 = a pool of genes from the African Taurine breeds, and P3 = a pool of genes from the European Taurine breeds.

ADMIXTURE1.2 software was used to determine the admixture levels of the cattle populations from Nigeria, Uganda and Nigeria and Uganda combined using 671,317 SNP data sets. ADMIXTURE programme estimated the cattle breeds ancestry in a model-based manner from large autosomal SNP genotype datasets, where the individuals were unrelated. ADMIXTURE's cross-validation procedure was used to determine the true number of clusters in the cattle breeds. A good value of K exhibited a low cross-validation error compared to other K values. Cross-validation was enabled by simply adding the Coefficient

of Variation (CV) flag to the ADMIXTURE command line to perform 5-fold CV reporting the cross-validation error in the output for K in 1, 2, 3, 4 and 5 (Alexander *et al.*, 2009).

3.5.3 Determination of the genetic relationship within and between the indigenous cattle populations from Nigeria and Uganda

Three different sets of cattle SNPs for the three different analyses (Nigeria, Uganda and Nigeria and Uganda), were used to do a network clustering analysis (phylogenetic trees. For all the analysis, sex chromosomes were excluded. After the quality control, F_{ST} values between the breeds were computed with R package Geneland (Weir and Cockerham, 1984) and the visualization of the relationship matrices in UPGMA was done with SPLITSTREE.4 software programme (Huson and Bryant, 2006).

CHAPTER FOUR

4.0 RESULTS

4.1. Genetic diversity in the indigenous cattle populations from Nigeria and Uganda

The experimental levels of genetic variability within the different indigenous cattle populations sampled in Nigeria and Uganda are shown in Table 4.1. Among the Nigerian breeds (N = 182), observed heterozygosity varied between 0.582 (Friesian × Bunaji) and 0.773 (Muturu) with a mean of 0.676. In the Ugandan breeds (N = 90), observed heterozygosity varied between 0.622 (Nganda) and 0.648 (Karamonjong) with a mean of 0.639. It was observed that the highest heterozygosity was expressed by Muturu (0.773), followed by the N'Dama (0.702) and Kuri (0.695) with the other breeds expressing statistically similar observed heterozygosities except Friesian × Bunaji which had the

lowest (0.582). Except for the Nganda which had the lowest (0.622), all the cattle populations from Uganda had statistically similar observed heterozygosities.

The expected heterozygosity ranged from 0.642 (for Muturu) to 0.638 (for Red Bororo) with a mean of 0.641 in the Nigeria cattle populations while it ranged from 0.635 (Karamonjong) to 0.632 (Serere) with a mean of 0.633 in the Ugandan cattle populations. The mean values of observed and expected heterozygosites in Nigerian cattle populations were generally higher than those in Ugandan cattle populations.

The gene flow (*N(nm)*) value combining all the 12 Nigerian breeds, amounted to 0.979 and that of Ugandan cattle breeds amounted to 0.953 (Table 4.1). In turn, when gene flow was examined within breed and country, gene migration in the Nigerian cattle population ranged from 0.981 (Muturu) to 0.975 (Red Bororo) with the greatest gene migration, observed in the Karamonjong (0.955), while the lowest was observed in the Serere (0.950) in the Uganda cattle populations. Generally gene flow was higher in the Nigerian cattle breeds than in the Ugandan cattle breeds.

Within population inbreeding coefficient (F_{IS}) per population in the Nigerian cattle populations ranged from 0.386 (Muturu) to -0.177 (Friesian × Bunaji), while the within population inbreeding estimates (F_{IS}) for the Uganda cattle population ranged from 0.041 (Karamanjong) to -0.033 (Nganda). The mean F_{IS} for the West African cattle populations was 0.105 with the East African breeds exhibiting a mean F_{IS} of 0.016. Muturu showed the highest inbreeding coefficient (0.386), with Friesian × Bunaji showing the least F_{IS} of

-0.177 while all the other breeds from Nigeria had statistically similar inbreeding coefficients. In cattle populations from Uganda, it was observed that except for the Nganda

which had the lowest inbreeding coefficient (-0.033), all the breeds had statistically similar inbreeding coefficients. Generally, the Nigerian and Ugandan cattle breeds had very low

Breeds	N	H_O	H_E	N(nm)	F_{IS}

inbreeding coefficients. The F_{IS} values for all the cattle breeds in both Nigeria and Uganda were significantly different ($P<0.05$) from zero and except for Friesian × Bunaji (-0.177) in Nigeria and Nganda (-0.033) in Uganda, positive F_{IS} were observed in this study.

All populations showed statistically significant deviations from Hardy–Weinberg Equilibrium (HWE) at one or more loci (Table 4.2). The majority of polymorphic SNPs were found to be in HWE, both across the whole sample and in each separate population. For the Nigerian cattle populations, only 36,674.036 (5.46%) of 671,317 SNPs markers were calculated to significantly deviate from HWE ($p < 0.01$), while in the Ugandan cattle populations, 491.130 (0.07%) of the markers appeared to be in disequilibrium.

Table.4.1. Summary statistics of the overall genetic diversity parameters on the indigenous cattle populations from Nigeria and Uganda

Nigeria					
Sokoto Gudali (SO)	21	0.661[c]	0.641	0.979	0.060[b]
Red Bororo (OR)	22	0.669[c]	0.638	0.975	0.092[b]
Adamawa Gudali (AG)	25	0.665[c]	0.642	0.980	0.068[b]
Kuri (KU)	2	0.695[b]	0.641	0.979	0.158[b]
Wadara (WD)	3	0.663[c]	0.641	0.979	0.063[b]
Bunaji (BJ)	23	0.670[c]	0.641	0.979	0.087[b]
Friesian × Bunaji (FB)	24	0.582[d]	0.641	0.979	-0.177[c]
N'Dama (ND)	23	0.702[b]	0.641	0.979	0.181[b]
Azawak (AZ)	2	0.678[c]	0.641	0.979	0.108[b]
Keteku (KT)	13	0.693[c]	0.642	0.980	0.151[b]
Yakanaji (YK)	12	0.667[c]	0.640	0.978	0.077[b]
Muturu (MT)	12	0.773[a]	0.642	0.981	0.386[a]
Total	182				
Mean		0.676	0.641	0.979	0.105
Uganda					
Karamonjong (KR)	16	0.648[a]	0.635	0.955	0.041[a]
Serere (ZS)	13	0.639[a]	0.632	0.950	0.022[a]
Sahiwal × Serere (SW)	13	0.640[a]	0.633	0.952	0.020[a]
Nganda (NG)	23	0.622[b]	0.633	0.951	-0.033[b]
Ankole (AO)	25	0.644[a]	0.635	0.954	0.030[a]
Total	90				
Mean		0.639	0.633	0.953	0.016

[a, b, c, d] For a given factor, means within the same column for a given country with different letters or superscripts differ significantly ($P<0.05$)

N = Number of individuals tested per population

H_o = Observed Heterozygosity

H_e = Expected Heterozygosity or gene diversity

N (nm) = Gene flow

F_{IS} = Fixation Index (Inbreeding coefficient within individual)

The Percentage Identities (PI) as computed from IBD (Identity by Decent) in the Nigerian and Ugandan cattle populations are shown in Table.4.3. Cattle breeds from Nigeria had a

percentage identity range of 0 to 0.406 for minimum percentage identity, 0.188 to 0.819 for maximum percentage identities, respectively. The highest percentage identities were observed in the Azawak, Muturu, Wadara and Kuri which had 0.407, 0.401, 0.304 and 0.358, respectively. For the Ugandan breeds, the minimum percentage identities (PI) ranged from 0 to 0.737 and the maximum percentage identities (PI) ranged from 0.540 to 0.842, respectively. The percentage identities (PI) in Uganda, was generally in a close range from 0.010 to 0.153.

4.2 Population structure and admixture levels in the indigenous cattle populations from Nigeria and Uganda.

4.2.1 Exploratory factor analysis of the indigenous cattle populations from Nigeria and Uganda

The exploratory factor analysis to study the population structure of the indigenous breeds of cattle from Nigeria and Uganda in the various principal component analyses are shown in Table 4.4. The various breeds of cattle from Nigeria significantly ($P<0.01$) influenced Factor 1, 2, and 3. There was no significant effect ($P>0.05$) on Factor 4 and Factor 5. It was observed that Sokoto Gudali (0.047), Red Bororo (0.053), Adamawa Gudali (0.053), Wadara (0.053), Bunaji (0.045), Azawak (0.056) and Yakanaji (0.055), explained the highest positive total variation at Factor 1 while Kuri (-0.521), Friesian × Bunaji (-0.030), N'Dama (-0.104), Keteku (-0.036), and Muturu (-0.147) gave lowest negative variations at Factor 1. All breeds gave high positive variation except for the Friesian × Bunaji (-0.176) at

Table. 4.2. SNP deviation from Hardy-Weinberg Equilibrium (HWE) in the cattle populations from Nigeria and Uganda

Breed ID	N	H_o	H_e	HWE	P<0.01
Nigeria					
Sokoto Gudali (SO)	21	444026.5	430347.2	434.768	**
Red Bororo (OR)	22	449390.2	428540.91	1014.347	**
Adamawa Gudali (AG)	25	446232.6	430800	552.844	**
Kuri (KU)	2	466394.5	430500	2984.156	**
Wadara (WD)	3	444867	431600	472.706	**
Bunaji (BJ)	23	449994	430404.35	910.368	**
Friesian × Bunaji (FB)	24	390419.2	430500	3731.639	**
N'Dama (ND)	23	471196.5	430217.39	3903.424	**
Azawak (AZ)	2	454896	430400	1394.178	**
Keteku (KT)	13	465047	430815.38	2720.030	**
Yakanaji (YK)	12	447682.5	429883.33	736.996	**
Muturu (MT)	12	518753.5	431108.33	17818.580	**
Total	182			36674.036	**
Uganda					
Karamonjong (KR)	16	435250.3	426394	188.125	**
Serere (ZS)	13	428686.5	424054	50.607	**
Sahiwal × Serere (SW)	13	429362.8	425115	42.445	**
Nganda (NG)	23	417752.1	424670	112.693	**
Ankole (AO)	25	432469.1	426032	97.261	**
Total	90			491.130	**

** P<0.01

N = Number of individuals tested per population,

H_o = Observed Heterozygosity

H_e = Expected Heterozygosity or gene diversity

HWE = Hardy Weinberg Equilibrium

Table.4.3. Percentage identities as determined by Identity by descent (IBD) in the indigenous cattle populations from Nigeria and Uganda.

Breed ID	N	PI	Min	Max
Nigeria				
Sokoto Gudali (SO)	21	0.076	0	0.538
Red Bororo (OR)	22	0.071	0	0.120
Adamawa Gudali (AG)	25	0.103	0	0.555
Kuri (KU)	2	0.358	0.358	0.358
Wadara (WD)	3	0.304	0.299	0.309
Bunaji (BJ)	23	0.064	0	0.106
Friesian × Bunaji (FB)	24	0.164	0	0.500
N'Dama (ND)	23	0.188	0	0.188
Azawak (AZ)	2	0.407	0.407	0.407
Keteku (KT)	13	0.104	0	0.819
Yakanaji (YK)	12	0.169	0	0.464
Muturu (MT)	12	0.401	0.340	0.812
Uganda				
Karamonjong (KR)	16	0.117	0	0.553
Serere (ZS)	13	0.153	0.093	0.553
Sahiwal × Serere (SW)	13	0.123	0.737	0.842
Nganda (NG)	23	0.106	0	0.586
Ankole (AO)	25	0.104	0	0.540

PI = Percentage Identity
Min = Minimum Percentage Identity
Max = Maximum Percentage Identity

Factor 2. Except for N'Dama and Bunaji (0.143 and 0.003) respectively, the rest of the breeds gave negative variations at Factor 3 with the lowest observed in Muturu (-0.158).

For cattle populations from Uganda, breed significantly ($P<0.01$) influenced Factor 1 and Factor 2. In the Uganda cattle populations, Karamonjong (0.074), Serere (0.138) and Sahiwal × Serere (0.103) gave the highest total variation at Factor 1 and Factor 2. The Karamonjong (0.007), Nganda (-0.079) and Ankole (-0.099) gave negative variations at Factor 1 while Karamonjong (-0.003) and Ankole (-0.112) gave negative variations with the exception of Nganda (0.100) which had a positive variation at Factor 2.

Communality ranged from 5.127 (Azawak) to -4.833 (Muturu) in the Nigerian cattle population while for the Ugandan cattle population, communality ranged from 4.025 (Serere) to -4.638 (Ankole). It was observed that four (Kuri, Keteku, Friesian × Bunaji and Muturu) and three (Karamonjong, Serere and Ankole) had negative communalities while the rest of the cattle populations from Nigeria and Uganda had positive communalities, respectively. Generally, the Nigerian cattle breeds showed superiority in their genetic uniqueness than the Ugandan cattle populations.

4.2.2 Population structure of the indigenous cattle populations from Nigeria

The Nigerian cattle populations were clearly separated according to their various population structures and subpopulation structures, with the first and second principal components (PC1 and PC2) explaining 10.72% and 4.82%, respectively, of the total variation (Figure 4.1). On clustering of breeds, PC1 and PC2 explained approximately 15.54% of the total variation where the African Taurine (N'Dama, Muturu and Kuri) breeds

were separated from indicine breeds (Zebu), while the admixed breed (Keteku) was intermediate.

Also the Friesian × Bunaji which are crossbreeds of the European Taurine (Holstein Friesian) and the African indicine (Bunaji) separated from the rest of the African Taurine and indicine breeds. PC1 and PC2 also revealed that the structure of Keteku (KT) being mostly an introgression of Muturu and Bunaji. It was also observed that Sokoto Gudali separated far from the other indigenous Zebus. The third principal component (PC3) accounted for approximately 2.94% of the variation and somewhat isolated the Muturu from the N'Dama animals. The results also indicated that the Keteku is introgressed from Muturu (Figure 4.2). No clear genetic distinction was evident between the humped breeds and no distinct clusters were observed in the Nigerian cattle populations at the first and fourth principal components (PC1 and PC4) (Figure 4.3), first and fifth principal components (PC1 and PC5) (Figure 4.4) and first and sixth principal components (PC1 and PC6) (Figure 4.5).

4.2.3 Population structure of the indigenous cattle populations from Uganda

There were distinct clusters in PC1 and PC2, PC1 and PC3, PC1 and PC4 (Figures 4.6a, 4.6b, 4.7 and 4.8). Clustering was based on the sub-populations and geographical regions where the breeds were sampled, from Western Uganda to the far north-eastern corner of the Ugandan cattle belt. The first and second principal component explained 2.06% and 1.60%, respectively (Figures 4.6a and 4.6b) hence, explaining approximately 3.66% of the total variation, whereas the third and fourth principal components (PC3 and PC4) accounted for approximately 1.19% and 0.98%, respectively. The Ankole (Sanga and from Western Uganda) clearly separated from the central widely-spread Nganda (Zebu × Sanga = Zanga

from Central Uganda) which was also separate from the Large East African Zebu (Karamonjong from Far North Eastern Uganda) and the Small East African Zebu (Serere from North Eastern Uganda). The Sahiwal × Serere × Zebus crosses (SW) clustered together with both the Large East African Zebu (Karamonjong) and the Small East African Zebu (Serere).

There was a clear evident cluster among breeds from the Sanga, Zanga, Large East African Zebus and Small East African Zebus with the Zebu admixed. Figure 4.6.b, clearly separated the four originally indigenous breeds of cattle in Uganda at PC1 vs PC2. No clear genetic clustering was evident among the Ugandan cattle populations at the first and fifth principal component (Figure 4.9) and first and sixth principal component (Figure 4.10).

4.2.4 Population structure of the indigenous cattle populations from Nigeria and Uganda

PCA evidently distinguished Nigerian cattle populations from Uganda, with the first and third principal components (PC1 and PC3) explaining 12.49% and 3.26%, respectively, of the total variation (Figure 4.13). On clustering of the populations according to their country of origin, PC1 and PC3 explained approximately 15.75% of the total variation, whereas the fourth principal component (PC4) accounted for approximately 3.06% of the variation (Figure 4.14). Figure (4.11) showed that Nganda had European Taurine like Friesian × Bunaji at the first and second principal components explaining 5.35% and 12.49%, respectively.

Table. 4.4. Exploratory factor estimates and communalities of indigenous cattle populations from Nigeria and Uganda.

Breed	N	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Communalities (%)
Nigeria							
Sokoto Gudali (SO)	21	0.047[a]	0.015[a]	-0.003[b]	0.003	-0.025	0.758
Red Bororo (OR)	22	0.053[a]	0.019[a]	-0.003[b]	-0.001	-0.031	0.746
Adamawa Gudali (AG)	25	0.053[a]	0.020[a]	-0.002[b]	-0.007	-0.034	0.581
Kuri (KU)	2	-0.052[b]	0.023[a]	-0.021[b]	0.014	-0.013	-0.972
Wadara (WD)	3	0.053[a]	0.019[a]	-0.001[b]	-0.010	0.118	3.577
Bunaji (BJ)	23	0.045[a]	0.019[a]	0.003[b]	-0.001	-0.019	0.923
Friesian × Bunaji (FB)	24	-0.030[b]	-0.176[b]	-0.013[b]	0.027	0.003	-3.763
N'Dama (ND)	23	-0.104[b]	0.036[a]	0.143[a]	-0.008	0.0003	1.347
Azawak (AZ)	2	0.056[a]	0.022[a]	-0.004[b]	-0.018	0.200	5.127
Keteku (KT)	13	-0.036[b]	0.040[a]	-0.068[b]	0.0002	-0.015	-1.582
Yakanaji (YK)	12	0.055[a]	0.021[a]	-0.004[b]	-0.016	0.155	4.234
Muturu (MT)	12	-0.147[b]	0.066[a]	-0.158[c]	-0.005	0.002	-4.833
Uganda							
Karamonjong (KR)	16	0.074[a]	-0.003[b]	-0.002	-0.080	-0.035	-0.931
Serere (ZS)	13	0.138[a]	0.029[a]	0.009	0.051	-0.025	4.043
Sahiwal × Serere (SW)	13	0.103[a]	0.012[a]	0.005	0.033	-0.014	2.779
Nganda (NG)	23	-0.079[b]	0.100[a]	0.026	-0.044	-0.014	-0.224
Ankole (AO)	25	-0.099[b]	-0.112[b]	-0.029	0.047	-0.038	-4.638

[a, b,] For a given factor, means within the same column for a given country with different letters or superscripts differ significantly ($P<0.05$)

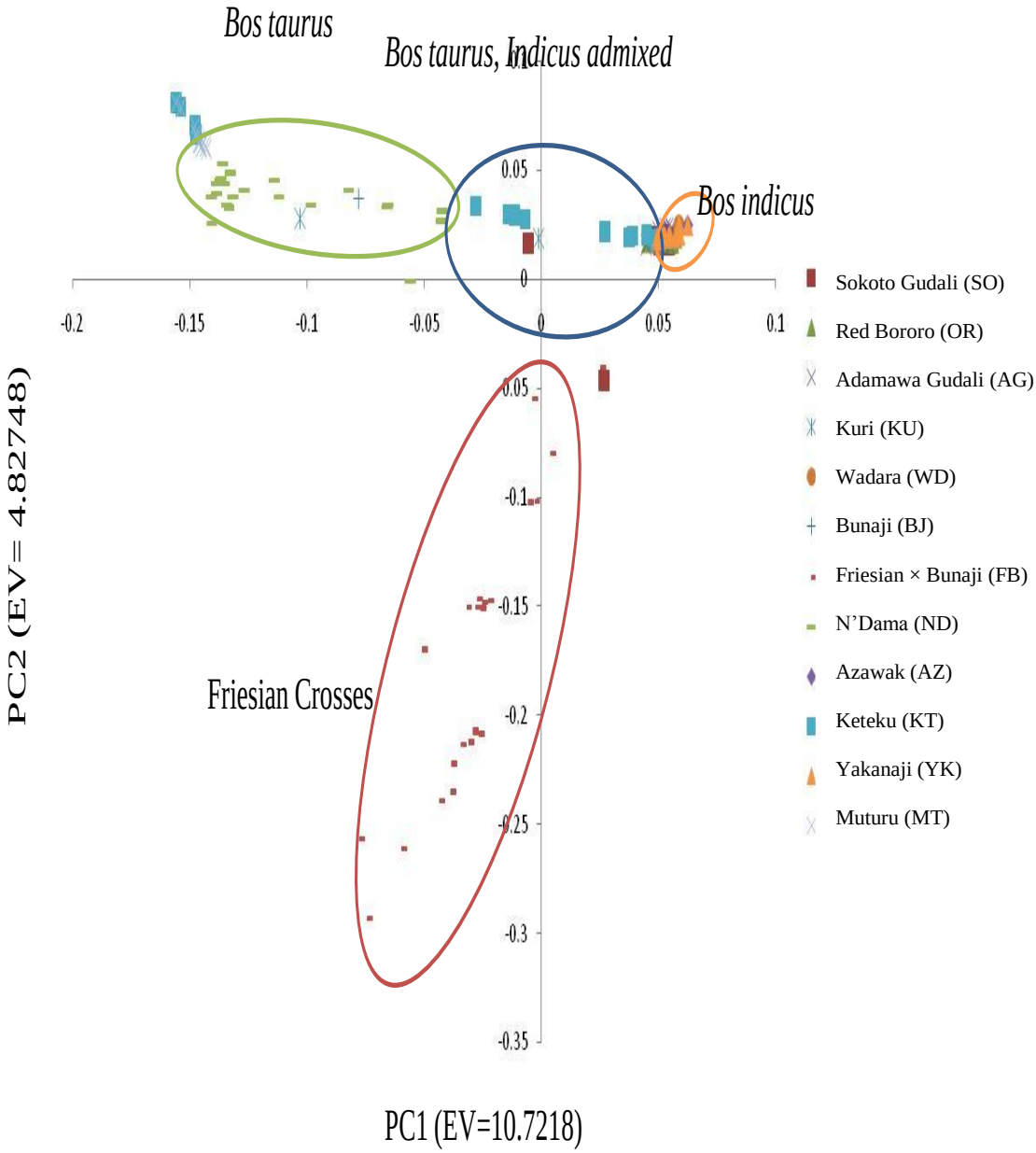


Figure 4.1 Bi-dimensional graph representing the relationship between PC1 vs PC2 using BovineHD genotypes derived from 12 indigenous cattle populations from Nigerian.

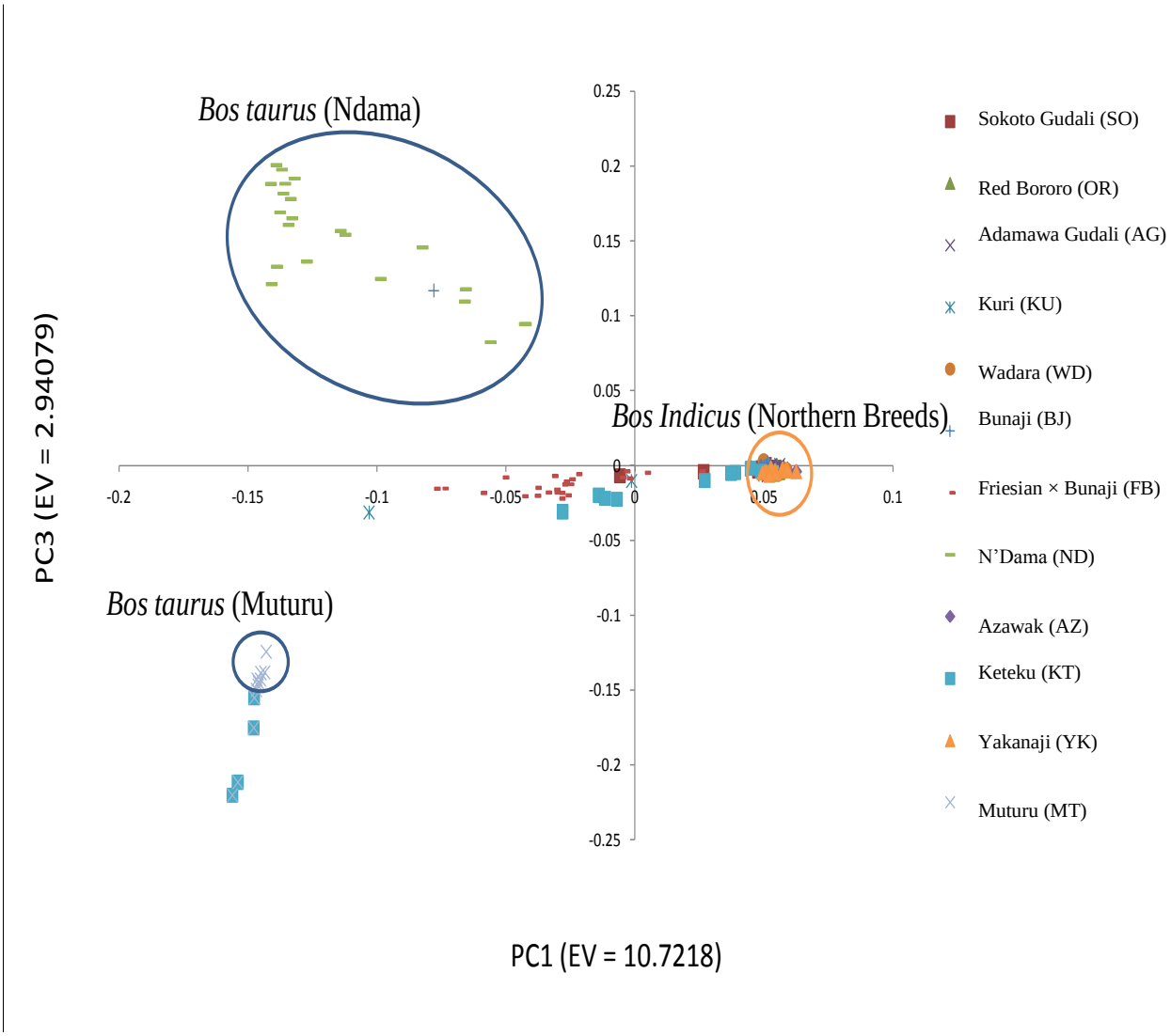


Figure 4.2 Bi-dimensional graph representing the relationship between PC1 vs PC3 using BovineHD genotypes derived from 12 indigenous cattle populations from Nigeria

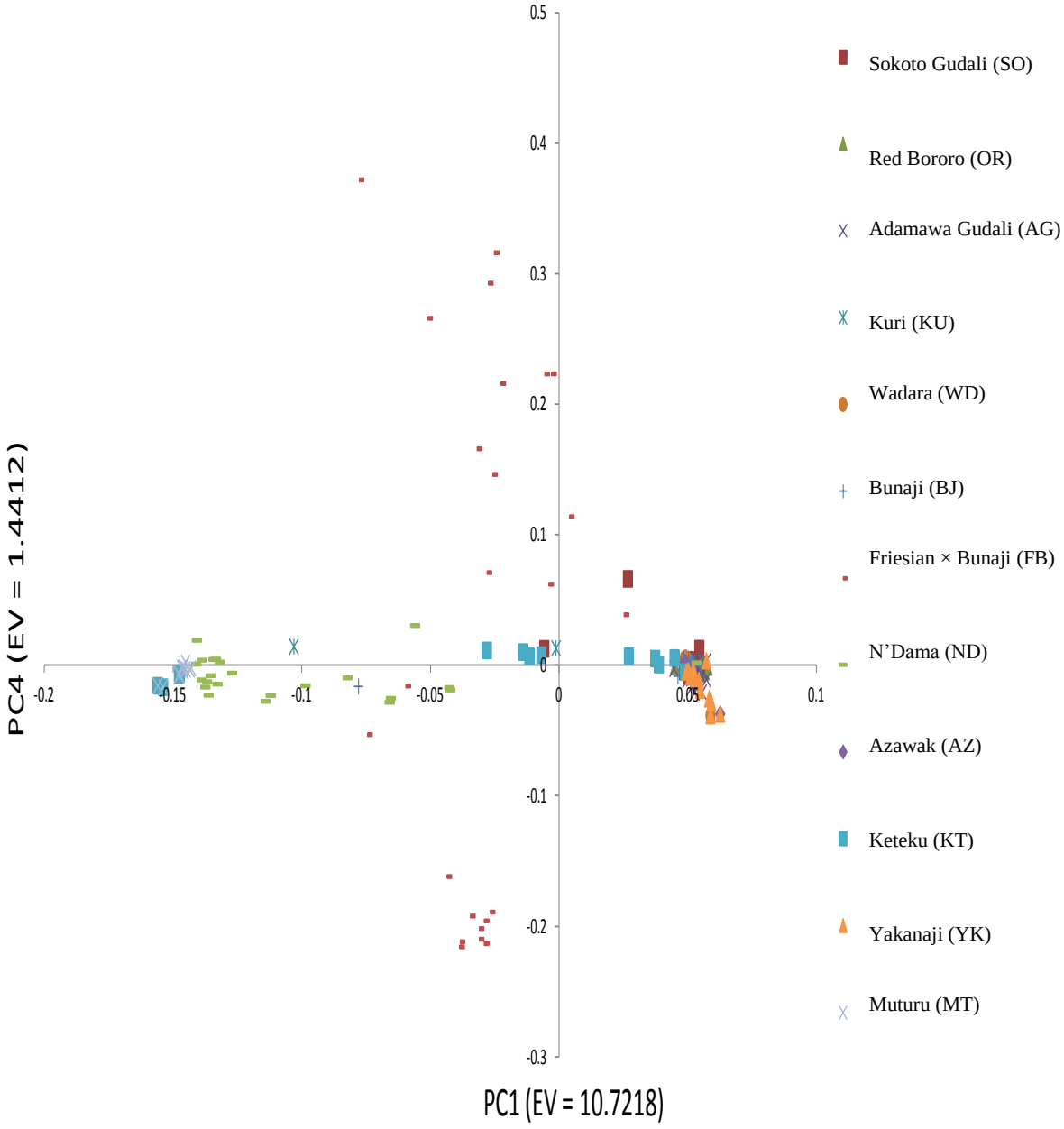


Figure 4.3 Bi-dimensional graph representing the relationship between PC1 vs PC4 using BovineHD genotypes derived from 12 indigenous cattle populations from Nigeria

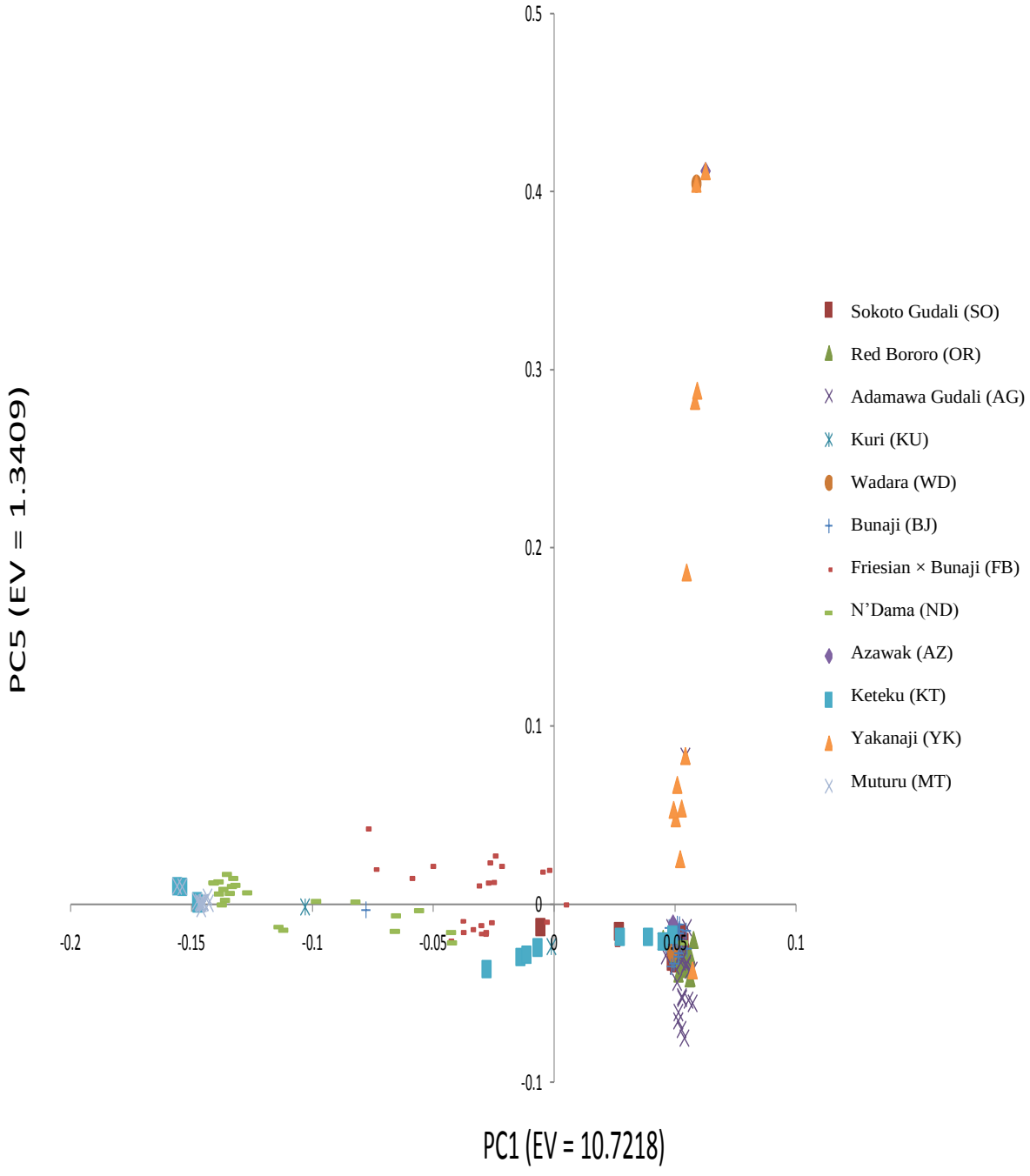


Figure 4.4 Bi-dimensional graph representing the relationship between PC1 vs PC5 using BovineHD genotypes derived from 12 indigenous cattle populations from Nigeria

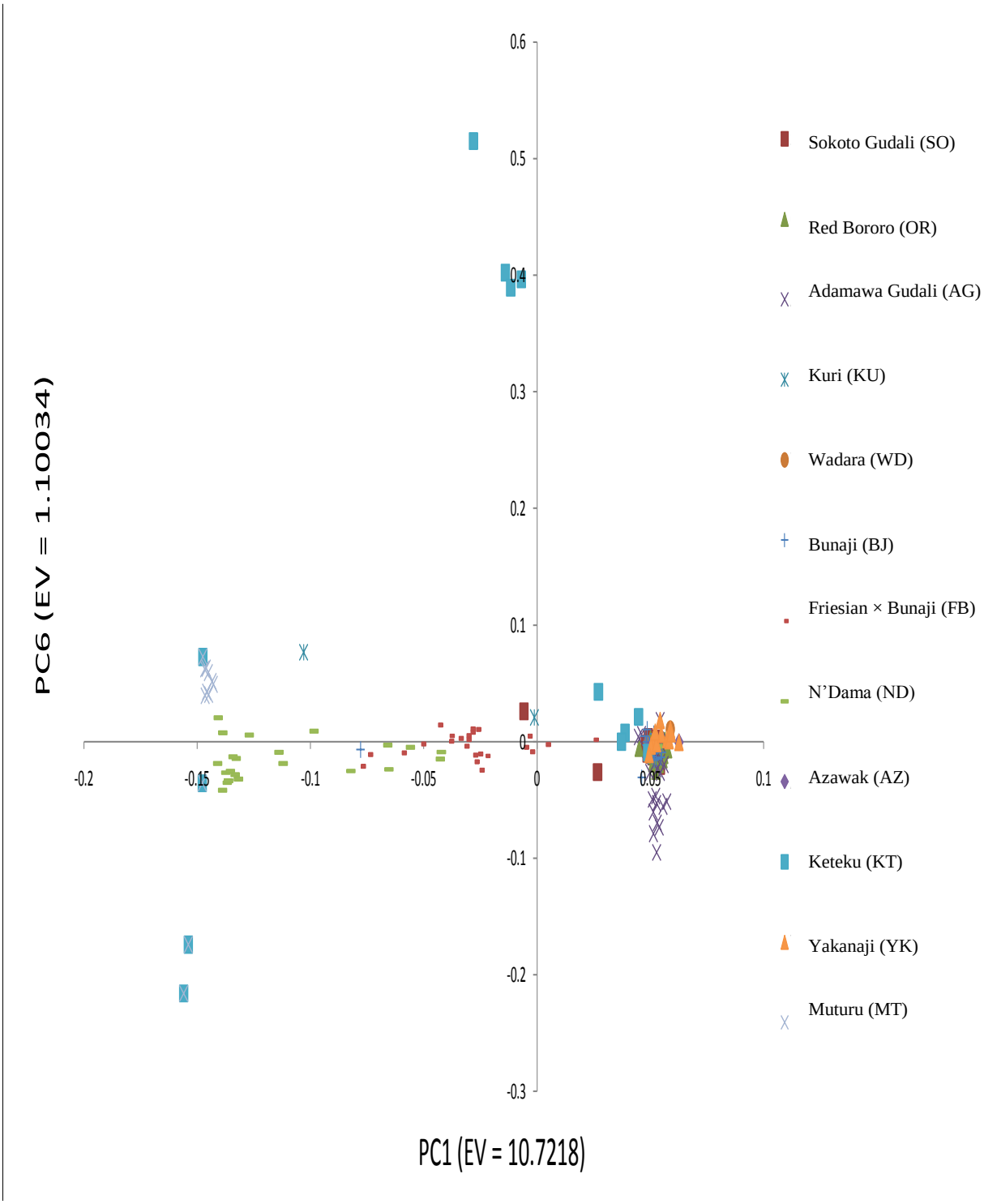


Figure 4.5. Bi-dimensional graph representing the relationship between PC1 vs PC6 using BovineHD genotypes derived from 12 indigenous cattle populations from Nigeria

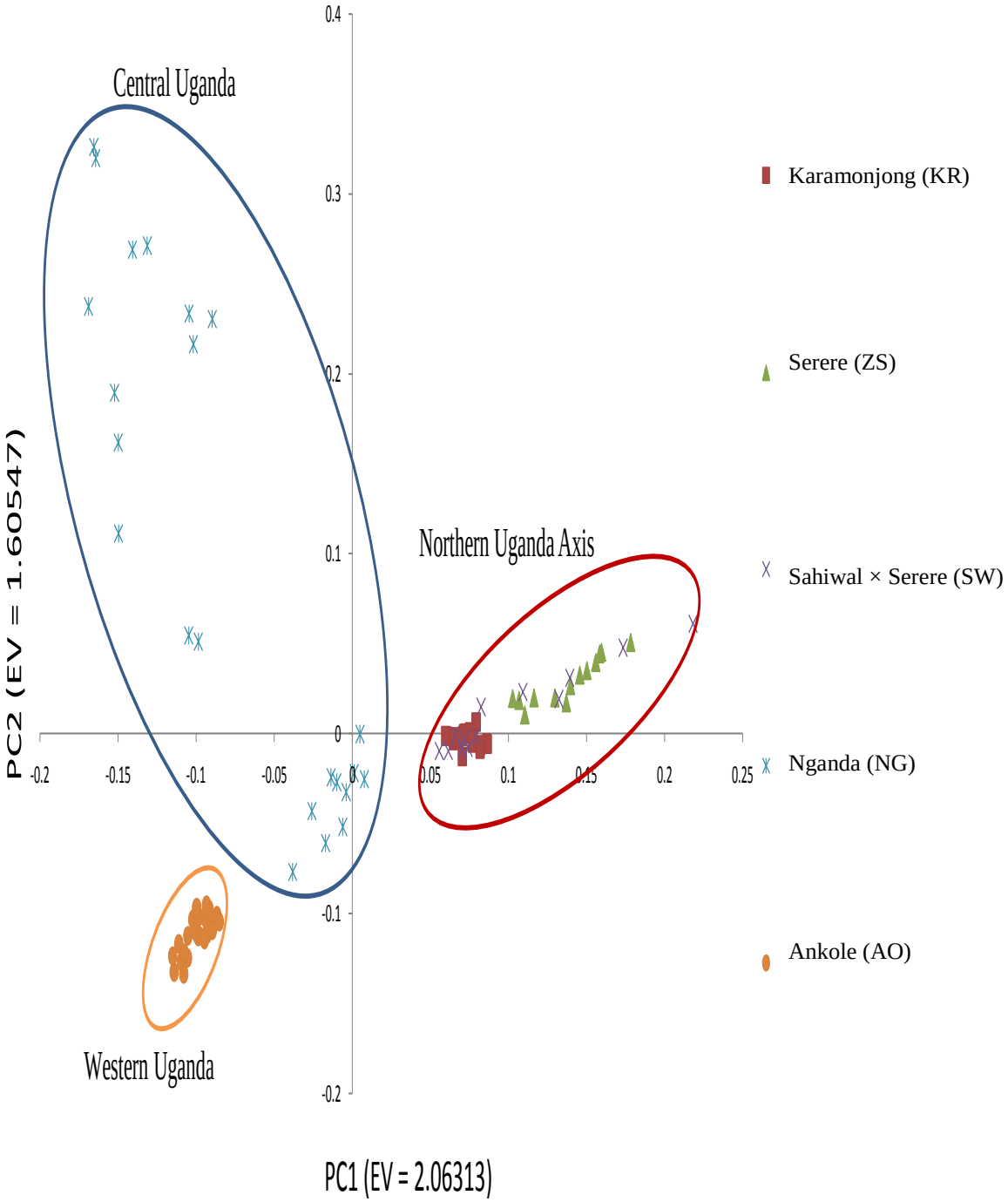


Figure 4.6a Two-dimensional graph representing the relationship between PC1 vs PC2 using BovineHD genotypes derived from 5 indigenous cattle populations from Uganda

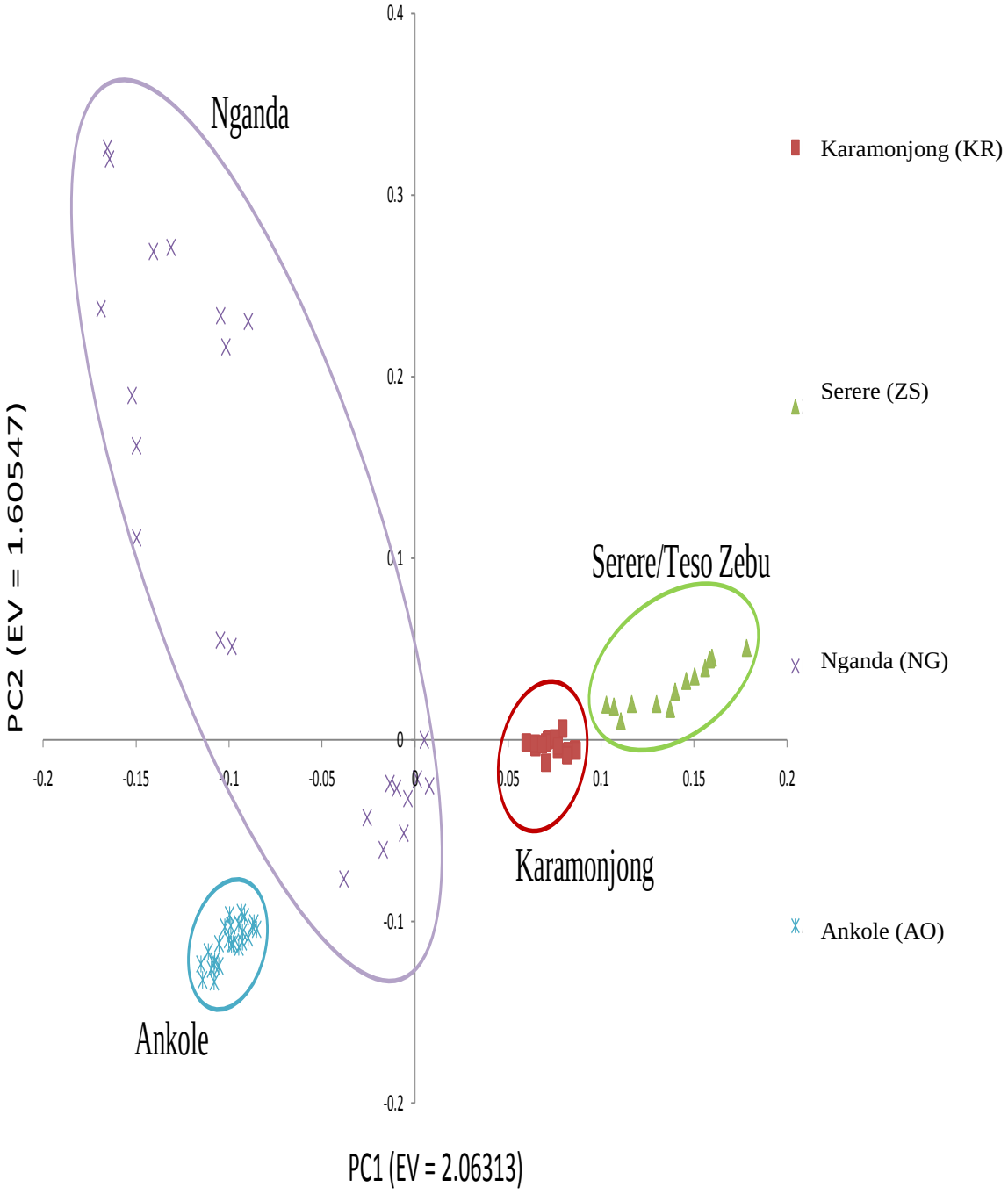


Figure 4.6b Two-dimensional graph representing the relationship between PC1 vs PC2 using BovineHD genotypes derived from 4 original indigenous cattle populations from Uganda

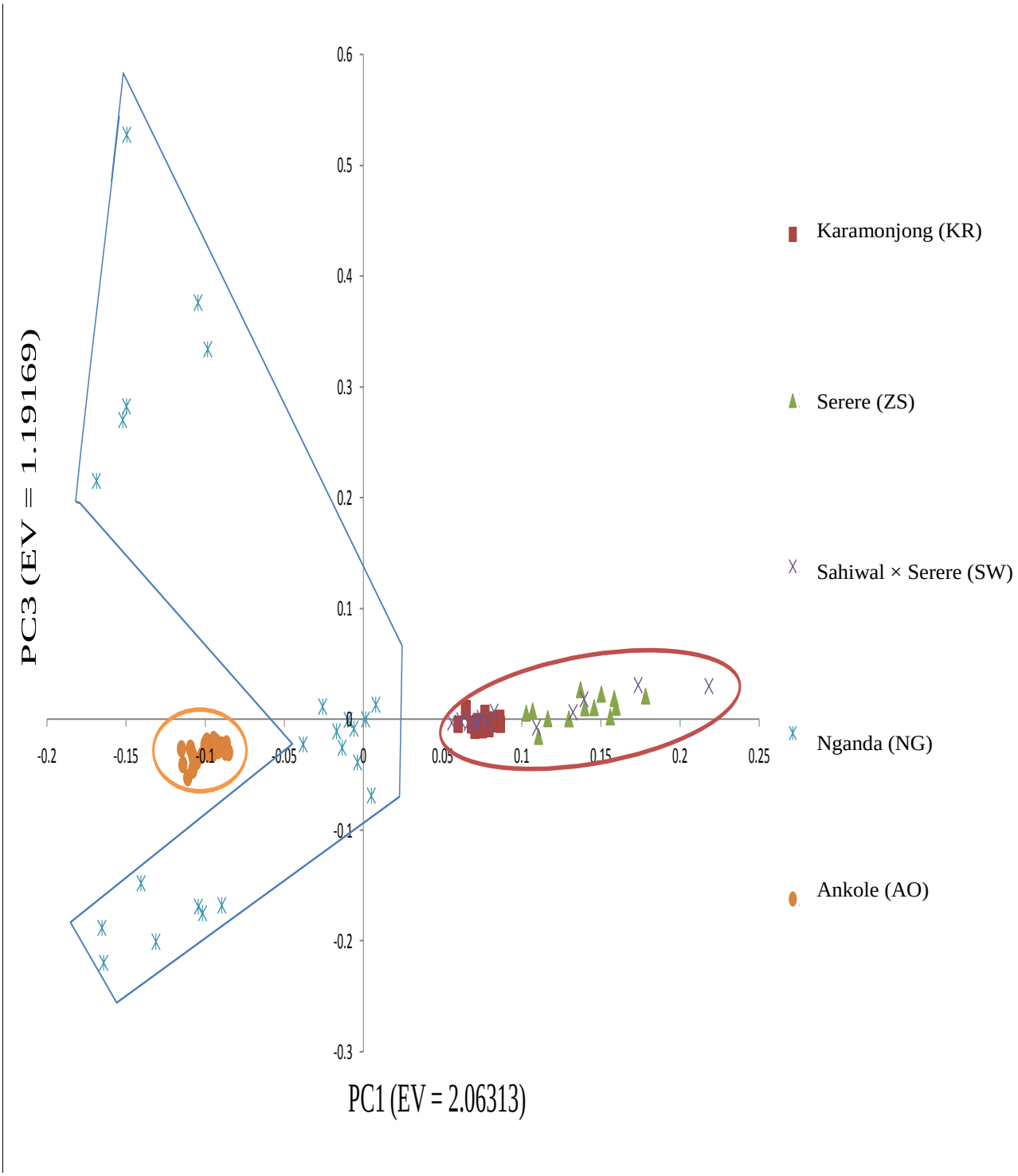


Figure 4.7 Two-dimensional graph representing the relationship between PC1 vs PC3 using BovineHD genotypes derived from 5 indigenous cattle populations from Uganda

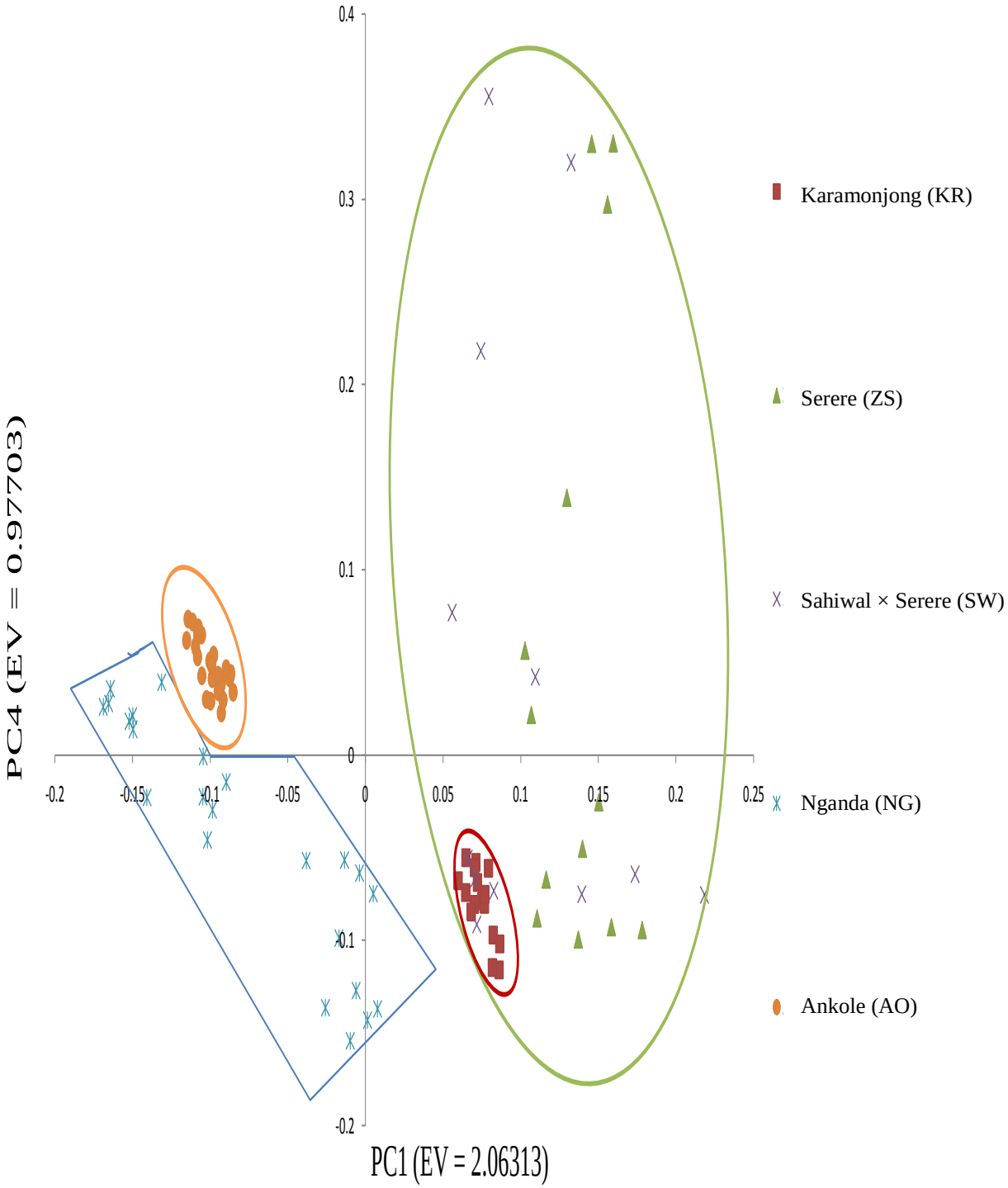


Figure 4.8 Two-dimensional graph representing the relationship between PC1 vs PC4 using BovineHD genotypes derived from 5 indigenous cattle populations from Uganda

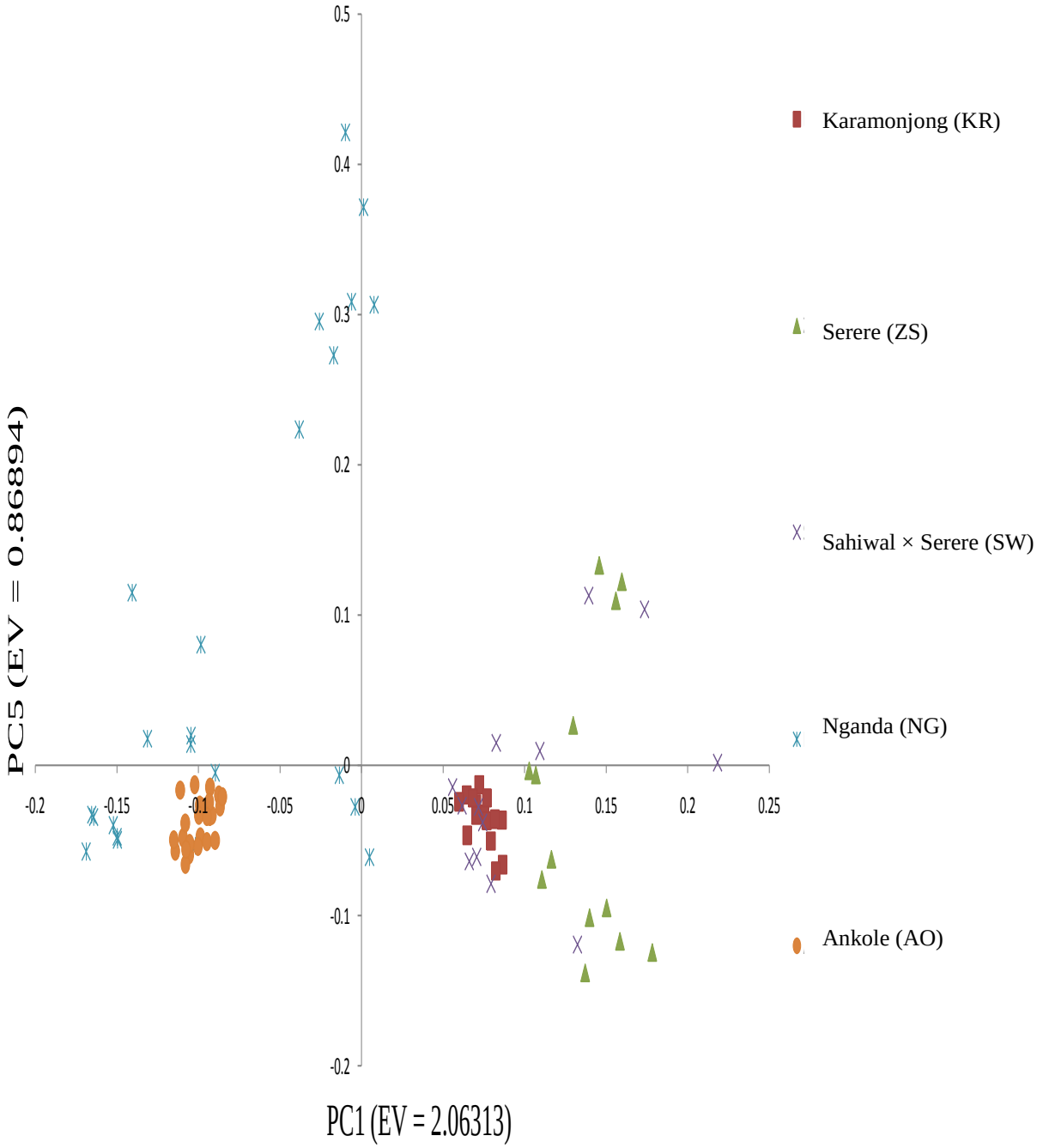


Figure 4.9 Two-dimensional graph representing the relationship between PC1 vs PC5 using BovineHD genotypes derived from 5 indigenous cattle populations from Uganda

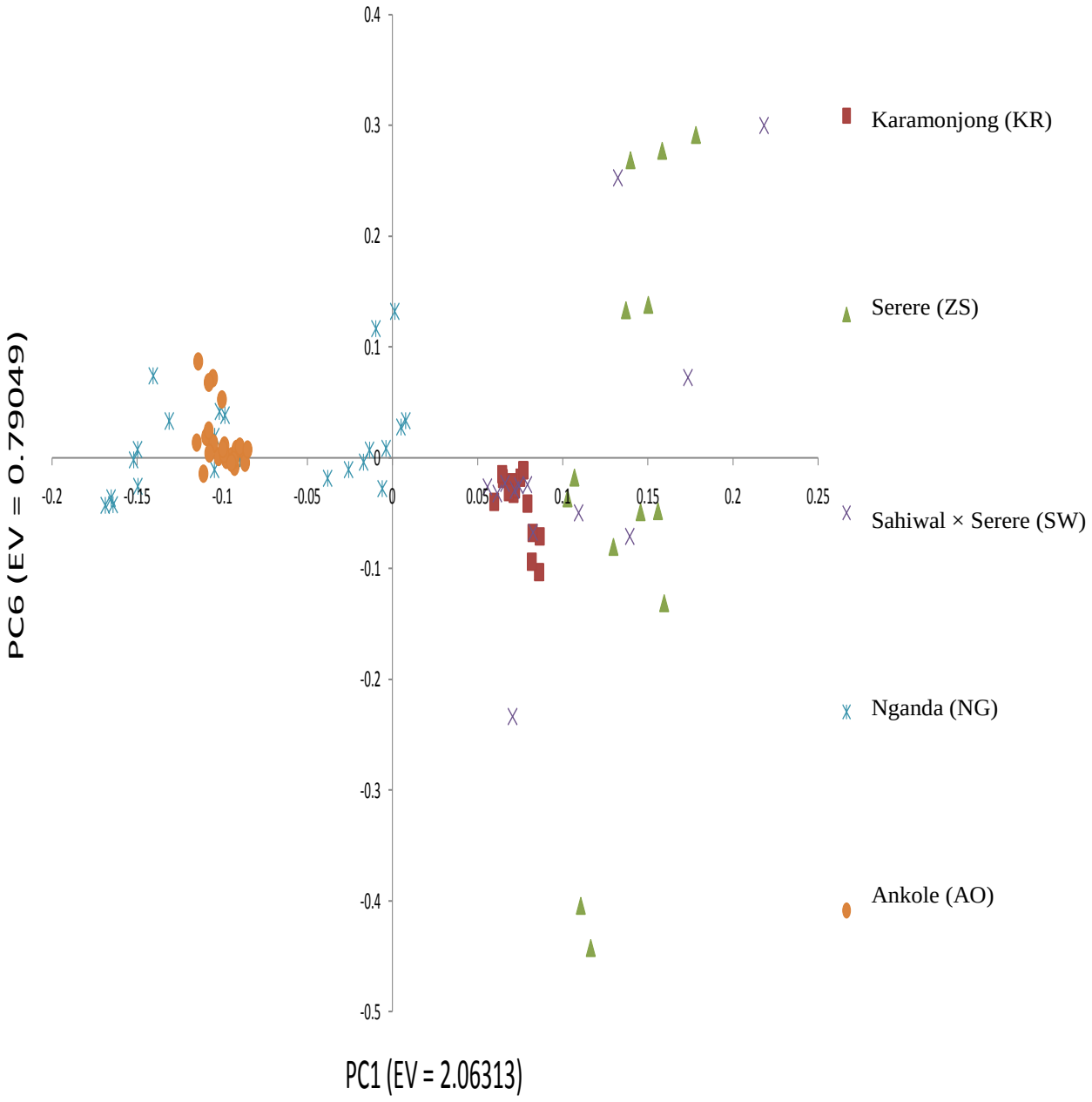


Figure 4.10 Two-dimensional graph representing the relationship between PC1 vs PC6 using BovineHD genotypes derived from 5 indigenous cattle populations from Uganda

This study revealed the differences among the Large East African Zebu, Small East African Zebu, Sanga, Zanga and the rest of West African Taurus and Zebus. No clear genetic distinction was evident between the Nigerian and Ugandan cattle populations at the first and fifth principal components (PC1 and PC5) (Figure 4.14) and first and sixth principal components (PC1 and PC6) (Figure 4.15).

4.2.5. Estimation of genetic admixture proportions in the indigenous cattle populations from Nigeria and Uganda

The effect of the indigenous cattle breeds on the estimated populations (almost half of the total number of breeds; respecting the existence of additional sub-populations, K) is shown in Table 4.5. The Nigerian indigenous cattle breeds showed a significant effect ($P<0.01$) on the population estimates at K = 1, K = 2, K = 3, K = 5, K = 7 while the population estimates at K = 4, K = 5 and K = 6 significantly ($P<0.001$) influenced the Ugandan cattle breeds. It was observed that Adamawa Gudali (0.380), N'Dama (0.627), Sokoto Gudali (0.557) and Friesian × Bunaji (0.630) had the highest population estimate followed by the other Nigerian cattle breeds having statistically similar population estimate for K = 1, K = 2, K = 3 and K = 5, respectively. For K = 7, the highest population estimates were observed in Azawak (0.517), Yakanaji (0.372), and Wadara (0.343) with the rest of the breeds having statistically similar population estimates. Ankole (0.688 and 0.293) showed the highest population estimate with the rest of the breeds of cattle from Uganda having statistically similar population estimates in K = 4 and K = 6, respectively. However, at K = 5, Nganda showed superiority over the other breeds from Uganda.

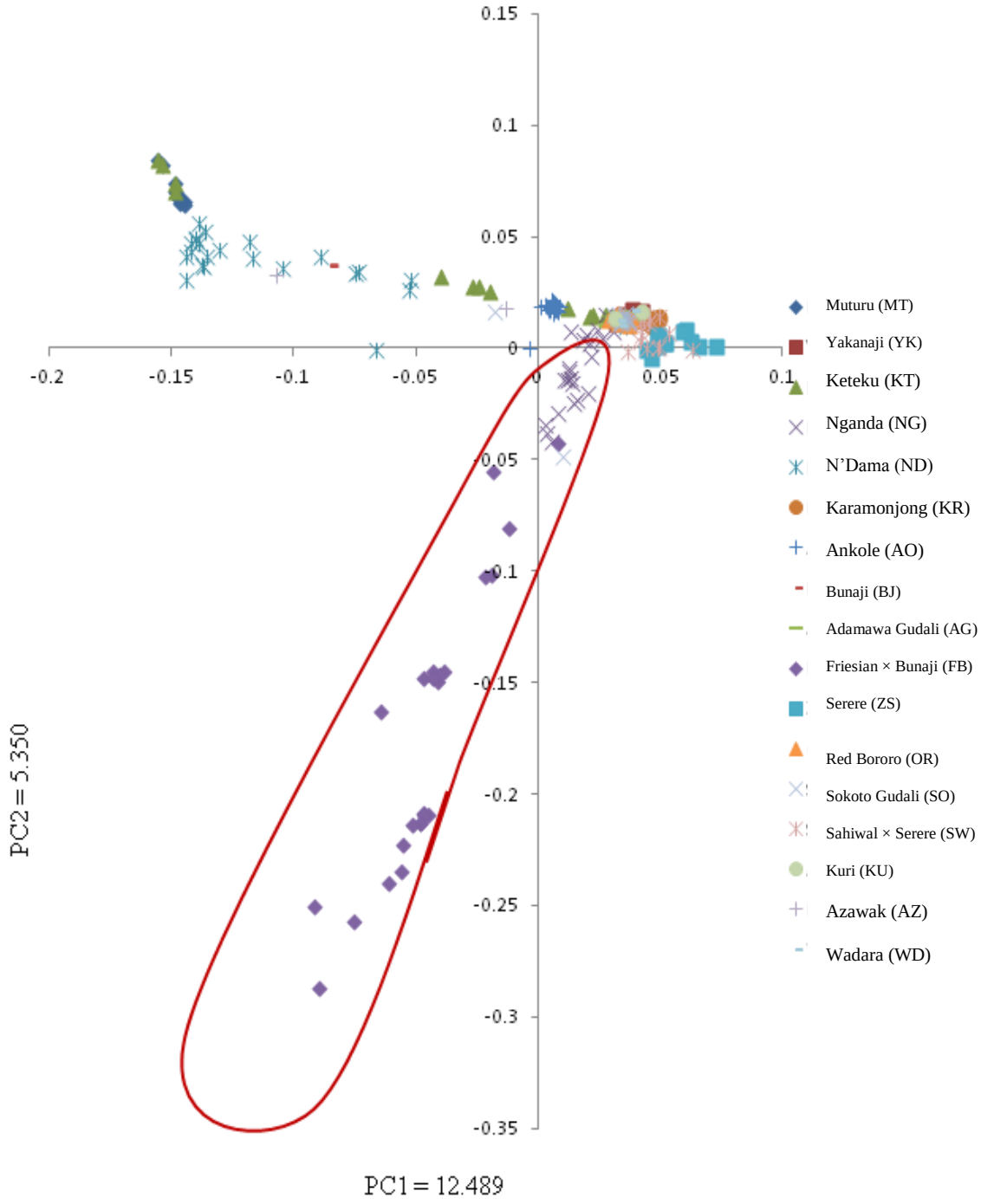


Figure 4.11 Two-dimensional graph representing the relationship between PC1 vs PC2 using BovineHD genotypes derived from 17 indigenous cattle populations from Nigeria and Uganda

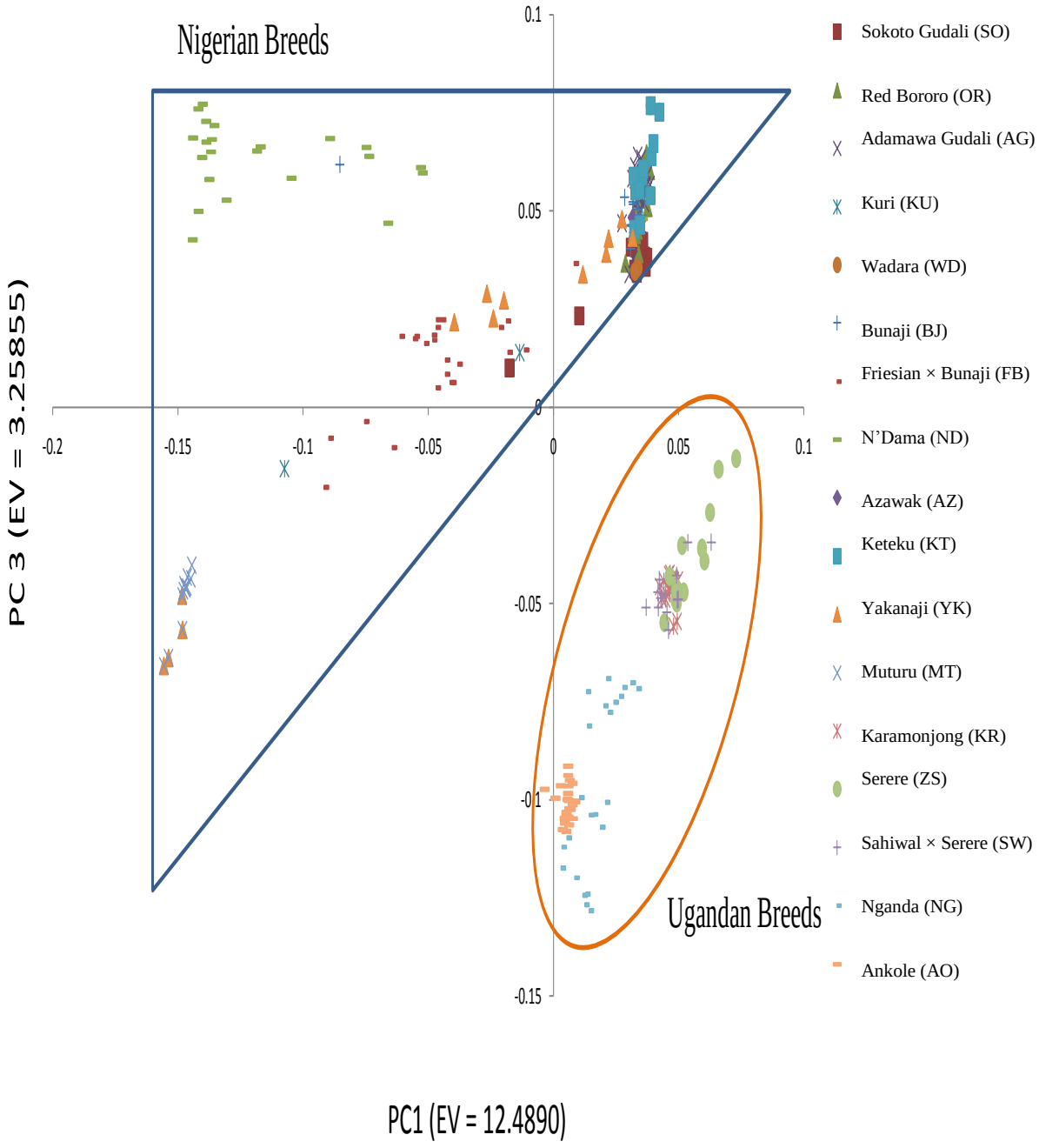


Figure 4.12 Two-dimensional graph representing the relationship between PC1 vs PC3 using BovineHD genotypes derived from 17 indigenous cattle populations from Nigeria and Uganda

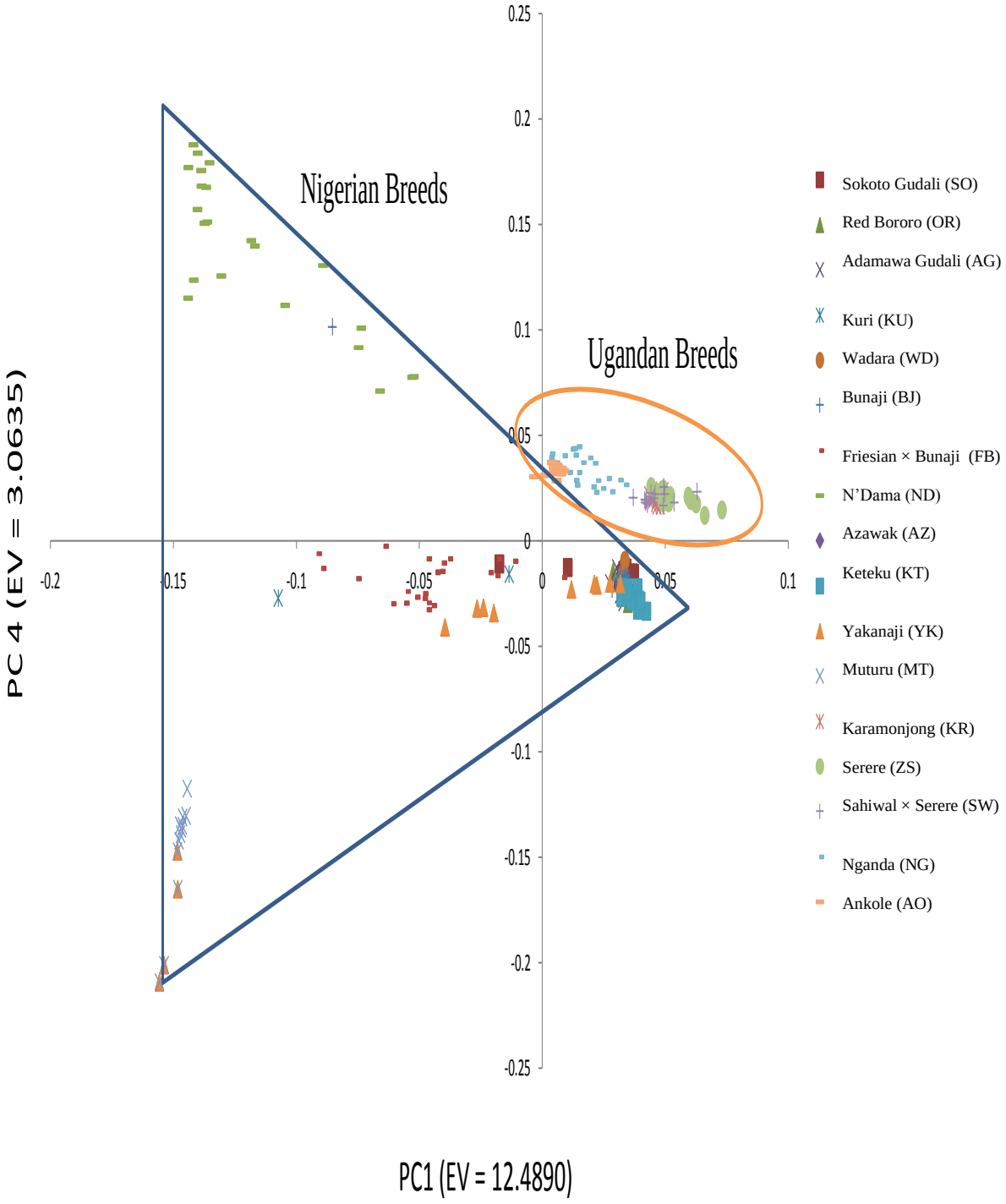


Figure 4.13 Two-dimensional graph representing the relationship between PC1 vs PC4 using BovineHD genotypes derived from 17 indigenous cattle populations from Nigeria and Uganda

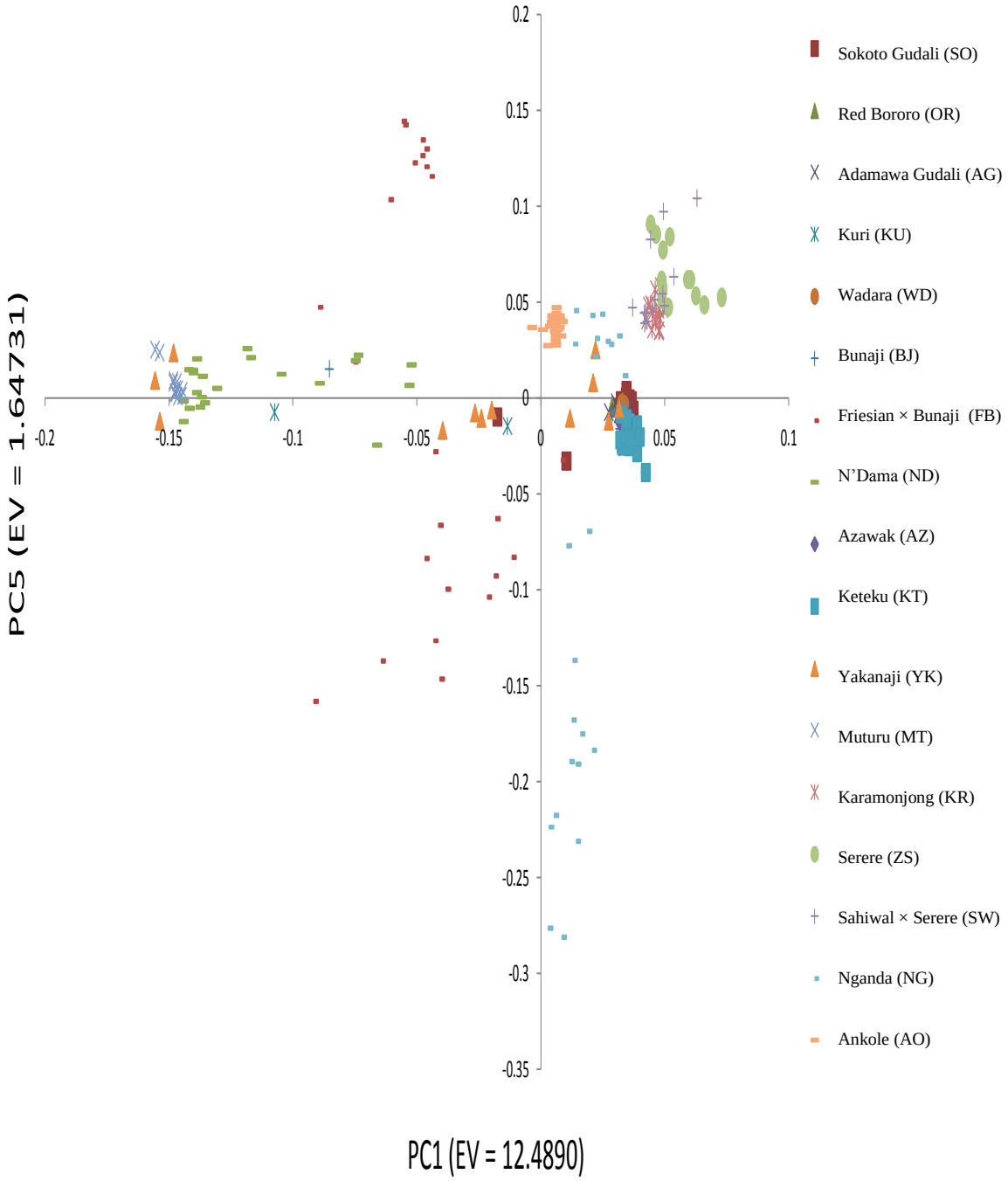


Figure 4.14 Two-dimensional graph representing the relationship between PC1 vs PC5 using BovineHD genotypes derived from 17 indigenous cattle populations from Nigeria and Uganda

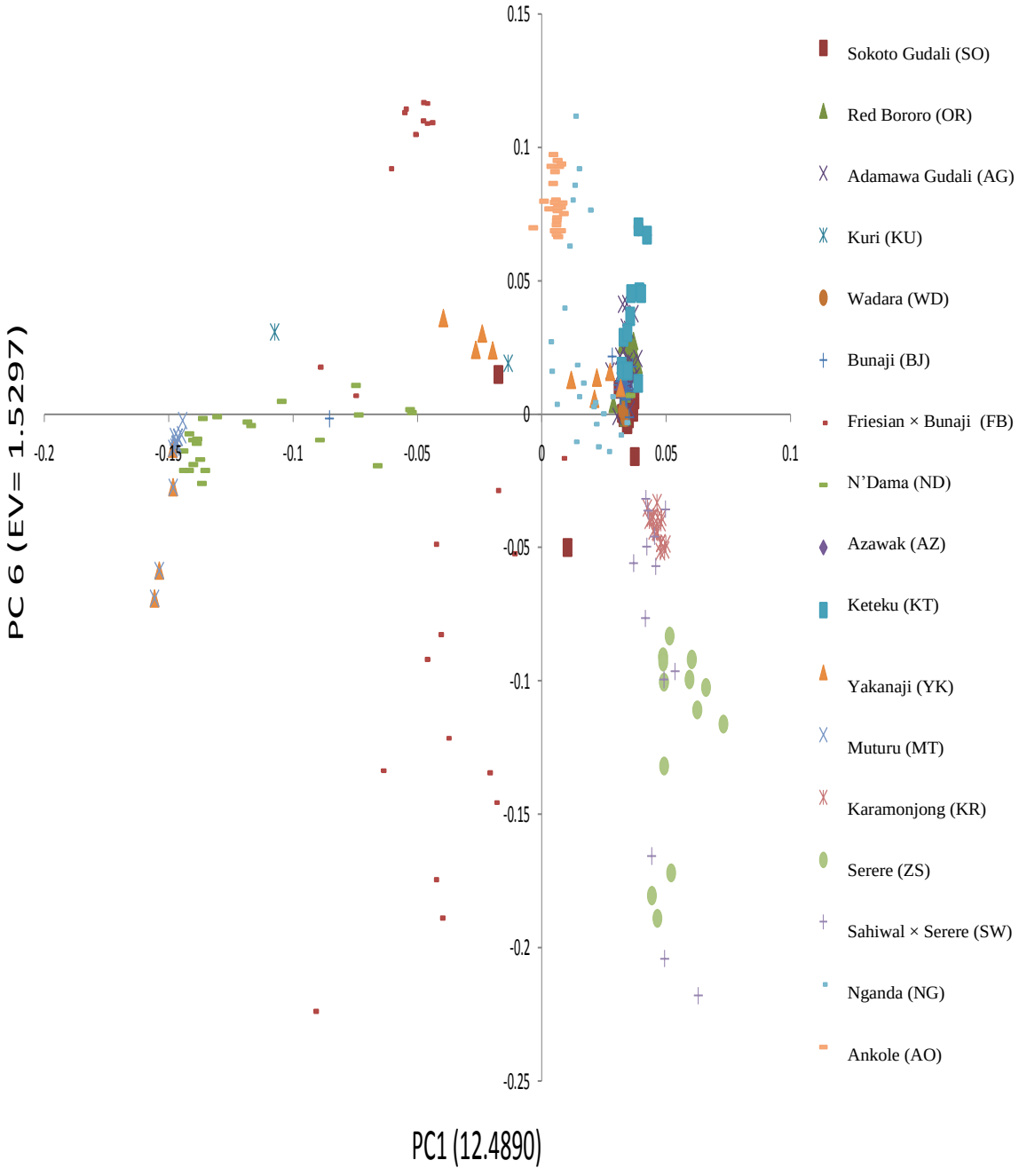


Figure 4.15 Two-dimensional graph representing the relationship between PC1 vs PC6 using BovineHD genotypes derived from 17 indigenous cattle populations from Nigeria and Uganda

4.2.6 Admixture levels in the indigenous cattle populations from Nigeria

The results obtained in the analysis with ADMIXTURE.1.2 showed an increase in the likelihood of the data with the number of clusters considered lower at K = 2 and 4. The lowest K were obtained at K = 2 (0.508) and K = 4 (0.507). Hence, the best genetic structure were detected at high values of K = 2 and K = 4 (Figure 4.16).

Population admixture levels were assessed by ADMIXTURE.1.2. Bar plot, generated by ADMIXTURE, depicts classifications with the highest probability under the model that assumes independent allele frequencies and inbreeding coefficients among assumed clusters. Each individual was represented by a vertical bar, often partitioned into coloured segments with the length of each segment representing the proportion of the individual's genome from K = 2, 3, or 7 ancestral populations. Breeds are separated by the symbols thus, Sokoto Gudali (SO), Red Bororo (OR), Adamawa Gudali (AG), Kuri (KU), Wadara (WD), Bunaji (BJ) Friesian × Bunaji (FB), Azawak, N'Dama (ND), Keteku (KT), Yakanaji (YK) and Muturu (MT).

Varying the number of presumed ancestral populations (K) within, ADMIXTURE revealed clusters consistent with the known history of cattle breeds (Figure.4.17). The first level of clustering (K = 2) reflects the primary, pre-domestication division of Taurine (N'Dama (ND) and Muturu (MT)) from indicine (Sokoto Gudali (SO), Red Bororo (OR), Adamawa Gudali (AG), Kuri (KU), Wadara (WD), Bunaji (BJ) and Yakanaji (YK) cattle. Consequently, breeds derived from indicine and taurine crosses (Friesian × Bunaji (FB) and Keteku (KT)) show signatures of admixture with both approaches.

Table.4.5. Estimates of coefficients of genetic admixture proportions of the indigenous cattle populations from Nigeria and Uganda

Breeds	N	K = 1	K = 2	K = 3	K = 4	K = 5	K = 6	K = 7	K = 8
Nigeria									
Sokoto Gudali (SO)	21	0.015^{b}	0.005^{b}	0.557^{a}	0.371	0.011^{b}	0.004	0.009^{b}	0.012
Red Bororo (OR)	22	0.026^{b}	0.0005^{b}	0.115^{b}	0.823	0.00001^{b}	0.005	0.007^{b}	0.009
Adamawa Gudali (AG)	25	0.380^{a}	0.001^{b}	0.069^{b}	0.479	0.00004^{b}	0.002	0.009^{b}	0.007
Kuri (KU)	2	0.027^{b}	0.164^{b}	0.069^{b}	0.201	0.053^{b}	0.028	0.003^{b}	0.076
Wadara (WD)	3	0.029^{b}	0.005^{b}	0.181^{b}	0.408	0.0006^{b}	0.005	0.343^{a}	0.012
Bunaji (BJ)	23	0.055^{b}	0.018^{b}	0.137^{b}	0.701	0.00001^{b}	0.026	0.021^{b}	0.018
Friesian × Bunaji (FB)	24	0.017^{b}	0.025^{b}	0.046^{b}	0.227	0.630^{a}	0.010	0.008^{b}	0.010
N'Dama (ND)	23	0.005^{b}	0.627^{a}	0.012^{b}	0.076	0.004^{b}	0.252	0.001^{b}	0.004
Azawak (AZ)	2	0.035^{b}	0.003^{b}	0.092^{b}	0.324	0.00001^{b}	0.00001	0.517^{a}	0.007
Keteku (KT)	13	0.018^{b}	0.007^{b}	0.067^{b}	0.241	0.00001^{b}	0.002	0.007^{b}	0.327
Yakanaji (YK)	12	0.021^{b}	0.0003^{b}	0.027^{b}	0.454	0.00001^{b}	0.0003	0.372^{a}	0.009
Muturu (MT)	12	0.00001^{b}	0.016^{b}	0.00001^{b}	0.00001	0.00001^{b}	0.00001	0.00001^{b}	0.00001
Uganda									
Karamonjong (KR)	16	0.059	0.004	0.618	0.109^{b}	0.0009^{b}	0.009^{b}	0.172	0.026
Serere (ZS)	13	0.005	0.002	0.232	0.017^{b}	0.0002^{b}	0.001^{b}	0.418	0.325
Sahiwal × Serere (SW)	13	0.032	0.003	0.319	0.108^{b}	0.005^{b}	0.008^{b}	0.273	0.252
Nganda (NG)	23	0.322	0.195	0.078	0.084^{b}	0.308^{a}	0.001^{b}	0.012	0.0003
Ankole (AO)	25	0.004	0.002	0.009	0.688^{a}	0.00001^{b}	0.293^{a}	0.002	0.001

[a, b] For a given factor, means within the same column for a given country with different letters or superscripts differ significantly ($P<0.05$)

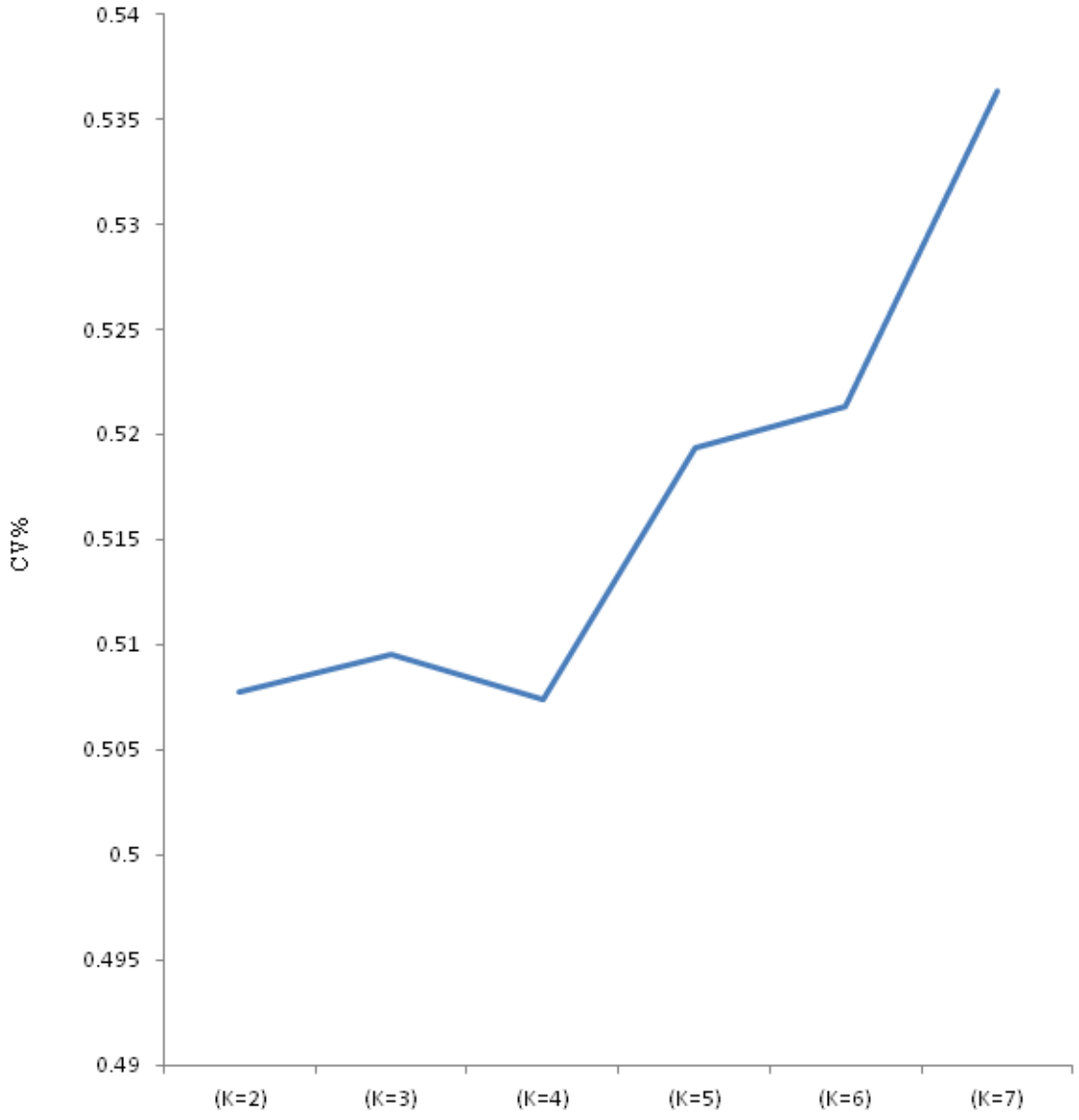


Figure 4.16 Coefficient of variation CV (%) as a function of L (K) for the indigenous cattle populations from Nigeria

At K = 4, the African breeds, N'Dama (ND) and Muturu (MT), separated from the European breeds Taurine (Friesian × Bunaji (FB)) - a division that reflects an early, possibly pre-domestication and divergence. At higher levels of K, clusters that identify single breeds as closed endogamous breeding units were observed. For example, as K increased from 5, 6, 7, each breed formed unique clusters.

4.2.7 Admixture levels in the indigenous cattle populations from Uganda

Figure 4.18 shows the results obtained in the analysis with ADMIXTURE.1.2 for Uganda. There was a clearly high likelihood of the data with the number of clusters considered, reaching a low plateau at K = 2 (0.537) to K = 3 (0.537) followed by a sharp decline in the likelihood of the data from K = 3. The best genetic structure was detected at high values of K = 2 and K = 3.

To examine relatedness among breeds, SNP genotype frequencies were analyzed with ADMIXTURE. 1.2. Each individual was represented by a vertical bar, often partitioned into coloured segments with the length of each segment representing the proportion of the individual's genome from K = 2, 3, or 7 ancestral populations from Uganda (Figure. 4.19). The first level of clustering (K = 2) reflected the primary, pre-domestication division of taurine from indicine cattle. There was a distinct cluster of the Sanga (Ankole (AO)) from the East African Zebus (Karamonjong (KR), Serere (ZS) and Sahiwal × Serere Zebus (SW). Also the Zanga which are Zebu × Sanga crosses clearly showed the signatures of admixture with both approaches. There was also a signature of admixture at K=2 in the East African Zebus used in this study.

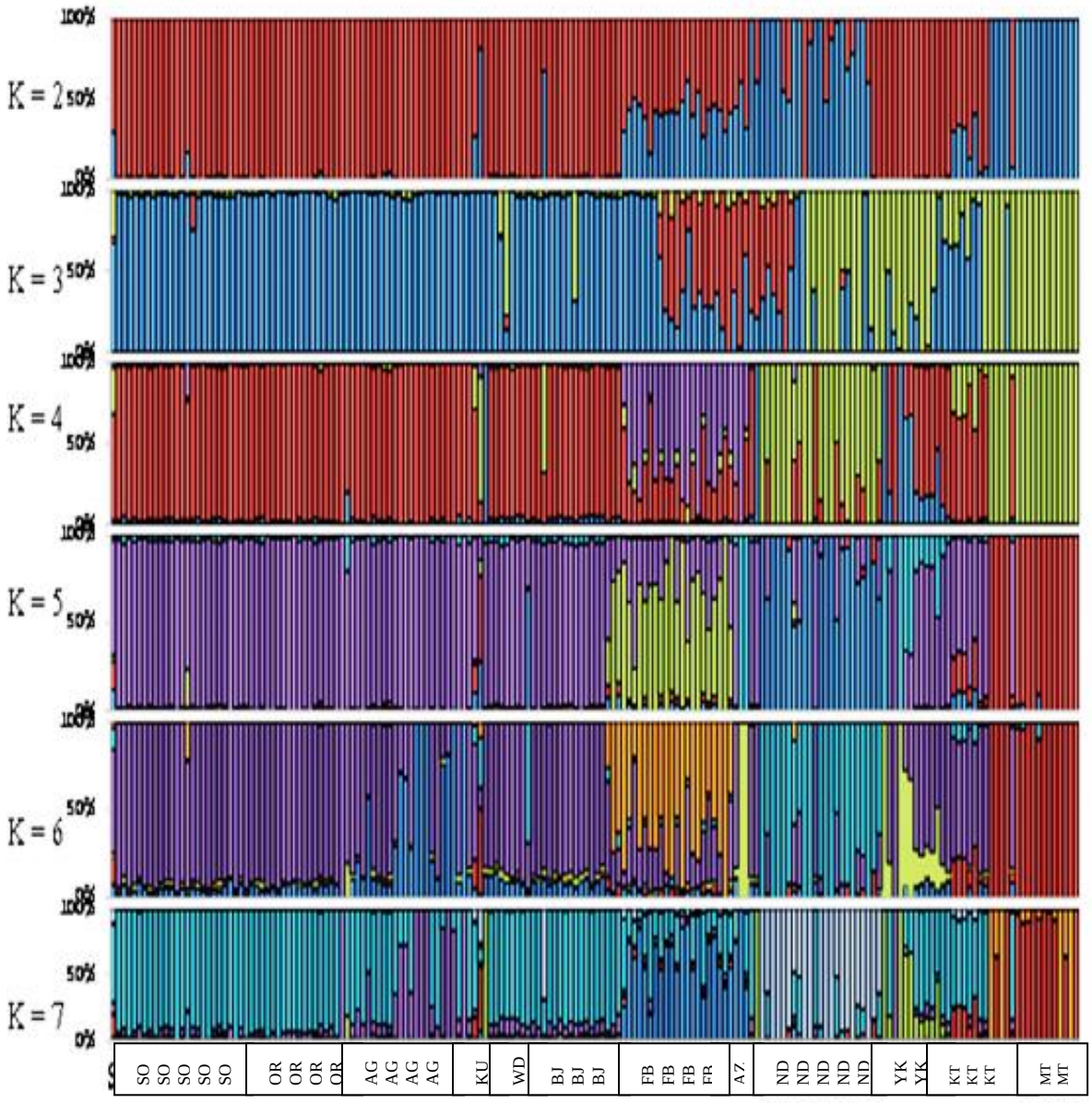


Figure 4.17 Clustering assignments of indigenous cattle populations from Nigeria as obtained by ADMIXTURE analysis.

SO (Sokoto Gudali)
OR (Red Bororo)
AG (Adamawa Gudali)
KU (Kuri)
WD (Wadara)
BJ (Bunaji)
FB (Friesian × Bunaji)
ND (N'Dama)
AZ (Azawak)
KT (Keteku)
YK (Yakanaji)
MT (Muturu)

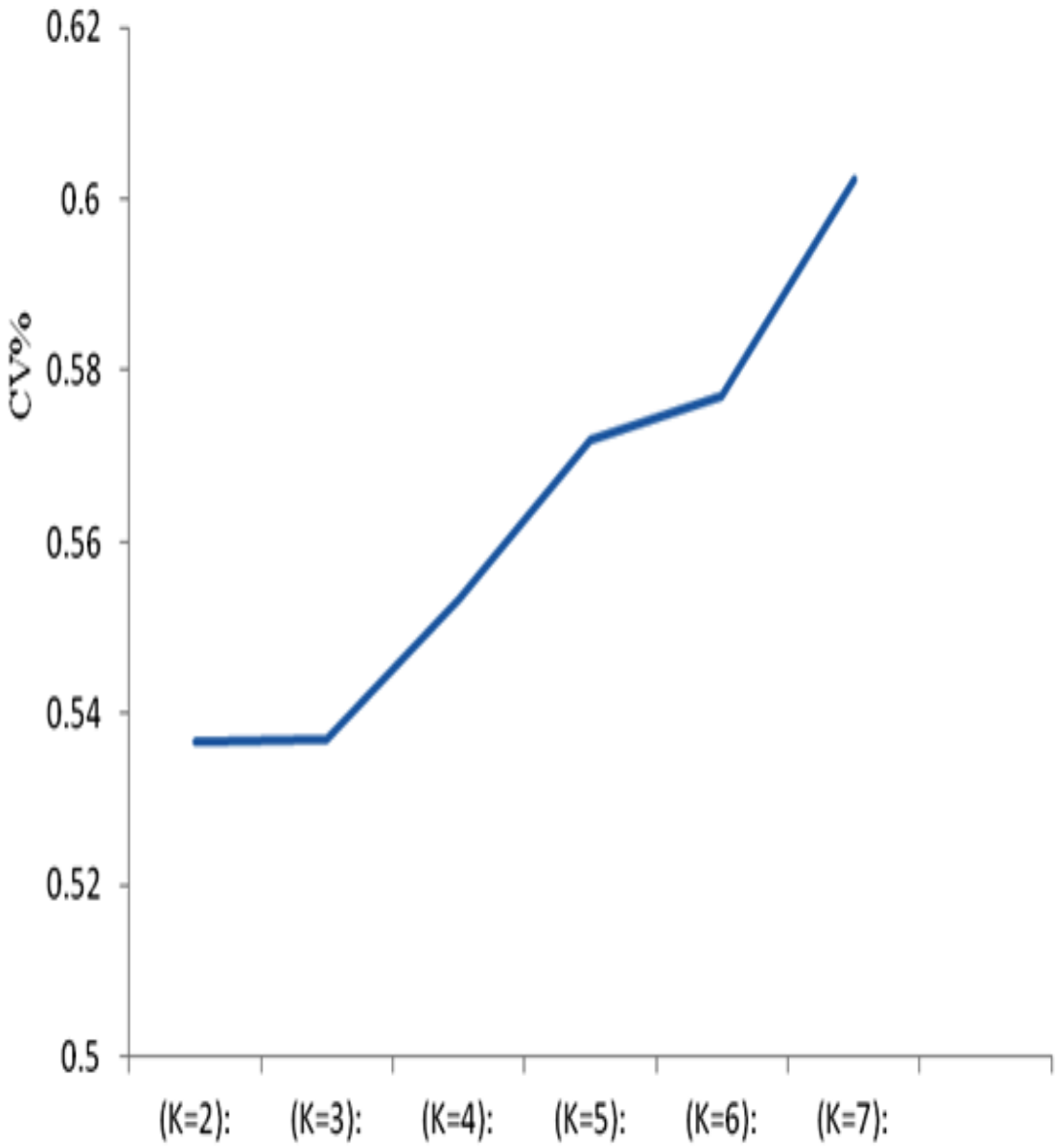


Figure 4.18 Coefficient of variation CV (%) as a function of L (K) for indigenous cattle populations from Uganda

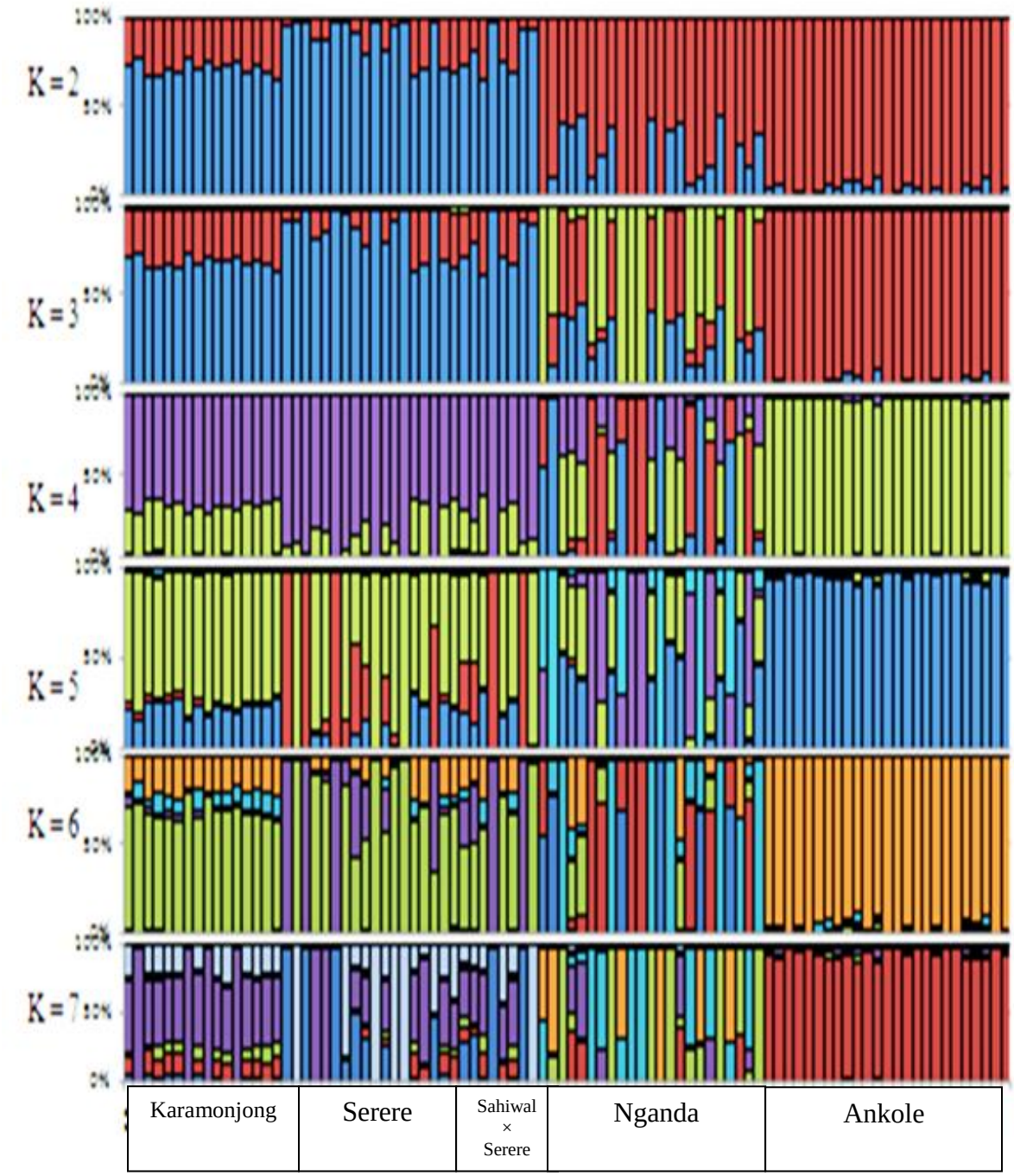


Figure 4.19 Clustering assignments of indigenous cattle populations from Uganda as obtained by ADMIXTURE analysis.

4.2.8 Admixture levels in the indigenous cattle populations from Nigeria and Uganda

For Nigeria and Uganda, the lowest K were at K = 2 (0.516) and K = 3 (0.516). The best genetic structure was detected at lowest values of k = 2 and k = 3 (Figure 4.20). The admixture analysis of the Nigerian and Ugandan indigenous cattle breeds is shown in Figure 4.21. At K = 2, most of the breeds had a higher percentage of Zebu genes for both Nigeria and Uganda cattle populations. Western Taurine admixture was clearly didtinguished at

K = 3.

4.3 Phylogenetic relationship among the indigenous cattle populations within and between Nigeria and Uganda

4.3.1 Phylogenetic relationship among the indigenous cattle populations with Nigeria

The phylogenic tree built on F_{ST} genetic distance, for the Nigerian breeds (Figure 4.22) revealed the following: The Muturu clustered together with the N'Dama breeds (*B. taurus* from Africa) but far apart from each other (cluster 1) while the Friesian × Bunaji (*Bos indicus* admixed with the European *Bos taurus*) clustered alone (cluster 2) just after the Muturu and N'Dama cluster (African *Bos taurus*). The Keteku and Kuri clustered together in-between the *Bos taurus* cluster and the *Bos indicus* cluster (cluster 3) reflecting their admixture. The remaining breeds, Sokoto Gudali (SG), Red Bororo (OR), Bunaji (BJ), Wadara (WD) Azawak (AZ), Yakanaji (YK) and Adamawa Gudali (AD) clustered together (*Bos indicus*) (cluster 4)

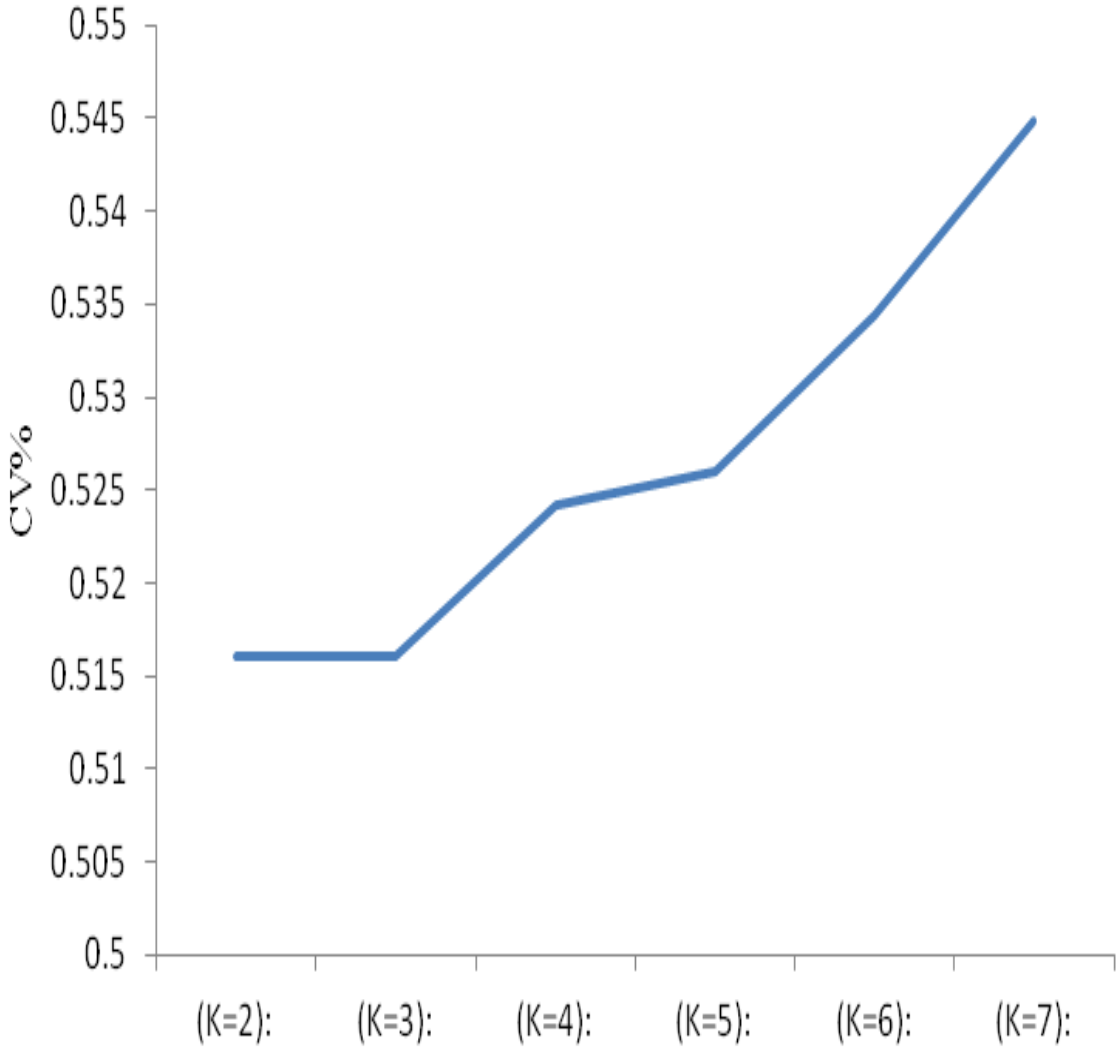


Figure 4.20 Coefficient variation CV (%) as a function of L (K) for Nigerian and Uganda indigenous cattle

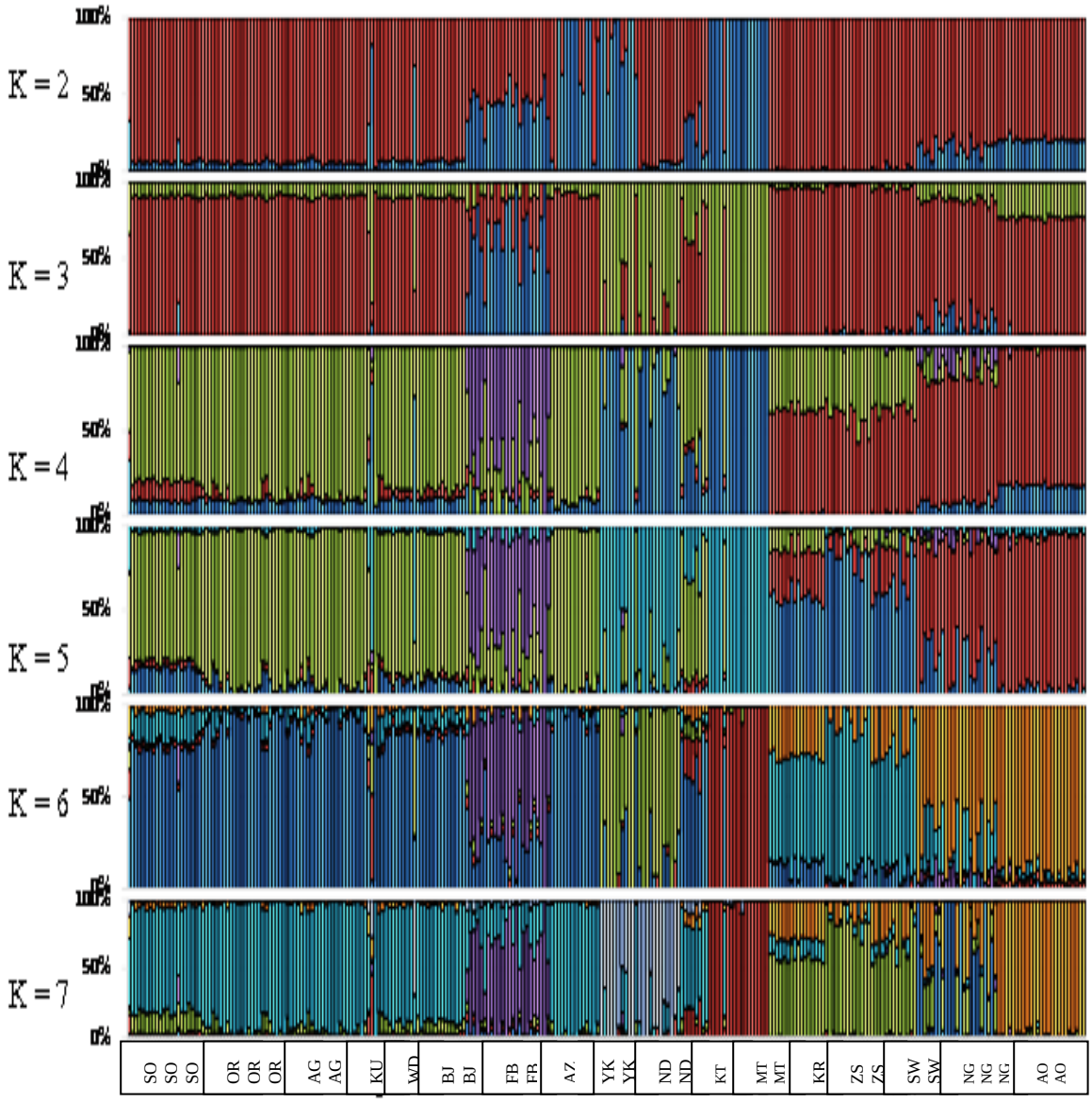


Figure 4.21 Clustering assignments of indigenous cattle populations from Nigeria and Uganda as obtained by ADMIXTURE analysis.

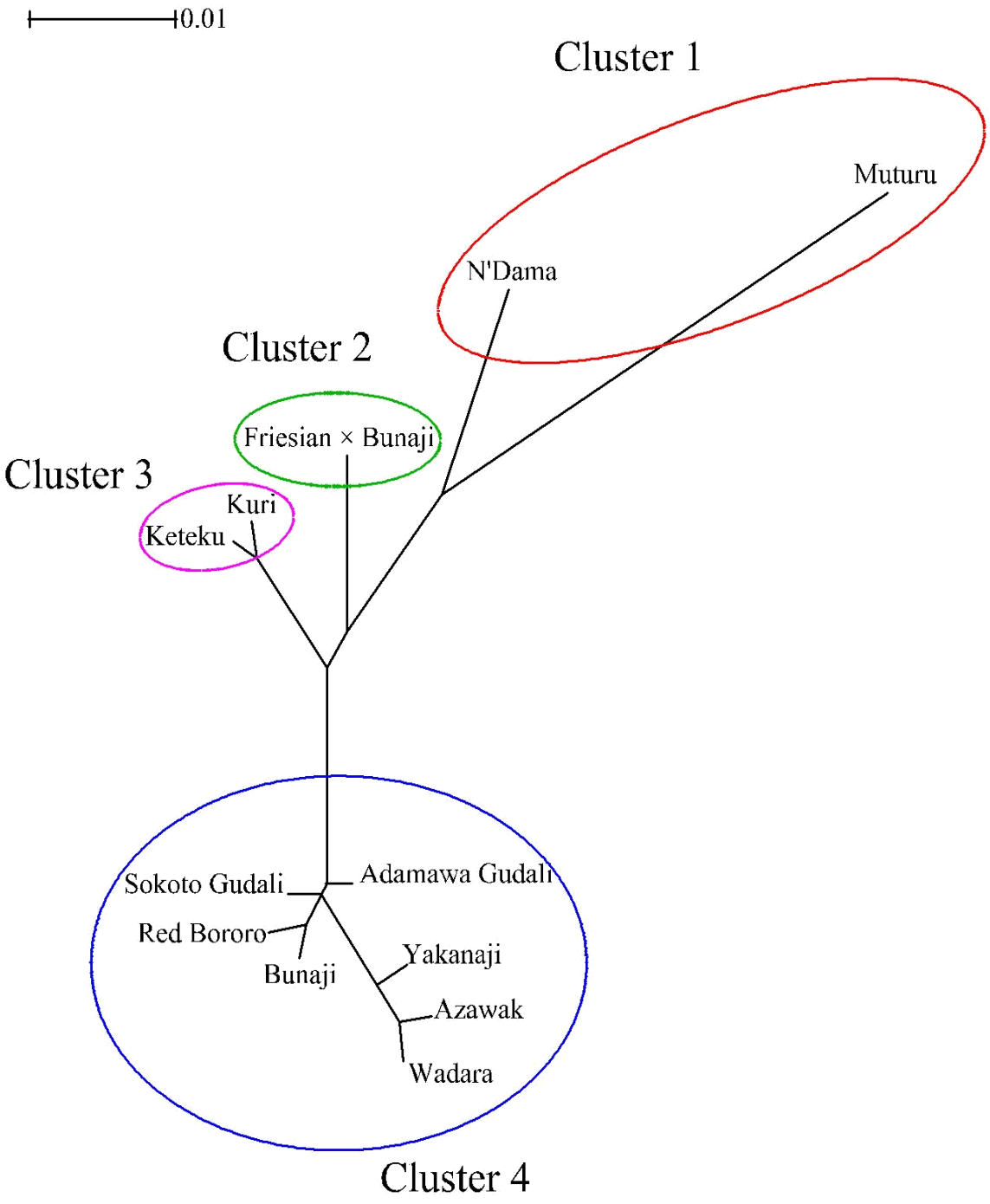


Figure 4.22 Phylogenetic relationships (UPGMA) among the indigenous cattle populations from Nigeria as constructed from D_A genetic distances

4.3.2 Phylogenetic relationship among the indigenous cattle populations within Uganda

In figure 4.23, the small East African Zebu (Serere (ZS)) and the Sahiwal × Serere Zebus crosses (SW) clustered together away from the Large East African Zebu (Karamonjong (KR)) (cluster A) while the Sanga (Ankole (AO)) and Zanga (Nganda (NG)) were also separately clustered but slightly to each other (cluster B). Generally, the East Africa Zebus clustered far away from the Sanga and Zanga breeds, respectively.

4.3.3 Phylogenetic relationship among the indigenous cattle populations within and between Nigeria and Uganda

Combining Nigeria and Uganda cattle populations in figure 4.24, the African Taurine (Muturu (MT) and N'Dama (ND)) clustered together far away from the rest of the breeds (cluster i). The admixed African Taurine and Indicine Keteku (KT) and Kuri (KU) clustered together from the European Taurine (Friesian Holstein) × African indicine (Bunaji), the Friesian × Bunaji (FB) (cluster ii). Also the Sanga (Ankole (AO) and Zanga (Nganda (ND)) clustered together (cluster iii). The East African Zebus clustered together and the Nigerian Zebus also clustered together separately (cluster iv and cluster v), respectively.

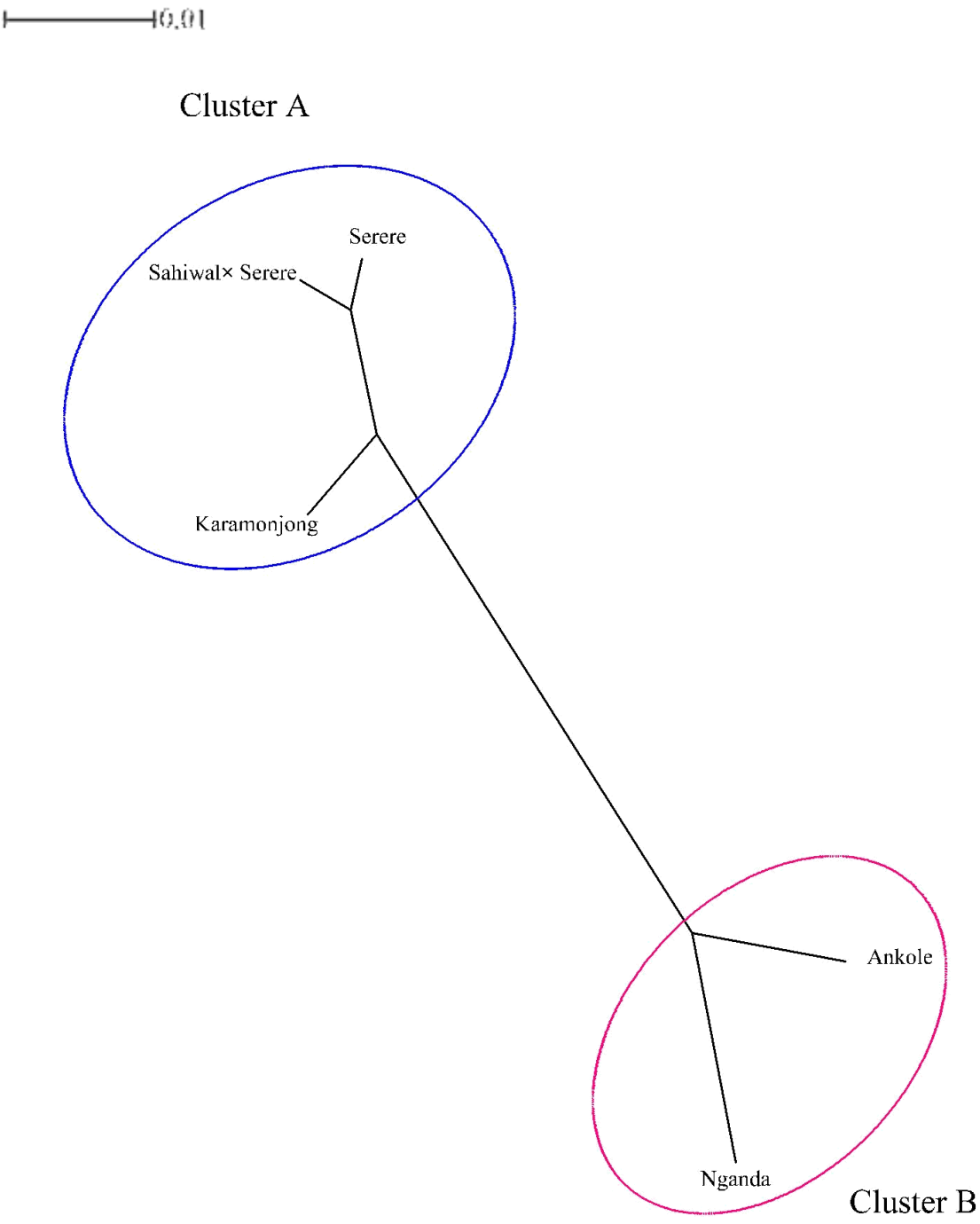


Figure 4.23 Phylogenetic relationships (UPGMA) among the indigenous cattle populations from Uganda as constructed from D_A genetic distances

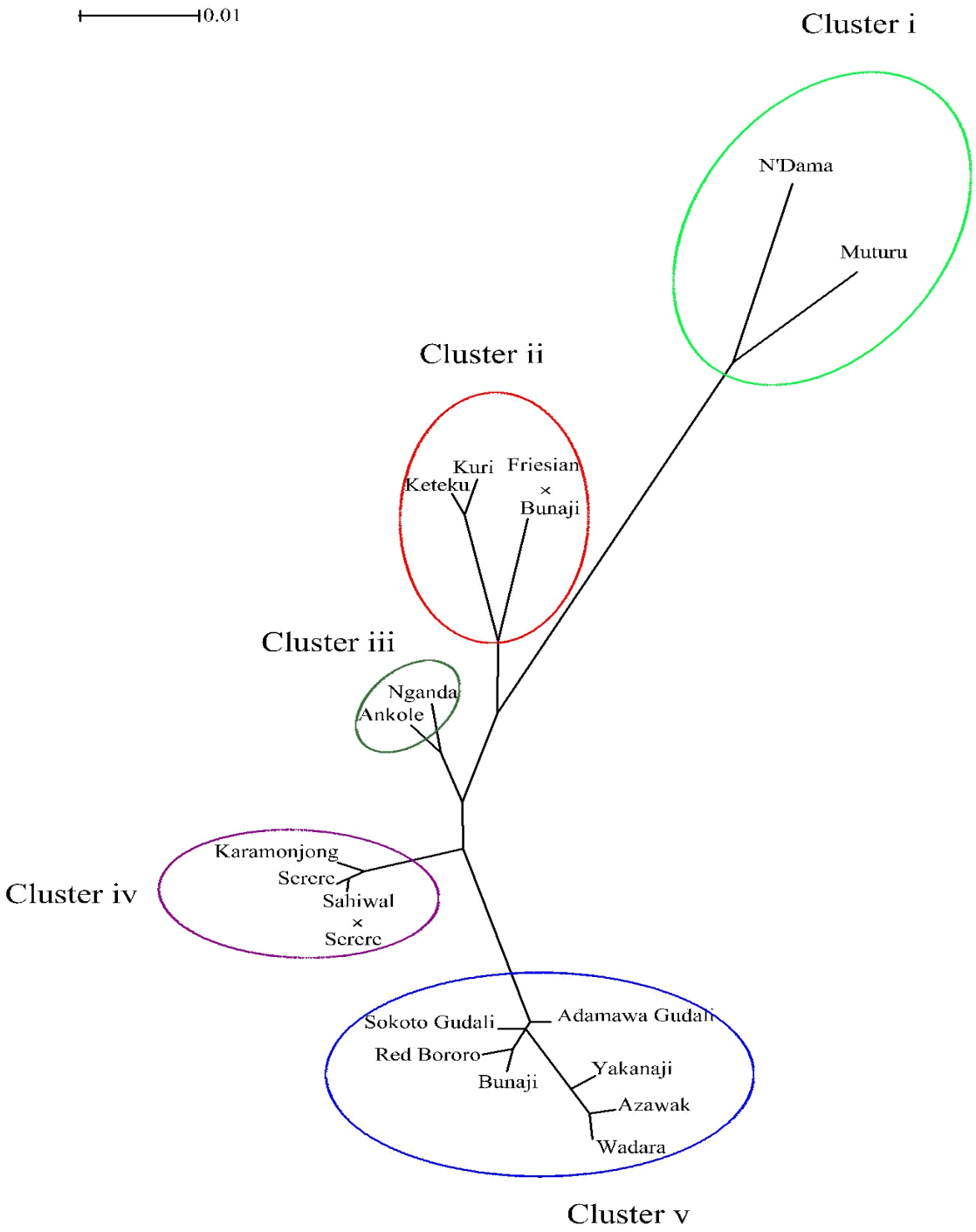


Figure 4.24 Phylogenetic relationships (UPGMA) among indigenous cattle populations from the Nigeria and Uganda as constructed from D_A genetic distances

CHAPTER FIVE

5.0 DISCUSSION

5.1. Genetic diversity in the indigenous cattle populations from Nigeria and Uganda

Among Nigerian and Ugandan cattle populations, observed and expected heterozygosity values were high in this whole genome SNP-based study compared to those estimated using microsatellite markers (Dadi *et al*., 2008) for 10 Ethiopian cattle populations. Similarly, Carruthers *et al.* (2011) reported higher values based on microsatellite marker studies, than those obtained using SNP analysis, in Angus and Brazilian cattle breeds. The difference in the results obtained using these two techniques could be a reflection of the higher mutation rate, lack of ascertainment bias and lack of power due to reduced sampling of the genome. Moreover, the results presented here are slightly higher than the 0.386 expected heterozygosity observed in Podolic cattle breeds by using SNPs (Pariset *et al*., 2010). Lower values of heterozygosity (0.332) have been described for the Angus breed (Carruthers *et al*., 2011), and heterozygosity values (He) of 0.25 for African cattle and 0.30 for European originated breeds, respectively, based on SNP analysis (Gautier *et al.*, 2007).

The higher genetic variability noted in the Nigerian and the Ugandan cattle is in harmony with the results of Lin *et al.* (2010), who observed lower genetic diversity in *Bos indicus*, yet linkage disequilibrium in indicus cattle suggest there is more diversity compared to taurine cattle due to admixture from more than one auroch species prior to breed formation. By contrast, a high level of genetic diversity was observed in Eastern Africa than in Western Africa and Europe (Hanotte *et al.*, 2002) microsatellite analysis. The differences in reported results may be explained by the application of different molecular markers or

differences in sampling. Ethiopia was thought to be the entry point for all cattle in East Africa and cattle in Uganda would be more limited in diversity based on this assumption.

The average observed heterozygosity estimations in this study (0.641, 0.633) were fairly comparable with those shown in seven Italian cattle breeds (0.60 − 0.68; Del Bo *et al*., 2001) and five Swiss cattle breeds (0.60 − 0.69; Schmid *et al*., 1999). Marginally lower levels of heterozygosity were reported in Deoni cattle breed (0.59) of India (Mukesh *et al*., 2004) and 12 West/Central African cattle breeds (0.506–0.697; Ibeagha-Awemu *et al*., 2004). Also lower heterozygosities (0.42 and 0.53) and lower numbers of alleles than in the indigenous cattle populations in this study have been reported in two Indian Zebu cattle breeds, namely Sahiwal and Hariana (Mukesh *et al*., 2004), whose populations are in rapid decline in India. Diversity could be also lowered based on limited importation due to a bottleneck of importation from India and other parts of Europe.

Genetic diversity is in fact important to allow genetic improvement and facilitate rapid adaptation to changing environments and breeding objectives (Notter, 1999). The higher variability observed within Nigerian and Ugandan cattle populations can potentially be attributed to the absence of strong artificial selection pressures, high levels of admixture in these populations (Rege, 1999; Dadi *et al*., 2008) causing increased heterozygosity, which is a distinctive trait of large traditional populations. The introduced Zebu cattle intermingled and crossbred with the original African longhorn and shorthorn taurine population to produce the various types of cattle found in West and East Africa today, as has been well documented (Payne and Wilson, 1999; Hanotte *et al.*, 2002).

It is clear from this study that *Bos taurus* (European and African) and *Bos indicus* cattle possess very distinct allelic distributions and that Zebu breeds in Nigeria and Uganda have a mixture of both African/European *Bos taurus* alleles, thus substantiating their high diversity status. Genetic diversity of African Zebu breeds is higher than the values reported for most pure European Taurine breeds (Hanslik *et al*., 2006; Loftus *et al.,* 1999; MacHugh *et al*., 1997) and Zebu breeds (Loftus *et al*., 1999; MacHugh *et al.,* 1997), and compares to reports on other African *Bos indicus* breeds (MacHugh *et al*., 1997; Kim *et al.,* 2002), breeds in Turkey, the Near East (Loftus *et al*., 1999) and Far East cattle (Moazami-Goudarzi *et al*., 2002). Loftus *et al*. (1999) interpreted high gene diversity in the Near Eastern breeds as having resulted from the retention of a high number of alleles by populations in a centre of origin and also an influence from Zebu breeds admixture through migrations. The higher genetic diversity in the Nigerian cattle breeds than in Uganda cattle breeds was expected because Nigeria is endowed with a larger number of indigenous cattle breeds than Uganda.

Number of migrants per generation (N(nm)) values indicates the relative strength of gene flow and genetic drift. Genetic differentiation will result in substantial differentiation where N(nm) < 1 but not where N(nm) > 1 (Slatkin, 1987). Migration values N(nm) can be interpreted in the context of the conservation and maintenance of the genetic variability of an animal as the upper limit of the number of migrants per generation, which would allow for the maintenance of the genetic differentiation observed between the breeds. Trexler (1988) showed that if N(nm) > 1 (in an infinite island model), gene flow is enough to attenuate the genetic differentiation between populations. In this investigation, the estimated number of migrants N(nm) was considerably higher than an earlier estimate for Ankole cattle (Kugonza *et al.,* 2011) and *Bos indicus* breeds (Mukesh *et al.,* 2004). The results in this study reflect considerable gene flow between populations, resulting in low measures of genetic differentiation and inbreeding. The N(nm) values estimated for Nigerian cattle breeds indicate the greatest exchange of the genetic material originating from the same geographical region, from the south-west of Nigeria (Muturu and Keteku), from north-western (Adamawa Gudali, Wadara and Kuri) with the lowest gene flow being in Red Bororo, Yakanaji and Bunaji breeds which are densely-populated and distributed all over northern Nigeria. The likely explanation for these results can be related to the fact that these breeds separated from their ancestral common trunk only a short time ago (MacHugh *et al.,* 1997).

Genetic migration would have played a relatively important role only between populations of close geographical vicinity. Although genetic migration between neighbouring populations could have been relatively important in some cases, isolation by distance between the Nigeria and Uganda cattle breeds has not been detected. Therefore, genetic

drift or migration appears as the most important factor of genetic differentiation among the analysed populations. Additionally, the inferred conclusions from indirect estimates of gene flow, integral to interpretation of micro-evolutionary patterns and geographical structure, should be taken with much caution (MacHugh *et al.,* 1997).

Values of F_{IS} in most natural populations are close to zero. The F_{IS} value provides the non-random union of gametes in population, i.e the mating among individuals in the population which is related more than average relationship. The more the F_{IS} value, the more the inbreeding. The negative values of F_{IS} point towards outbreeding. For populations mating at random, genes are equally related-within or between individuals and in this case $F_{IS} = 0$. Therefore, estimates of F_{IS} that differ significantly indicate departures from random mating. Any avoidance of mating of relatives will cause F_{IS} to exceed 0 and to be negative. More commonly, F_{IS} is positive, which could be interpreted as evidence of inbreeding (Weir, 1996).

In this study, F_{IS} (0.386 to -0.177; mean = 0.105) for Nigerian and (0.014 to -0.033; mean = 0.016) for Ugandan cattle breeds were close to zero, which indicates very little admixture between the breeds. Except for the Friesian × Bunaji (-0.177) and Nganda (-0.033) which were slightly outbred populations, low and positive F_{IS} values were observed indicating very low inbreeding in the Nigerian and Ugandan cattle populations. Neglecting the effects of migration, and assuming a low contribution of mutations to the genetic diversity between these breeds, the differences in allele frequencies may be interpreted as primarily the result of random genetic drift. The genetic differentiation in the Friesian × Bunaji and Nganda may be seen as the result of a decreased mean inbreeding coefficient F_{IS} over a rather short period of time leading to outbreeding because of the different *Bos taurus* and *Bos indicus*

for the Friesian × Bunaji and Sanga and *Bos taurus* for the Nganda (Lin et al., 2010). Hence it is considered that the relatively low mean F_{IS} value (-0.177 and -0.032) indicated that a larger reproductive isolation existed among the Friesian × Bunaji in Nigeria and Nganda in Uganda; to be the result of an increase of heterozygosity within the Friesian × Bunaji and Nganda studied.

Genetic characterisation of Ethiopian cattle breeds using microsatellites reported within population inbreeding of 0.071 and a total inbreeding of 0.083 (Dadi *et al*., 2008). A negative F_{IS} value, indicating observed heterozygosity values greater than expected, was significant in Friesian × Bunaji (-0.177) and Nganda (-0.033) for a 95% confidence interval which was expected due to the admixture because crossbreds are usually not inbred. The shortage of heterozygotes and excess of homozygotes ($F_{IS} > 0$) might be attributed to a number of factors including assortative mating, linkage with loci under selection, population heterogeneity or null alleles. The absence of serious significant inbreeding effects may be a reflection of the high gene flow between the populations, as supported by high N(nm) values, the large population from which the samples were drawn, and the fact that related individuals were purposely avoided. The high inbreeding detected in Muturu is likely to be a manifestation of diminished population size and confinement to a small breeding territory, coupled with insufficient number of breeding males in the breeding region.

The Hardy-Weinberg Equilibrium (HWE) was tested for all breed combinations and the results obtained in this study demonstrated that all the sampled bovine populations deviated from equilibrium as reported by Georgescu *et al.* (2009). The variation in proportion of SNPs displaying deviation from HWE among the sampled populations could be explained

by migration and mutation. Selection pressure (the genetic hitchhiking effect, being close to some morphological or productive trait of selective interest) and population structure (stratification, admixture; the presence of population substructure within the breed may lead to Wahlund's effect) would be also implicated in this case.

The high percentage by identities observed in the few Nigerian breeds (Kuri, Wadara and Azawak) in this study might have been caused by the smaller number of individuals used in this study. However, Muturu cattle had some inbred individuals, which is reflected in the F_{IS} (0.386). That all the Ugandan cattle breeds had relatively low percentage identities might be due to the variety of geographical areas sampled. The populations included in this study were characterised by (a) widespread regional distribution, (b) small population sizes and (c) breeds linked to traditional production systems.

5.2. Population structure and admixture levels in the indigenous cattle populations from Nigeria and Uganda.

The largest amount of variance in the genotypic data is explained by the first component (Factor 1) which differentiates sub-species. The second "component" or factor that is identified explains the variance in the data and is perpendicular (thus, uncorrelated) to the first component. The remaining components, or factors, are found in a similar manner (Thompson *et al*., 2000). In this study, in Factor 1 only *Bos indicus* breeds had higher loading than all the *Bos taurus,* explaining the high variance in the Zebu breeds in Nigeria and Uganda. When the second component (Factor 2) was further analysed, the rear or uncommon genetic uniqueness of the Western *B*os *taurus* (Holstein Friesian) was exhumed in the Friesian × Bunaji from Nigeria while the Ankole and Karamonjong were inferior with low variation among the Ugandan cattle populations. The genetic uniqueness of

N'Dama was realized in Factor 3. This factor analysis provided a general understanding of the unique genetic dimensions that exist in the cattle breeds from Nigeria and Uganda (Rege, 1999).

In this study, communalities showed the percentage variance of the breeds that is accounted for by each factor. Except for the Kuri, Friesian × Bunaji, Keteku and Muturu from Nigeria and Karamonjong, Nganda and Ankole from Uganda, all cattle populations used in this study exhibited positive genetic uniqueness implying that they have common genetic variation hence a common centre of origin (MacHugh *et al.,* 1997). The negative communalities detected the percentage of breed with rear and uncommon genetic uniqueness with the Muturu having the highest negative communality (-4.833), followed by the Friesian × Bunaji (-0.376) with Keteku (-1.582) and Kuri (-0.972) from Nigeria and Ankole (-4.638), Karamonjong (-0.931) and Nganda (-0.224) from Uganda.

The results observed in this study were expected. Friesian × Bunaji animals had rare and uncommon genetic uniqueness because it was a cross between Western Taurine (Holstein Friesian) and the African indicine (Bunaji) in the course of improving the Bunaji breed for milk production (Oni *et al.,* 2001). Being a Nigerian Middle Belt breed, Keteku is admixed with various Zebu breeds from Northern Nigeria and the Muturu and N'Dama (African Taurine) from Southern Nigeria making its genetic make-up complex and unexceptionally unique. In areas where the tsetse challenge is not too severe, Zebu characteristics are perceived as desirable by farmers and crossing humpless females to Zebu bulls has become a common practice (Rege, 1999).

The unique genetic composition of Kuri from the other breeds may be due to their geographical locations where they are isolated from the Zebu introgression. The Kuri cattle breed is found on the shores and islands of Lake Chad. Its main habitat is in southern Chad and north-eastern Nigeria but the breed is also found in northern Cameroon, in Niger and, to a limited extent, in the Central African Republic (Mpofu and Rege, 2002). The importance of the Kuri lies not only in its unique physical characteristics but also in its meat and milk production potentials. The Kuri is rustic and well-adapted to its habitat, but is unable to thrive outside the environs of the lake (Mpofu and Rege, 2002).

The genetic uniqueness of Ankole and Karamonjong is attributed to the geographical location where the animals are indigenous coupled with the traditional production systems practised by the respective pastoralists and the usual abundance of pastures which limit animal migration in search of water and pasture. Nganda's rare genetic composition is due to the European Taurine contamination or crossbreeding with the European Taurine (Kugonza *et al.*, 2011).

Multivariate analysis of allele frequencies has been a powerful tool to reveal the underlying evolutionary history and admixture among distantly-related Taurine and Zebu populations from Europe, Asia and Africa (MacHugh *et al*., 1997; Freeman *et al*., 2006). PCA in this study clearly revealed a cline of Zebu introgression. The Nigerian cattle, the Muturu, N'Dama and the Friesian × Bunaji breeds form a gradient between the Zebu and the African Taurine breeds. The N'Dama and the Friesian × Bunaji were undoubtedly more influenced by Zebu genes than Muturu breed in this study. On the other hand, in Uganda, the Nganda had a lower influence from Zebu genes and Sahiwal crosses had a lower influence from Sanga breed which are situated in the north-east and Western Uganda,

respectively, which is in agreement with a widespread movement of Zebu cattle from east to south (MacHugh *et al*., 1997; Hanotte *et al*., 2002; Freeman *et al*., 2006).

Relative to the origins of the Nganda, the data reported here support the hypothesis of a crossbreeding between Zebu and Sanga breeds, as suggested by Regé and Tawah (1999). The Nganda breed had a higher percentage of Sanga genes than the Zebu breeds analysed, which points towards an Ankole derivation. However, the results do not show whether Nganda was derived from crossbreeding between Ankole and Serere or between Ankole and Karamonjong. The later hypothesis seems more reasonable given the geographic proximity between the parental breeds. Secondly, the recent breeding programmes in Uganda centred more in the central region of the country where the Nganda is predominant and this has seen the Nganda being rapidly crossbred with the Western taurine which may also be the reason for its derivation.

Relative to the indigenization of the various breeds from Nigeria and Uganda, the data reported here support the hypothesis of the Nigerian Indicine and the African Taurus from West Africa and the Sanga, the crossbreeding between Zebu and Sanga breeds for Nganda, the Large East African Zebu (Karamonjang) and the Small East African Zebu (Serere) as suggested by Regé and Tawah (1999).

Estimation of genetic admixture proportions using a coalescent approach (mY) (Bertorelle and Excoffier, 1998) showed that for the Nigerian cattle populations, the Adamawa Gudali had the highest probability (0.380) of representing one cluster (K = 2) followed by N'Dama at K=3, Sokoto Gudali at K = 4, Friesian × Bunaji at K = 6. Ankole had the highest

probability (0.688) of representing five (K = 5) and seven (K= 7) clusters while Nganda represented the highest probability at six clusters (K = 6) in the Ugandan cattle populations.

Based on the assignment of individuals without previous population information, this estimation gave a split of the Adamawa Gudali and Sokoto Gudali breeds to different clusters (K = 2 and K = 4) and N'Dama and Friesian × Bunaji to cluster three (K = 3) and cluster six (K = 6) in the Nigerian Breeds while the Ankole breed were split to two clusters (K = 5 and K = 7) and the Nganda to the other cluster (K = 6) in the Ugandan breeds.

This pattern corresponds effectively to an indicine and a taurine clustering. Higher K values might indicate a more ancestral subdivision of cattle populations that could precede breed formation. An examination of the K = 1 and K =4 admixed coefficients gave credits to the Gudalis (Adamawa Gudali and Sokoto Gudali), respectively, proving them to be considered among the true shorthorn Zebus of West Africa (Tawah and Rege, 1996). This study was in contrast with the results of Ibeagha-Awemu *et al.* (2004) in whose study; admixed coefficients gave this credit rather to the Nigerian Red Bororo and observed that Adamawa Gudali was the Zebu that diverged most from the others. Its low sample size and the high genetic contribution (K 2 = 38.2%) from the African Taurine could be responsible for this behaviour. Close relationships for the Zebus may be the consequence of management practices that promote high gene flow between them while slight differences could be attributed to genetic drift, which is in line with the migratory histories of the Fulani people.

Between two and seven clusters (K values) were tested using the admixture model, assuming that each individual does not necessarily have a genetic background originating from one of the K populations. Consistent results across runs were obtained. A good value

of K exhibited a low cross-validation error compared to other K values (Alexander *et al.*, 2009), concluding that 2 and 4 were the optimal Ks. A graphic representation of the estimated membership coefficients to the clusters for each individual, obtained running ADMIXTURE setting K from 2 to 7, showed each individual represented by a single vertical line broken into K coloured segments, whose lengths are proportional to each of the K inferred clusters. Differentiation within the Nigerian and the Ugandan cattle populations is first observed for K = 2 which reflected a Zebu, Taurine and Sanga cluster pattern before differences at the breed level are detected, respectively.

At K = 4, differentiation within the Friesian × Bunaji from Nigeria and Nganda from Uganda are assigned to a separate cluster. All individuals from both countries showed the highest level of genetic admixture and could not be differentiated for K values from 5 to 7. Muturu was assigned to a Taurine group in K = 2 and very much remained apart from the other Taurine breeds in K = 4. This might be attributable to the high level of inbreeding in the population analysed (F_{IS} = 0.386). Generally, the breeds of cattle used in this study were admixed with the Indicine and Taurine proportions. Individuals of Friesian × Bunaji from Nigeria and Nganda from Uganda showed the highest level of genetic admixture, revealing that the genotype distributions of these breeds was more similar than that of the other breeds examined (reflection of the European Taurine contamination). This is not surprising, in that these breeds, besides having a different history, underwent specific selection for milk production (Kugonza *et al.*, 2011; Oni *et al.*, 2001).

From this analysis, despite their different morphology, Large East African Zebu and the Small East African Zebu were clearly identified as genetically-distinct breeds as earlier reported by Rege and Tawah (1999). This could be attributed to either a different origin of

the breeds or a consequence of the recent history that led to the selection and probably fixation of genes. There was definitely admixture of some levels by admixture and the Nganda have European Taurine contamination and the N'Dama has admixture from indicus.

5.3. Phylogenetic relationship among the indigenous cattle populations from within and between Nigeria and Uganda

Thus, the grouping of *Bos taurus* (cluster 1) and *Bos indicus* (cluster 4) differently, reflects the effect of the geographic origin on the genetic structure of the Nigerian cattle populations. This could arise from a lower gene migration between breeds originating from different geographical zones and a greater probability of common ancestors for breeds maintained in a geographical proximity. However, the fact that Keteku grouped with Kuri cattle (cluster 3) indicated a certain degree of gene migration between these breeds. The Friesian × Bunaji clustered near the African Taurine populations.

Nevertheless, various Taurine phylogenetic analyses performed with a higher number of microsatellites also evidenced a weak topology in Taurine breeds (Moazami-Goudarzi *et al.,* 1997; Martı´n-Burriel *et al.,* 2001). As discussed by Moazami-Goudarzi and Laloe (2002), another way of understanding the lack of structure is assuming that populations have differentiated according to a radiative scheme of divergence. According to this model, it is expected that genetic distances between breeds would be equivalent, and any casual differences among them might be due to random genetic drift. The grouping of the *Bos taurus* is likely to be due to a high level of admixture and similarity of production environments.

The clustering of Muturu and N'Dama (cluster 1) separately from other Nigerian cattle populations could be attributed to their unique genetic compositions (African Taurine), geographical isolation, and ecological differences. Thus, it is the ancient Taurine component which emigrated from Iberian Peninsula. The detected signature of admixture of the two breeds and separation from the rest of the groups could be due to the fact that the two breeds share input from the African shorthorn Taurus populations. Similarly, Keteku is an intermediate type, formed by hybridization of the indigenous humpless cattle with Zebu (Rege, 1999). These findings are also in contrast with the hypothesis that livestock facing selection pressure from environmental conditions, such as drought, are expected to show higher genomic divergence across habitats, compared to a neutral genome background (Hanotte *et al*., 2010). The grouping of the Nigerian *Bos indicus* (Cluster 4) is likely to be due to a high level of admixture and similarity of production environments.

For the cattle populations from Uganda, Ankole formed cluster with Nganda and confirming direct or indirect gene flow between the mountain cattle breeds and Nganda is known to be influenced by Ankole (Rege and Tawah, 1999), which explains their relatively short genetic distance. Karamonjonag, Serere and Sahiwal Zebu crosses are all from North-eastern Uganda and also appear to be relatively close. Similar results were also reported by Okomo (2002) in various European, African and Indian cattle breeds. Summarizing the results obtained from an analysis of the genetic distances in both countries, it might be stated, that the breed structure of the analysed populations of West and East African cattle is principally a reflection of the geographic origin of individual breeds.

The clear differentiation between the Nigerian and Ugandan cattle populations according to their conventional classification is in good contrast with the already established facts

showing that since the introduction of the Zebu breeds along North to West Africa and along the Nile to East Africa, there has been extensive hybridization between the Zebu and original African longhorn and shorthorn Taurine cattle (Epstein, 1971; Rege, 1999; Hanotte *et al*., 2002; Freeman *et al*., 2006). The present study was also in line with the separate clustering of *Bos indicus* and *Bos taurus* breeds from principal component, phylogenetic, and ADMIXTURE analysis (Lin *et al*., 2010). Principal component, ADMIXTURE and phylogenetic analyses clearly separated within and between the Nigerian and Ugandan cattle populations, which is in accordance with their separate geographical origins, domestication, and divergence long before domestication of *Bos indicus* and *Bos taurus* subspecies (Loftus *et al*., 1994, Loftus *et al*., 1999; Bradley *et al.*, 1996). Furthermore, the high divergence of Ugandan from Nigerian cattle is consistent with the recent reports of independent cattle domestication in Africa (Hanotte *et al.*, 2002).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

A significant amount of genetic variation is retained within indigenous cattle populations from Nigeria and Uganda. Population structure analyses, genetic distance, phylogenetic tree, clearly differentiated the cattle populations according to their historical origins and represented the genetic distinctiveness of Nigerian cattle populations from the Ugandan cattle breeds. The high within- population genetic diversity and the unique adaptation of the current populations to wider environmental factors (disease, heat stress, drought, and feed shortage), might be a consequence of the peculiar admixture among the different cattle breeds. Hitherto, these populations have represented a unique genetic resource and

unexploited opportunity that warrants initiatives for their sustainable conservation and utilization. Nontheless, these data provide a strong initial framework to find signatures of selection maintained between admixed breeds to identify some of the genetic factors conferring adaptation in various production environments of Nigeria and Uganda.

The clustering of populations by ecological distribution is an insight suggesting that further investigation of the association between genetic markers and geo-environmental parameters could better enable exploitation of valuable genetic material. Apparent partition of the populations (Nigerian and Ugandan) according to their historical origins, and corroboration of the established details of the genetic diversity and composition of Nigerian and Ugandan cattle populations, suggest that analyses using 600K SNP markers provided sufficient genetic information to properly assess the genetic structure.

The most important conservation aspect of the single nucleotide polymorphism variation survey presented here has been the capacity to accurately identify and characterise the extent and pattern of genomic introgression from Zebu cattle into valuable trypanotolerant N'Dama and Muturu (African Taurine) populations in the Southern equatorial regions of Nigeria and the Ankole and Nganda in West and Central Uganda. There are however numerous other African Taurine breeds in Nigeria (Muturu, N'Dama and Kuri) and the Nganda in Uganda which are under serious imminent threat of absorption and replacement with Zebu populations migrating from the Sahel.

6.2 Conclusion

This study investigated the genetic diversity, population structure and admixture levels and the phylogenetic relationship among the Nigerian and Ugandan indigenous cattle breeds

using 600K SNP markers. On genetic diversity, the cattle populations from Nigeria and Uganda expressed high genetic variability within the breeds (0.641 for Nigeria and 0.633 for Nigria), with relatively high (0.979) genetic material exchange in cattle populations from Nigeria, compared to 0.953 in cattle populations from Uganda. Despite, the high genetic material exchange, it appears that they retain some of their genetic identity (0.105 for Nigeria and 0.016 for Uganda). The level of genetic variation observed in this study highlighted that the 600K SNP markers can be potentially used for genetic studies in African cattle populations

In the second objective of this study, the genetic structure of the cattle populations from Nigeria and Uganda was based on their indicine and taurine origin of the breeds studied.

For Nigeria, Kuri (-0.972), Friesian × Bunaij (-3.763), Keteku (-1.582) and Muturu (-4.833) while Ankole (-04.638), Nganda (-0.224) and Karamonjang (-0.931) for Uganda exhibit rare and uncommon genetic uniqueness from the rest of the West and East African cattle breeds used in this study. The Zebu breeds in this study are not genetically pure *Bos indicus*, as they all exhibit a marginal proportion of taurine backgrounds and a significant proportion of Zebu background relative to taurine in the West and East African breeds except the Ankole from Uganda. In the third objective of the study, these indigenous cattle populations were found to form relatively homogenous and genetically unique groups of populations, that were distinct to the pure *Bos indicus* and *Bos taurus* breeds. The admixed breeds were shown to be closely related or intermediate, while the *Bos indicus* and *Bos taurus* were different. Relationships among Nigerian and Ugandan cattle reflect their geographical distribution rather than phenotypic breed groups.

6.3 Recommendations

The local breeds, perfectly adapted to the environmental conditions in their foundation area, are preserved as gene pools for the next generations. The unique genetic background of West and East African cattle and their known adaptation to local environmental conditions support the need for their conservation and utilization in local farming systems.

- The high variability in the cattle populations from Nigeria and Uganda makes them suitable candidates for conservation and improvement. Controlling gene flow between breeds, by adopting effective breeding and management practices, will facilitate the conservation of historical breeds and preserve special characteristics of each breed.
- The observed genetic differentiation of the breeds may deserve more investigation. Further analysis could help in tracing an unbiased picture of past events and provide the basis for a rational exploitation of livestock, suggesting appropriate cross-breeding plans based on genetic distance, or breeding strategies that include the population structure.
- Commercial European breeds as well as African cattle have contributed to the genetic composition of the Friesian × Bunaji in Nigeria and Nganda in Uganda. Therefore, further work in a wider context of a meta-analysis of European/African cattle is necessary to better understand breed relationships and admixture.
- More research needs to be done on the Nigerian and Ugandan native cattle breeds and the choice of breeds for conservation must take into account any available information on productivity traits of economic value, specific adaptive features (tolerance to heat, low quality feeds, disease etc.), presence of unique genes or

phenotypes, local or regional importance of a breed in production systems, and the availability of resources and infrastructure in the two countries for animal production.

- Both *in situ* conservation of animals with uncommon genetic variation and use of animal breeds must play an increasingly important role in the future of genetic resource conservation. *In situ* conservation will enable animal populations to continue to adapt, evolve and be selected for use in their natural environments. This method conserves not only the livestock, but also the traditional systems of which they are a part.

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