1. Introduction

The current genetically diverse cattle populations are the result of long-term evolutionary processes. The diverse populations are adapted to different environmental conditions and to a wide range of human needs. All domesticated cattle of the world are of a single species derived from the wild aurochs, *Bos primigenius* Bojanus (1827), which has been extinct about 379 years ago: based on archaeological evidence, the last cow died in a forest in Poland in 1627 (Epstein and Masson, 1984).

Out of 1.2 billions estimated number of the world cattle population; about 188 million head are found in Africa and of this about 31 million, representing 16% of African cattle population, are in Ethiopia (FAO, 1994). Hall (1992) showed the existence of 873 cattle breeds in the world, of which 178 are considered indigenous to Africa. DAGRIS (2006) reported that there are 27 cattle breeds/types in Ethiopia (Appendix I).

A very basic and extremely important work that is needed for a country and to the world is to obtain an inventory of genetic resources and characterization of these resources. Genetic variation studies provide an understanding of the current and historical evolutionary processes that have generated biodiversity patterns, the preservation of which should be an important component of conservation plans (Smith and Wayne, 1996) and to develop future breeding plan.

Due to the various changes in agricultural technology and mostly as a result of the needs of high-input farming system, local cattle breeds are being displaced by commercially high productive exotic cattle breeds like Holstein Friesian. Many traditional breeds have disappeared because of

farmers focus on the new cattle breeds. Around 16% of them have become extinct and 15% are rare (FAO, 2000). Many breeds become rare either because their characteristics do not meet contemporary demand or because their qualities have not been recognized. However, the multi purpose local and traditional breeds are often well adapted to local conditions, climate, diseases, and nutritional environment (Jolanta *et al.*, 2002). According to Jolanta *et al.* (2002) such breeds may also be better adapted to locally produced forage, or be more resistant to geographically localized pathogens and pests. The native breeds have been also selected for different objectives, depending on the traits that were important to the local human communities.

Domestic cattle are the major component of the world's economy. Through milk and milk products, they provide the bulk of animal protein consumed by many human societies. They also contribute other important commodities including meat, hides, traction and dung for fertilizer as well as energy source. Cattle production in Ethiopia is an integral part of nearly all farming systems in the crop producing area of the country and it is the major occupation in the lowlands, it forms the basis for millions of life. The role of livestock in general and cattle in particular in the national economy is significant, the sector is even more important than the figures would suggest when their contributions such as work, fuel and manure are considered (Mekonen, 1992).

Despite the significant contribution of livestock to the country, little attention is given to identify, characterize and conserve the diversity of the various classes of livestock types. The current classification of the Ethiopian cattle breeds is primarily based on some morphological and production characters such as body size, horn shape and size, skin/coat color, hump etc. and using geographic area where the cattle are inhabited. However, in the absence of proper genetic

identification, the possibilities for the loss of the genetic diversity of the indigenous cattle population are very high.

Ethiopian cattle genetic diversity is currently under threat mainly due to crossing among the local populations and with exotic breeds. The increased human movements, trade, cultural and social reasons are the main factors for the first, while commercialization of live stock industry and seeking for better productive temperate type breed are the possible reasons for the latter, which result in loss of valuable genetic resources which causes much economic loss to the producers. Beside this, genetic dilution of the indigenous stock is at risk to their survival since temperate breeds and their crosses are limited in their ability to survive and produce in tropical environments.

In Ethiopia for the development of appropriate breeding strategies and sustainable use of the genetic diversity, the various cattle breeds and/or types, which are distributed in the different agro-ecological zones, need to be characterized. Otherwise there will be much more loss in the genetic variation and animals, which can thrive and produce in local conditions might be lost. Such trends already exist in Africa and the pure indigenous cattle populations are likely to admonish as a result of cross breeding or neglect (Rege, 1992).

In the past, morphological and production characters have been studied. However, the usefulness of phenotypic traits to study the genetic variation of the divergence between populations is less. Marker genes, which are assumed to be neutral to the selection forces, are appropriate in the study of genetic relationship between breeds. Classification using genetic markers provides a large unbiased basis for the estimates of average breed similarities and/or differences.

2. Objectives

The overall objective of this study was to assess the between and within breed genetic variability of five indigenous Ethiopian cattle breeds.

Specific Objectives

- To determine the magnitude of genetic diversity of each breed.
- To determine genetic distance between the breeds.
- To see the relation between genetic and geographic distances.

3. Literature Review

3.1 Origin, Classification and Domestication of Cattle

3.1.1 Classification of Bovinae

The sub family Bovinae of family Bovidae is classified in to five genera viz. Bubalus (Asiatic buffalo), Sincerus (African buffalo), Bibos (the North Cambodian forest ox, kouprey), Bison (Europian and American bison) and Bos, which comprises two sub genera, namely the extinct urus and the yak (Epstein, 1971). Domesticated cattle were evolved from genus Bos (Payne, 1978).

3.1.2 Origin and Domestication of Cattle

The most common assumption about the history of domestication of cattle is that all modern cattle breeds are the result of domestication events, which took place in the Middle East (Epstein, 1971; Manwell and Baker, 1980; Epstein and Masson, 1984). The original domestication of cattle (Hamitic longhorns) probably occurred in Turkistan around 8000 B.C. and later about 6000

B.C. Hamitic shorthorns were evolved (Williamson and Payne, 1974). However, in light of modern archeological and molecular results, Meghen *et al*. (1994) indicated that cattle were originally domesticated in at least two primary locations approximately 10,000 years ago. One of these was concentrated in the nascent civilization of the near east and the other probably in north India and Pakistan. But in contrast to above, through a continent- wide sampling of indigenous African cattle (50 populations from 23 African countries), Hanotte *et al*. (2002) reported that people living in central Africa developed cattle domestication on their own, and that the techniques or the herders themselves gradually migrated towards the west and south, spreading domestication across the continent.

3.1.3 Classification of the Domestic Cattle

Epstein (1971) suggested that domestic cattle through out the world are of one species, which are evolved from the now extinct wild urus (*B. primigenius* B. 1827). In the beginning of the 19th century many bones of aurochs were excavated and one complete skeleton existed. Bojanus in 1827 named a new species from this skeleton: *Bos primigenius* (http://www.petermaas.nl/extinct/speciesinfo/aurochs.htm). Oliver (1983) also indicated that cattle were probably derived from one ancestral strain or urus, the wild ox, which was found all over Eurasia and North Africa. Humped cattle (zebu) are then thought to have been produced at a later date through breeding and selection from humpless (taurine) cattle (Epstein, 1971; Epstein and Mason, 1984; Payne, 1990). However, Manwell and Baker (1980) based on protein polymorphism analysis and MackHugh *et al*. (1997) based on micro satellite DNA variation, suggested that a separate origin for domesticated humped (zebu) and humpless (taurine) cattle. Through analysis of mitochondrial DNA Loftus *et al*. (1999) demonstrated that taurine and zebu cattle probably diverged at least 210,000 years ago, well outside the range required for human

mediated development of zebu cattle from taurine progenitors. The hypothesis that humpless (taurine) and humped (zebu) cattle have separate domestic origins was also supported by the microsatellite survey where a large genetic divergence being apparent between the non hybrid taurine and zebu groups (Loftus, et al., 1999).

3.2 Origin, Classification and Distribution of African Cattle

3.2.1 Origin of African Cattle

The history and biogeography of cattle populations in Africa represent a complex interaction of ecological, genetic and anthropological factors. The original indigenous cattle of Africa are universally considered to have been taurine (MacHugh *et al*., 1997). The populations are thought to have arisen from migrations of early pastoralists from the near east (Epstein, 1971; Payne, 1990). Recent archeological evidence has, however, questioned this viewpoint, suggesting that the African aurochs (*Bos primigenious opisthonomus*) may have been domesticated independently somewhere on the African continent which gave rise to African taurine cattle (Wendorf and Schild, 1994). Hanotte *et al*. (2002) also reported that people living in central Africa developed cattle domestication on their own.

3.2.2 Classification and Distribution of African Cattle

African domestic cattle are divided in to two main groups viz. humpless and humped (Epstein, 1971). The humpless cattle are sub-divided in to longhorn and shorthorn, while the humped cattle are classed in to zebu proper and zebu crossbred types (Epstein, 1971).

Humpless longhorn: Humpless longhorn cattle occur in the southwest of West Africa (N'Dama) and in the Chad region (Kuri). Formerly they were ubiquitous in North Africa, Ethiopia, Kenya and Uganda; and in the mountains and oases of the Sahara (Epstein, 1971).

Figure 1. Kuri cattle. Source: DAGRIS

Humpless shorthorn: These classes of cattle occur in the northern half of Africa in a narrow belt skirting Sahara. They are bred in Egypt, with a vanishing offshoot in the Nuba Mountains of the Sudan, and extend from Libya through the Atlas countries into Morocco. In West Africa they are found mainly in the coastal regions from Gambia to Cameroon, with a resent offshoot in the

western extension of the Congo (Epstein, 1971). In sub-Saharan Africa humpless shorthorn cattle is restricted to the tsetse-infested areas (DAGRIS, 2006). It is represented by 14 breeds/strains of pure shorthorn and five breeds, which have been derived from zebu-shorthorn interbreeding.

Figure 2. Sheko cow. Source: DAGRIS

Zebu: Although, zebu cattle are thought to have been first introduced in to Africa about 4000 years ago, they only started to become widespread about 700 AD with the Arabic migrations into North and East Africa (MacHugh *et al*., 1997). Meghen *et al.* (1994) suggested that zebu cattle have a more Eastern origin. Although zebu cattle first appeared in Egypt in ancient times, only started to spread comprehensively through West Africa along with Arabic influences with in the last 1400 years (Meghen *et al*., 1994). In Africa the zebu type is represented by great varieties of

different forms. The hump is the most important and persistent zebu feature, although it is not equally well developed in all zebu breeds, and not all cattle in which it occurs are true zebus (Epstein, 1971).

Figure 3. Zebu cattle.Source: International Miniature Zebu Association (http://www.miniature-zebu-cattle.com/).

Sanga: The term 'sanga' is an Ethiopian word meaning 'bull' and it relates to the origin and centre of dispersal of this group of cattle breeds (DAGRIS, 2006). It is in this part of East and Northeast Africa where Sanga cattle first evolved as a result of the interbreeding of the longhorn humpless, shorthorn humpless and zebu type cattle, commencing about 3000 to 4000 years ago, a process that has continued up to the present time (Payne and Wilson, 1999). Therefore at present, Africa represents a mosaic of cattle morphologies with zebu (hump and dewlap) and humpless cattle (horns and no humps), often referred to as sanga, predominating over the continent (Payne, 1978; Epstein, 1971). The present distribution of the 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. Its evolution probably began in Ethiopia or Uganda after the introduction of cervico-thoracic-humped zebu (Epstein, 1971).

Figure 4. Africander cow. Source: DAGRIS

3.3 Domestication and Classification of Cattle in Ethiopia

3.3.1 Domestication

Humpless cattle: The conventionalized and schematic paintings and engravings of pastoral scenes (rock paintings of herds' men and humpless longhorn cattle at Genda - Biftou, Sourre, in Harar, Ethiopia) and Somalia indicate cattle raising has been a later date activity (Epstein, 1971). Epstein (1971) referred to historical evidences that suggesting the cattle of punt as belonging to two different cattle types, long horned and short horned, and inferred that these cattle had penetrated to the Ethiopian region by the middle of the second millennium B.C. There are different suggestions that humpless shorthorn cattle may at one time have been introduced in to Ethiopia. The Sheko breed is believed to be the last remnants of the original humpless shorthorn cattle in eastern Africa.

Zebu (humped cattle): Zebu cattle were introduced in to the horn of Africa and Ethiopia in the 4th century A.D. and later (Epstein, 1971). Hetzel (1998) suggested that thoracic humped zebu cattle arrived in Africa from the Indian subcontinent via Somalia and Ethiopia around 400 A.D and constitute the majority of the cattle population of Ethiopia. However, the data given seems to be wrong since the diversity of such cattle in the area mentioned is quite high indicating a longer period of time to accumulate such a magnitude of diversity. Epstein and Masson (1984) also stated that all domestication occurred long before any records were kept.

3.3.2 Classification

According to Epstein (1971), Ethiopian cattle breeds have originated from the migration of Hamitic longhorn and shorthorn, and from the later introduction of the zebu. Inter breeding among these cattle type resulted in the formation of some breeds such as sanga and other varieties. Epstein (1971) classified all Ethiopian cattle breeds and/or types in to three main groups viz. zebu, sanga, and humpless (Table 1). However, (Albero and Hailemariam, 1982; Tesfaye *et al*., 1994) identified additional type of cattle group showing intermediate phenotypic characteristics between zebu and sanga and called sanga-zebu (Table 1). However, Beyene and Bruk (1992), Tesfaye *et al*. (1994) indicated that at present the indigenous cattle are not properly categorized to belong to specific breed groups, thus, the classification is based or named after the tribal owners and the area they occupy. In general, (DAGRIS, 2006) reported that there are 27 indigenous cattle breeds/types in Ethiopia (Appendix I) representing humpless shorthorn, zebu, sang, and zenga (sanga-zebu).

Table 1: Classification of indigenous cattle of Ethiopia (based on Epstein, 1971; Albero and Hailemariam, 1982 and Tesfaye *et al*., 1994).

Breed Groups				Source
Zebu	**Sanga**	**Sanga-zebu**	**Humpless**	
Wagera (Fogera), Walega zebu, zebu of central highland, black highland, Jiddu	Danakil/Adal or Keriya, Galla Azebo, Raya Azebu, Abigar/Nuer/Agnuak		Sheko	Epstein, 1971
Arsi, Borana, Arab (Adani, Berbera, Bahari), Abyssinian zebu (Short horn zebu), Shorthorn zebu-Harer, Highland-zebu (Bale), Black-zebu (Jem-Jem), Small zebu (Jijiga), Fellata, Barka	Danakil (Adal, Raya, Kariyu, Afars), Abigar (Nilotic)	Horro, Fogera (Wagara), Arado, Jiddu	Sheko (Mitzan Goda) short horn, Kuri (Kouri) longhorn	Alberro and Haile-Mariam, 1982
Borana, Arsi, Arab (Aden), Abyssinian zebu (shorthorned zebu, Jijiga, highland zebu, Jemjem zebu)	Danakil, Abigar	Horro, Fogera, Arado	Sheko (Mitzan or Goda)	Tesfaye *et al*., 1994

3.4 Diversity in Cattle Breeds and Its Importance

3.4.1 Diversity

Cattle are found through out the world. Through Asia to Europe, from pacific cost in the east to the Atlantic in the west, from the north to the cost lands of the Indian ocean and through Africa from the Mediterranean countries to the cape of Good Hope. Only in certain exceptional ecological regions they are replaced by other Bovidae or other domesticated animals (Epstein, 1971). Worldwide there are more than 790 breeds of cattle (FAO, 1995).

The genetic variation both between and within breeds is described as the diversity with in species (Kantanen *et al*., 2000). The variation is generated by mutations such as nucleotide substitution, insertions/deletions, gene conversion, and inter allelic recombination. However, most of these new mutations are eliminated from the population by genetic drift or purifying selection, and only minority of them, neutral or positive, are incorporated in to the population by chance (Nei and Kumar, 2000).

3.4.2 Importance of the Diversity

The biological diversity represented by multiplication of different breeds of cattle enables productive agriculture to be carried out in a wide range of environments than would be the case if there were genetic uniformity (Global Biodiversity, 1992). Livestock genetic diversity in general has provided the material for the very successful breeding improvement programs of the developed world in the 19th and 20th century. It represents a unique resource to respond to the

present and future needs of livestock production both in developed and developing countries (Hanotte and Jianlin, 2005; Hall and Bradley, 1995).

Local cattle breeds are considered, for many reasons, precious genetic resources that tend to disappear due to a new market and agricultural demands (Beja-Pereira *et al*., 2003). Many traditional breeds have disappeared because of farmers focus on the new cattle breeds. Around 16% of them have become extinct and 15 % - are rare (FAO, 2000). Many breeds became rare either because their characteristics do not meet contemporary demand or because their qualities have been not recognized. However, the multipurpose local and traditional breeds are often well adapted to local conditions, climate, diseases, and nutritional environment throughout many years. Such breeds may also be better adapted to locally produced forage, or be more resistant to geographically localized pathogens and pests. The native breeds have been also selected for different objectives, depending on the traits that were important to the local human communities (Jolanta *et al*., 2002). One can assume that livestock breeds represent the outcome of socioeconomic development and are unlikely to survive outside the social and agricultural systems having formed them.

The genetic diversity of cattle is decreasing. Local cattle breeds have become almost totally displaced by high productive exotic breeds, mostly as a result of the needs of high-input farming system. Conflicts and other forms of socio-political instability led the owners to move their cattle out of their original area, which increased the possibility of extinction and mixing animals with other breeds, thereby potentially losing local specific breed (s). As identified by (FAO, 2000),

there is a serious risk that most of these breeds will disappear before they have been fully identified, and this applies particularly to the breeds of developing countries.

3.5 Characterization of Cattle Breeds

The animal-oriented definition recognizes that breeds differ by the totality of average differences observed in many quantitative and qualitative traits (Meghen *et al*., 1994). The differences may overlap, but they have genetic bases, and these differences taken together provide a unique description (Baker and Manwell, 1991). The primary aim of population genetics is to detail in an unambiguous and often numerical manner all parameters that are useful in describing a population. The studies of genetic variation provide an understanding of the current and historical evolutionary processes that have generated biodiversity patterns, the preservation of which should be an important component of conservation plans (Smith and Wayne, 1996; Mitra *et al*., 2002).

Different cattle breeds may have similar phenotypic characters, but still can be different genetically; conversely, breeds may look very different but be genetically closely related (Jolanta *et al*., 2002). Such differences and/or similarities can be detected using varieties of genetic markers ranging from morphological (phenotypic) characters to protein and DNA-based markers including RFLP, AFLP, variable number of tandem repeats (VNTRs), RAPDs, and SNPs (Meghen *et al*.,1994; Mitra *et al*., 2002; MacHugh *et al*; 1997; Vignal *et al*; 2002; Suzan *et al*; 2002; Seranno *et al*; 2004; Parker *et al;* 1998; MacHugh *et al*; 1998; Kantanen *et al*., 1995).

3.5.1 Characterization based on morphological markers

Traditionally, phenotypic (internal or external) characters have been used to ascribe a given animal to a breed (Meghen *et al*., 1994). These extremely varied characters have ranged from

skin color to size of testes, from the presence or absence of the bifid process to milk butterfat content. One of the problems associated with this type of analysis is the difficulty in combining different measures in order to provide a useful tool for the description of a given breed. Because of the influence of both natural and artificial selection on phenotypic traits, it is unlikely that any such comparison would always produce meaningful results. Furthermore, morphological markers usually show low degree of polymorphism and hence are not very useful for genetic characterization (Mitra *et al*., 2002).

3.5.2 Characterization using molecular markers

In contrast to morphological traits molecular markers are less influenced by natural and artificial selection and enable to test the genome better. As a result nowadays they became markers of preference for characterization. With the advent of different techniques, diverse array of new molecular genetic tools have become available for high-resolution genetic studies of population-level processes (Kantanen *et al*., 1995). Which technique is most appropriate for a particular question depends up on; the extent of genetic polymorphism required to best answer the question, the analytical or statistical approaches available for the technique and the pragmatics of available time and costs of materials (Parker *et al*., 1998).

3.5.2.1 Protein electrophoresis

A protein solution is electrophoresed through a gel and an enzyme specific reaction highlights one locus whose alleles (allozymes) may migrate different distances because of differences in size, shape, and/or charge (Queller *et al.,* 1993; Parker *et al*., 1998; Glazko, 2003). Obtaining

protein data is relatively inexpensive and straight forward once the basic procedures have been optimized for a given species. Large numbers of data can be processed with far less time per sample than the DNA-based methods (Parker *et al*., 1998). Furthermore protein (isozyme) electrophoresis data are codominantly inherited. However, a great amount of genetic variations at protein loci remain undetected, since changes in the underlying nucleotide sequences may not necessarily lead to corresponding change in the amino acid owing to degeneracy in the genetic code (Mitra *et al*., 2002), some species are monomorphic for most allozymes, and the number of allozymes that can be screened by standard procedures is limited, narrow endemic species and others that have experienced genetic bottlenecks often lack polymorphic loci (Parker *et al*., 1998).

3.5.2.2 Nucleic acid markers

DNA markers provide a means for powerful, fine grained analysis of individual genotypes. In many situations DNA markers are essential. Moreover, the range of questions that can be addressed using DNA markers is expanding rapidly as more efficient techniques are developed. However, the challenge for population biologist is to find the appropriate method that reliably reveals adequate genetic variation for a particular question, with a minimum amount of effort and expense.

Restriction Fragment Length Polymorphisms (RFLPs): Restriction Fragment Length Polymorphisms (RFLPs) were the first DNA markers to be used by population biologist (Parker *et al*., 1998). A study of restriction fragment length polymorphisms (RFLPs) involves comparing the number of band size of deoxyribonucleic acid (DNA) fragments produced by the digestion of DNA with various restriction enzymes and detection by Southern blot hybridization (Meghen *et al*., 1994). The banding pattern is generated by the presence or absence of restriction cutting sites.

The fragments are blotted to membranes and probed with cloned radio labeled DNA that binds to a single locus. The variations can sometimes be visualized directly by staining with ethidium bromide following electrophoresis of the DNA in an agarose gel (Parker *et al*., 1998).

A more efficient approach to RFLP analysis may be to use PCR to amplify random fragments of the genome. Fore one or more clones of ~2 kb chosen from a DNA library, sequences are determined for the 100-200 bases at each end of the clone. The amplified product can then be treated with restriction enzymes and the fragments separated on an agarose gel and visualized by ethidium bromide staining to identify RFLPs (Karl *et al*., 1992). Although, RFLPs have wide range of application in population studies, they still require a great deal of labor, including the construction of genomic DNA library, some initial DNA sequencing, time consuming and fine tuning of the PCR conditions (incase of PCR RFLPs) (Karl *et al*., 1992). Moreover, since mutational events are generally the product of base substitutions, however, the rate of mutation is likely to be extremely low and this results in a similar problem to that of proteins, which is a lack of resolving power when dealing with very closely related groups (Meghen *et al.,* 1994). This has been demonstrated by Theilamann *et al*. (1989), who carried out a study of nine RFLPs in six breeds of cattle, with the result that only Brahman (*Bos indicus*) cattle differed significantly from other breeds, which were all *Bos taurus* Linnaeus (1758).

Amplified fragment length polymorphism (AFLP): In amplified fragment length polymorphism assay (Zabeau and Vos, 1993), template DNA is digested with two restriction enzymes (REs), and the resulting restriction fragments are then ligated with adapters and, subsequently, PCR amplification is carried out using specially designed primers which comprise (I) a unique part corresponding to selective bases, and (II) a common part corresponding to the

adapters and the RE sites. Ajmone-Marsan *et al*. (1997) reported that AFLP patterns are reproducible in independent experiments and polymorphic fragments segregate in cattle families according to Mendelian rules.

Microsatellites: Simple tandom repeats (STRs) or microsatellites are a relatively new class of genetic markers. Microsatellites consist of tandom repeats of very short nucleotide motifs from one to six base pairs long, the dinucleotide repeat 'CA' being the most common in mammalian genomes (Meghen *et al.,* 1994). When the unique sequence flanking both ends of the repeated sequence is known, the microsaatellite can be preferentially amplified using the polymerase chain reaction (PCR). Different length classes (alleles) vary in the number of repeats and can be separated using polyacrylamide gel electrophoresis (PAGE).

Microsatellites are extremely useful for population studies because they are highly polymorphic, displaying many different alleles for a given locus, and reproducible (Parker *et al*., 1998; Meghen *et al*., 1994; Vignal *et al*., 2002; MacHugh *et al*., 1997). The disadvantages of microsatellites are mainly associated with the requirement of prior knowledge of DNA sequence. As a result the technique can be relatively time consuming and costy.

DNA sequencing: The most detailed analysis of DNA differentiation can be obtained by sequencing the region of interest from different individuals. Until recently, extensive use of DNA sequencing for population studies has not been practical because the DNA fragments to be sequenced for each individual had to be isolated from sub genomic DNA libraries after identification by Southern blotting and hybridization. Using PCR, however, specific regions can be targeted for amplification if the sequence of the conserved regions flanking the region of

interest is available (Parker *et al*., 1998). Deoxyribonucleic acid sequencing can be used to address virtually any systematic problem and many problems in population genetics, but it is not necessarily the most efficient or cost effective method (Meghen *et al*., 1994; Park *et al*., 1998).

Random amplified polymorphic DNA (RAPD): Random amplified polymorphic DNA (RAPD) is another recently identified genetic technique (Williams *et al*., 1990). Unlike the sequence-tagged PCR assay, randomly designed single primer is used to amplify a set of anonymous polymorphic DNA fragments. It is based on the principle that when the primer is short (usually 8 to 10 mer), there is a high probability that priming may take place at several sites in the genome that are located with in amplifiable distance and are in inverted orientation (Mitra *et al*., 2002). RAPD markers generally have high levels of genetic variation and unlike microsatellite a prior knowledge of DNA sequence is not necessary (Meghen *et al*., 1994).

Different RAPD patterns arise when genomic regions vary for the presence/absence of complementary primer annealing sites. Allelic variation consists of the presence or absence of particular amplification products, which can be separated on agarose gels stained with ethidium bromide. The RAPD process typically reveals several polymorphic genetic segments per primer within populations; other segments may appear as monomorphic bands with in or across populations (Hadrys *et al*., 1992). The degree of variability observed for many primers suggests that the technique will be useful for a variety of questions, including individual identification, paternity analysis, strain identification, and phylogenetic analysis (Parker *et al*., 1998).

Although there are some drawbacks (Meghen *et al*., 1994) in using RAPD as a tool for genetic characterization, such as lack of full understanding of how the genetic variation is generated,

vulnerability for inconsistency of results even under minor differences in experimental conditions that can produce erratic results and their dominant pattern of inheritance, presently the PCR-based RAPD assays are being used for characterization of cattle, sheep and chicken breeds (Gwakisa *et al*., 1994; Rincon *et al*., 2000; Bielawski *et al*., 1995; Glazko, 2003; Kantanen *et al*., 1995; Ali, 2003; Plotsky *et al*., 1995).

Single nucleotide polymorphisms (SNPs): SNP marker is a single base change in a DNA sequence, with a usual alternative of two possible nucleotides at a given position. For such a base position with sequence alternatives in genomic DNA to be considered as SNP, it is considered that the least frequent allele should have a frequency of 1 % or greater (Vignal *et al*., 2002). No such extended studies have yet been made for farm animals, but from limited data available, indications of high densities of SNPs in defined regions can be found and high level of diversity is observed in *Bos taurus* and *Bos indicus* (Vignal *et al*., 2002).

3.6 Genetic variation and Genetic Distance

3.6.1 Genetic variation

To understand the influence of selection, mating systems and other factors on population genetics, first one must be able to describe and quantify the amount of genetic variation in a population and the pattern of genetic variation among populations (Hedrick, 1983). Nei (1987) indicated that one of the main objectives of population genetics is to describe the amount of genetic variation in populations and study the mechanisms of maintenance of variation. Genetic variation may be measured at various levels; one of which is measurement of allelic variations at structural loci, particularly variation identified by electrophoresis.

Genetic variation can be studied in several methods for instance Wright (1965) showed that the variation in gene frequency among populations may be analyzed by the fixation indices of F-statistics. Nei (1972, 1973) also proposed other methods of measuring gene diversity or heterozygosity based on the gene or allele frequency variation which can be applied to any population regardless of the number of alleles at a locus or to the pattern of the evolutionary forces such as mutation, selection, and migration. It is also applicable to any organism, whether it is diploid or non- diploid, as far as gene frequencies can be determined (Nei, 1973).

3.6.2 Genetic distance

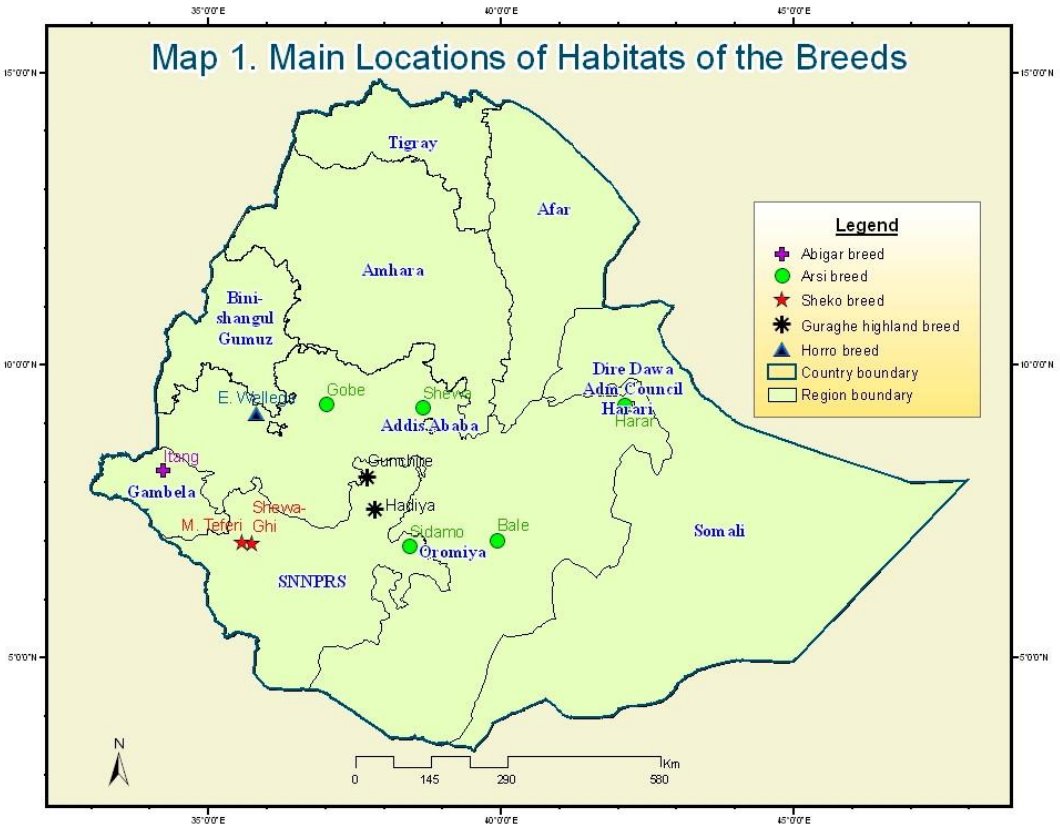
Genetic distance is the extent of gene (genomic) differences between populations or species that is measured by some numerical quantities (Nei, 1978). Thus, the number of nucleotide substitutions per nucleotide site or the number of gene substitutions per locus is a measure of genetic distance. The concept of genetic distance was first used by Sanghvi (1953) for an evolutionary study based on the concept of measuring population differences in terms of quantitative characters. But during 1960th the concept of genetic distance interms of gene frequency data was first constructed by Cavalli-Sforza and Edwards (Nei, 1978).

Since Cavalli-Sforza and Edwards (1964) published their work, many geneticists were interested in this problem, and various measures of genetic distance were proposed. Among these, Nei's genetic distances such as minimum genetic distance (D_m), standard genetic distance (D), and distance measures for the case of unequal rates of gene substitution (D') are widely used methods (Nei, 1971; Nei, 1972; Nei, 1978). In Nei's genetic distances estimate one can estimate the number of gene or codon substitutions per locus between two populations.

4. Material and Methods

4.1 Description of the breed

Sheko, Abigar, Horro and Guraghe highland breeds originated from their respective localities Sheko, Gambella, Easertn Wollega and Guraghe highland (Gunchire) (Map 1) were obtained from Tollaye cattle breeding station of ILRI/EARO in the Ghibe Valley primarily collected for trypanosomiasis tolerant breed selection, where as Arsi was obtained from Gobe cattle breeding and improvement ranch. As the information obtained from the ranches, physical characteristics of the animals, herd composition of the supplier to confirm whether there is exotic breed in the herd, specific village where the animal was brought from and other information were included while purchasing the animals from different markets of their localities.



Horro breed (Fig. 5): The Horro cattle are found in the western part of Ethiopia. Horro drives its name from the sub-province of Horro-Guduru, from where it has spread widely through Welega then to Ilubabor and Kefa, and to some extent to Shoa. It was developed out of interbreeding of various Abyssinian Highland Zebu and Nilotic Sanga, particularly the Abigar**.** The breeds that emerged from these crosses have been classified in a separate group of "zenga" cattle (DAGRIS, 2006). Albero and Haile-Mariam (1982) and Tesfaye *et al*. (1994) also grouped the Horros in to sanga-zebu (zenga) type (Table 1). They are very good looking animals being uniform in color and body conformation. They are of medium to large size, with small and finely shaped head, a straight profile and medium to large horns. The hump is small to medium in size. The Horro cattle have a uniform brown color which is slightly lighter around the muzzle and on the flank

(Albero and Haile-Mariam, 1982; Tesfaye *et al*., 1994). Alhough the total number of population is not yet recorded DAGRIS (2006) reported that the breed is not at risk of genetic erosion.

Figure 5. Horro breed. Bull (left) and cows (right). (Curtsy of DAGRIS)

Sheko breed (Fig. 6): The Sheko cattle are believed to be the last remnants of the original humpless shorthorn (*Bos taurus,* Linnaeus 1758) cattle in eastern Africa. They were first reported in 1929 from south-western Ethiopia, and later in 1982 (DAGRIS, 2006). At present some of the Sheko cattle manifest small humps that they inherited from zebu introgression. The breed is now considered affected by gradual interbreeding with local zebu and sanga (Abigar) and its population size is estimated to be 31,000 (Rege, G. E. O., 1999 as sited in DAGRIS, 2006). These short horned humpless cattle are found around Shoa_Ghimmira and Sheko region of southwestern Ethiopia and believed to have some level of trypanotolerance. They are small sized either without hump or with very small hump. They have small horns and many are polled. Their color is brown or black and white. This group of cattle may be the only remaining representative of the humpless cattle in Eastern Africa (Alber o and Haile-Mariam, 1982; Tesfaye *et al*., 1994).

Figure 6. Sheko breed (cow). (Curtsy of DAGRIS).

Arsi breed (Fig. 7): Descended from the recent introductions of zebu into Africa from West Asia. Probably developed from a group of small shorthorn Abyssinian zebu by the highland Oromo people (DAGRIS, 2006). Arsi cattle are mainly found in the central highlands of Ethiopia especially in Arsi, Shoa and Bale administrative regions. Their number is estimated over 2,012,000 (Rege, G. E. O., 1999 as sited in DAGRIS, 2006). They are small, short and compact. Red, with a black muzzle, is the predominant color although many animals are black, light gray or white with black spots and classified in to zebu cattle type (Albero and Hail-Mariam, 1982; Tesfaye *et al*., 1994; Epstein, 1971 and personal communication).

Figure 7. Arsi breed. Bull (left) and cow (right) (Curtsy of DAGRIS)

Abigar breed (Fig. 8): It is classified in to sanga type in which the original sanga stock evolved in Ethiopia, and the Nilotic sanga appear to have developed from early migrations and spread across south-western Ethiopia into southern Sudan as a result these cattle are found around the White Nile in the Sudan and adjacent lowlands of south west of Ethiopia where they are mainly kept by the Nuer people in the Akobo area of the Gambella region (DAGRIS, 2006). They are similar to the adjacent Aliab Dinka in the Sudan, and both have retained the large body size, long horns and small humps of the true sanga. They have straight profile head and the horns vary in length and shape but in general are very long and project outward and upward or are oval. Typical coat colors are light in shade, white with red and gray coat colors are also found (Albero and Haile-Mariam, 1982). Their population size is estimated to be 548, 600 (Rege, G. E. O., 1999 as sited in DAGRIS, 2006) and not at risk.

Figure 8. Abigar breed. Bull (left) and cow (right). (Curtsy of GAFRIS)

Guraghe highland breed (Fig. 9): These cattle types are not well studied and characterized as a breed. They were classified in to Abyssinian short horned zebu or Ethiopian highland zebu (Rege *et al*., 2001). The small Abyssinian shorthorned zebu inhabits the higher altitude, wetter agricultural areas, and hence they are also referred to as Ethiopian highland zebu (DAGRIS, 2006). The Guraghe highland is one of this on the wet central and south-western highlands of Ethiopia, mainly with the Guraghe and Hadiya people (DAGRIS, 2006; Rege *et al*., 2001). They are small- sized, usually with red, chestnut or roan coat color cattle and are mainly found in Guraghe and Hadiya areas (Rege *et al*., 2001; DAGRIS, 2006). Population size and status (risk level) is unknown (DAGRIS, 2006).

Figure 9. Guraghe highland breed. (Curtsy of DAGRIS)

4.2 Sampling animals and blood collection

Because of the fact that there were very few and/or no male animals in the ranches, only female animals were considered. Thirty matured unrelated individuals were taken randomly and blood samples were taken from each. 5 ml blood was drawn from jugular vein using 10 ml capacity EDTA coated vacutainer tube. The blood was mixed gently by inversion 5 times (Bruford *et al*., 1992) to distribute the anticoagulant and put in the ice-box containing ice. The blood was transported to Addis Ababa University molecular genetic research laboratory on next day morning and stored at -20 ^{o}C.

4.3 Preparation of DNA extraction buffer

The following DNA extraction buffers were prepared according to Bruford *et al*. (1992). EL buffer: 0.155M NH_4Cl; 10mM $KHCO_3$; 1mM EDTA, pH 7.4, KL buffer: 10mM Tris; 2mM EDTA; 0.4M NaCl, pH 8.2, TE buffer: 10mM Tris-HCl; 0.1mM EDTA, pH 7.4, 20 % SDS, Proteinase K (20 mg/ml) and 6 M NaCl.

4.4 DNA extraction

Because of several reasons such as scarcity of critical laboratory reagents, financial problems and breakage of some of the blood containing vacutainer tubes during storage, only 10 blood samples per breed were taken for DNA extraction. Genomic DNA was extracted from blood samples according to Bruford *et al*. (1992) with some modification (reducing the amount of proteinase K from 100 µl to 25 µl). This procedure is based on extraction of DNA from white blood cells (leukocytes) since mammalian red blood cells lack nucleus and hence nuclear DNA. In this technique the red blood cells (erythrocytes) are disrupted using lysis buffer (EL) in which WBCs are isotonic to this specific buffer followed by density gradient centrifuge separation of WBC from lysed cells (RBC).

1 ml blood was taken using pasture pipette and added to 10 ml capacity macro centrifuge tube. Three times by volume (3 ml) cold lysis (EL) buffer was added, mixed by inversion 5 times and placed on ice for 15 minutes, mixing occasionally, to disrupt the red blood cells (RBC) and then centrifuged for 10 minutes at 3000 rpm using macro centrifuge. The supernatant was discarded. Again 1 ml EL buffer was added to the precipitate and centrifuged for 10 minutes at 3000 rpm and the precipitate was discarded. This step, addition of buffer to the precipitate, centrifugation (for 5 mts at 3000 rpm) and discarding the supernatant, was repeated 2-3 times until there was no

visual sign of hemoglobin (redish color). The white blood cell pellet was resuspended using 3 ml KL buffer. 25 µl proteinase K (20 mg/ml) and 150 µl 20 % SDS were added accordingly, mixed carefully and incubated over night at 37 ^{o}C.

The solution was found to be viscous in he next day morning. About half volume (1.6 ml) of 6 M NaCl was added to the solution, shaked vigorously for about 15 seconds and centrifuged for 15 minutes at 4000 rpm. The supernatant was saved to another 10 ml capacity test tube with maximum care in order not to take any of the foam together with the clear supernatant. Approximately two volume of 100 % ethanol added, the top of the tube was closed, and mixed by inversion until white and clear fibre like precipitate (DNA) appeared. The DNA was spooled out using micropipette tips and rinsed in to 2 ml capacity eppendorph tube pre-filled with 1 ml 70 % ethanol for 20 minutes at 4 ^{o}C. The alcohol was totally drained and the DNA was transferred in to 2 ml capacity eppendorph tube pre-filled with 0.5 ml TE buffer and stored at 4 ^{o}C overnight in order to dissolve the DNA. Finally the DNA samples were stored at -20 ^{o}C.

4.5 Test for presence and quality of genomic DNA

4.5.1 Preparation of electrophoresis buffer and gel

The quality of genomic DNA, if present, was tested using agarose gel electrophoresis. Stock solution of 10X Tris Borate EDTA (TBE), commonly used electrophoresis buffer: 108 gm Tris base; 55 gm Boric acid; 7.44 gm EDTA, pH 8.57 components per liter was prepared and stored at room temperature. From the stock, working solution of 1X TBE enough both to pour the gel and fill the electrophoresis tank was prepared. 1 gm molecular grade agarose to prepare 1 % agarose concentration was measured and 100 ml of 1x TBE was then added in a blue top bottle together with magnetic steerer. The vessel was covered to minimum evaporation but not tightly closed in

order to avoid build up of pressure. The slurry was heated in a microwave for 3 minutes. After the agarose solution was cooled down to about 55 ^{o}C, it was poured on to the gel tray and the comb was inserted immediately after the agarose was poured and the gel was left for 35 minutes to dry properly. The comb was carefully removed and put the gel tray in to the electrophoresis tank properly filled with electrophoresis buffer.

4.5.2 Loading genomic DNA sample

2 µl of genomic DNA sample was added to 6 µl 2x loading dye (Bromophenol blue) and mixed very well. The mixture was loaded to the well of the gel. The electrode was connected. The power supply was turned on, the voltage was adjusted at 80 V and left for about an hour.

4.5.3 Visual analysis of genomic DNA on the gel

To allow visualization of the genomic DNA sample, the gel was stained with ethidium bromide, the most commonly used intercalating dye. The gel was stained in 1.75 µg/ml of water solution of ethidium bromide for 30 minutes (Quiagen, 2003) and destained by rinsing the gel with water for 20 minutes to remove excess ethidium bromide. The genomic DNA band photograph was taken from the gel using cannon power shot G5 camera connected with BidocAnalyzer (BidocAnalyz^{TM}) and computer. The presence/absence of genomic DNA, quality (presence/absence of smear to the extent that affects the amplification program), intensity of genomic DNA bands (to determine whether further dilution is needed or not) was carefully observed. As a result some of the genomic DNA samples were diluted and further observed for any improvement in quality of bands.

4.6 Primer screening and optimization

Seven random amplified polymorphic DNA (RAPD) primers (OPC16, OPC10, OPC4, OPB15, OPB14, OPB11 and OPB8) were screened using some randomly taken samples (Table 2). All the seven primers were tested under four different mixture and two different PCR profile conditions. As a result, three primers (OPC16, OPC10, and OPB15) which produced relatively more number of reproducible and polymorphic bands were selected for the study.

Table 2: Primers used for screening and their sequences

Name of the primer	Primer sequence	GC content
OPB-07	5'-GGTGACGCAG-3'	70 %
OPB-11	5'-GTAGACCCGT-3'	60 %
OPB-14	5'-TCCGCTCTGG-3'	70 %
OPB15**	5'-GGAGGGTGTT-3'	60 %
OPC-04	5'-CCGCATCTAC-3'	60 %
OPC16**	5'–TGTCTGGGTG-3'	60 %
OPC10**	5'-CACACTCCAG-3'	60 %

** The selected primers

4.7 Amplification condition

Amplification reactions were performed in 2 µl of 10X PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 15 mM KCl_2, 0.01% NP-40 and 0.01 % triton X-100), 1.6 µl of 0.1 mM of each dNTP, 2.8 µl of 3.5 mM $MgCl_2$, 2 µl of 10 ng/µl primer, 0.12 µl of 0.6 UI, *Taq* DNA polymerase (PeQLab), 2.5 µl template DNA and 8.98 µl ddH_2O in a final volume of 20 µl. Desired volume of

master mix for single primer consisting PCR buffer, dNTPs, $MgCl_2$, specific primer, ddH_2O and *Taq* DNA polymerase was prepared and mixed very well. 17.5 µl master mix was distributed to each 0.5 ml capacity ependorph tube prepared. Finally 2.5 µl template DNA was added in to the master mix. Ice was used while the reaction mix was prepared.

4.8 PCR amplification

DNA amplification was performed in a thermal cycler, T3PCR Biometra (Biometra ™) programmed as preheating 94 ºC for 3 min. followed by 45 cycles consisting of Denaturation at 94 ºC for 1 min, annealing at 37 ºC for 1 min, extension at 72 ºC for 2 min. and a final extension at 72 ºC for 10 min. followed by holding at 4 ºC until the amplicons were taken out for the next step.

4.9 Gel electrophoresis and visualization of the gel

4.9.1 Agarose gel electrophoresis

Samples were prepared with 2 µl of 6x loading dye and 9 µl of PCR product and mixed very well. The total mixture was pipetted and applied to the wells of the gel. The electrodes were connected. The power supplier was turned on and run the gel at 90 V (~5V/cm of plate distance) for 1 hour and 47 minutes.

4.9.2 Visualization of the gel

To allow visualization of bands, agarose gel was stained with Ethidium bromide. 70 µl of 10 mg/ml ethidium bromide solution was added in an automatic staining apparatus pre filled with 400 ml of distilled water. The gel was soaked in to the solution for 30 minutes and rinsed with

distilled water for about 20 minutes to remove excess ethidium bromide. The gel image was photographed. Different photograph using different lens apparture were taken and saved for scoring.

4.9.3 Data scoring

Although a large number of fragments were generated from each primer, only clearly distinguishable and reproducible bands were considered and data was entered in a computer file as a binary matrix "0" coded for absence and "1" for presence of a band.

4.9.4 Data analysis

I. Number of total and polymorphic loci were obtained by direct count, and the size of bands were compared with 100 bp DNA ladder (Roth).

II. Percent polymorphic loci were calculated as follows:

- Percent polymorphic loci over the three primers was calculated as:

  PPL = (TNPL/TNL) x 100

  Where, PPL = Percent polymorphic loci over all primer

  TNPL = Total number of polymorphic loci over the three primers

  TNL = Total number of loci observed

- Percent polymorphic loci per primer was calculated as:

  PPLP = (NPLP/NLP) x 100

  Where, PPLP = Percent polymorphic loci per primer

  NPLP = Number of polymorphic loci per primer

NLP = Number of total loci per primer

- Percent polymorphic loci per breed was calculated using POPGENE version 1.31 (Yeh *et al*., 1999) as proprtion of number of polymorphic loci counted for each breed over the three primers and total number of polymorphic loci as:

  PPLB = (NPLBATNPL) x 100

  Where, PPLB = Percent polymorphic loci per breed

  NPLB = Number of polymorphic loci per breed over the three primers

  TNPL = Total number of polymorphic loci

III. The genetic variability was calculated according to Nei (1987) using the ARLQUIN 3.01 software package (Excoffier, 2006). The degree of genetic variation between all breed pairs was compared using Student's *t* test as described by Nei (1987).

$$H = \frac{n}{n-1} \left(1-\sum_{i=1}^{k} p_i^2\right)$$

Where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the i-th haplotype.

IV. The degree of genetic differentiation averaged over all populations and population pairwise comparison for genetic differentiation and their significant test over all loci were tested using ARLQUIN version 3.01 (Excoffier, 2006).

V. Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was used to separate the variance between and within populations and F values were computed using the ARLEQUIN version 3.01 program package (Excoffier, 2006).

VI. Population pairwise genetic distance matrix was calculated using POPGENE version 1.31 (Yeh *et al*., 1999) based on Nei's (1978) genetic distance.

VII. A tree was constructed using Unweighted Pair Group Method using Arithmetic Averages (UPGMA) and was expressed as dendrogram. NTSYS software version 2.1 was used to construct the tree (Sneath and Sokal, 1973).

VIII. Pairwise correlation between genetic and geographic distances was calculated using Mantel test of ARLQUIN version 3.01 (Excoffier, 2006).

5. Results

5.1 RAPD markers

The three primers produced a total of 33 loci with 884 bands ranging from 300 to 1000 bp. Example of the RAPD profile is illustrated in figure 10. Primer OPB15 produced the highest number of loci (16) followed by OPC10 which produced 9 loci and the least (8) from OPC16. A total of twenty polymorphic loci, constituting 60.61 % of the total loci, were investigated (Table 3). When percent polymorphic loci per primer is considered, the highest percentage was observed from OPC10 (66.67 %) followed by OPB15 (62.5 %) and the least from OPC16 (50 %). Among

the five breeds studied, the highest number of polymorphic loci (17) was observed in Sheko, followed by Horro and Guraghe highland (16), Arsi (15), and Abigar (14).

Table 3: Characteristic summery of the three RAPD primers

	Characteristic information					
Primer	TNL	NPL	PPL	PS	GC content	NBP
OPC16	8	4	50	TGTCTGGGTG	60 %	219
OPC10	9	6	66.67	CACACTCCAG	60 %	233
OPB15	16	10	62.5	GGAGGGTGTT	60 %	432
Total	33	20	60.61			884

TNL: Total number of loci, NPL: Number of polymorphic loci, PPL: percent polymorphic loci, PS: primer sequence, GC: Guanine and Cytosine content, NBP: Number of bands produced

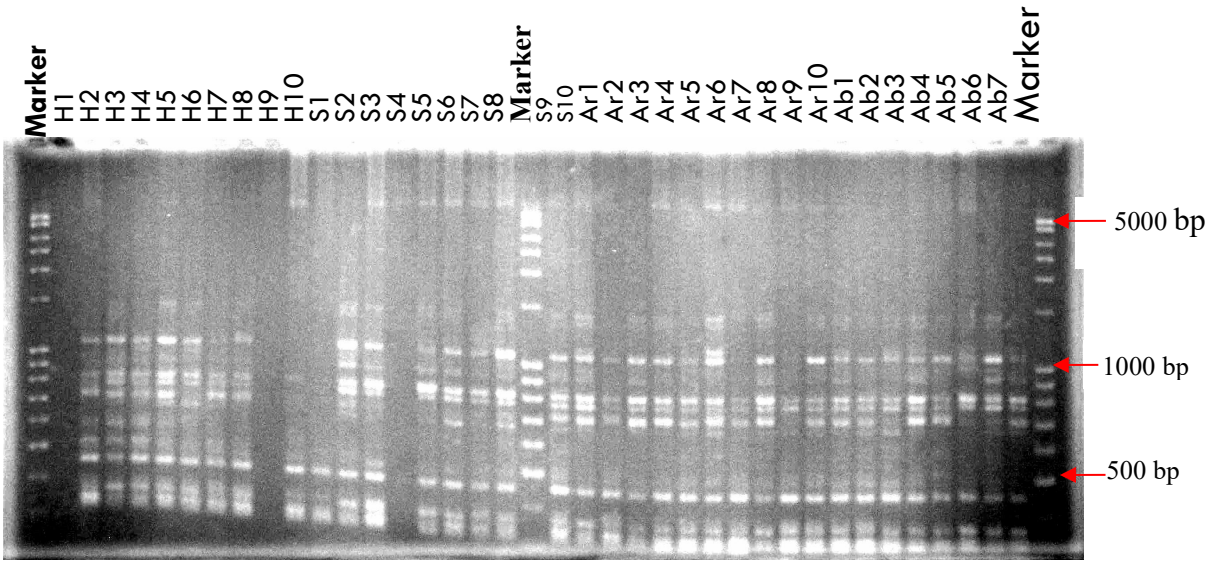


Figure 10: Agarose (1.5 %) electrophoresis of amplicons obtained with primer OPB15 together with molecular marker (100 bp DNA ladder) in three lanes. H-Horro, S-Sheko, Ar-Arsi, Ab-Abigar.

5.2 Genetic variability with in breed

Within breed genetic variability was estimated over polymorphic loci. The gene diversity value for each breed vary from 0.25 (0.15) in Arsi to 0.35 (0.20) in Sheko and the remaining three

breeds were found between this range; 0.32 (0.19), 0.31 (0.18), and 0.27 (0.16) in Horro, Guraghe highland and Abigar respectively.

5.3 Population differentiation

F_{ST} was performed using to see the degree of genetic differentiation among the breeds. The average F_{ST} value (0.1186) is highly significant ($P \leq 0.05$). Pairwise F_{ST} comparison between breeds was computed and the difference was highly significant ($P \leq 0.05$) except the lowest value observed (-0.0156) between Guraghe highland and Abigar, and 0.0716 between Guraghe highland and Arsi ($P > 0.05$). The highest F_{ST} value (0.1644) was observed between Guraghe highland and Sheko (Table 4).

Table 4. Population pairwise F_{ST}

Breeds	Horro	Sheko	Arsi	Abigar	G. highland
Horro	0.0000				
Sheko	0.1385*	0.0000			
Arsi	0.1515*	0.1516*	0.0000		
Abigar	0.1424*	0.1094*	0.1196 *	0.0000	
Guraghe	0.1280*	0.1644*	0.0716**	-0.0156**	0.0000

* Significant at $P \leq 0.05$; ** Insignificant at $P > 0.05$

Results of the Analysis of Molecular Variance (AMOVA) from haplotype frequencies of the five cattle breeds tested was presented in Table 5, and showed that although there is a considerable genetic variation among the different breeds, the within breed variation is much higher. Among breeds variation accounts 11.86 % where as within breed variation accounts 88.14 %.

Table 5. Analysis of molecular variance (AMOVA) obtained from haplotype frequencies of five indigenous cattle breeds.

Source of variation	Df	Sum of squares	Variance components	Percentage of variation
Among populations	4	27.324	0.39652	11.86
Within populations	44	129.656	2.94672	88.14
Total	48	156.980	3.34324	
Fixation Index F_{ST} :	0.1186			

5.4 Genetic distance

The unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) in pairwise breed comparison are presented in Table 6. The smallest distance was found between Guraghe highland and Abigar (0.0039) and the largest was between Guraghe highland and Sheko (0.0354).

Table 6. Unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among five indigenous cattle breeds.

Breeds	Horro	Sheko	Arsi	Abigar	G. highland
Horro	*****	0.9809	0.9807	0.9876	0.9832
Sheko	0.0193	*****	0.9770	0.9862	0.9652
Arsi	0.0194	0.0233	*****	0.9855	0.9869
Abigar	0.0125	0.0139	0.0146	*****	0.9961
G.highland	0.0169	0.0354	0.0132	0.0039	*****

The resulting genetic distance was used to construct a dendrogram using Unweighted Pair-Group Method using Arithmetic Averages (UPGMA). The resulting tree and the coefficients are presented in Figure 11. The Sheko breed formed a distinct branch from the rest of the breeds. Horro breed formed a cluster with Arsi, and sub cluster containing Abigar and Guraghe highland.

The mantel test of correlation between genetic distance and geographic distance matrices showed low level of correlation (r = 0.22) and is insignificant (P>0.05).

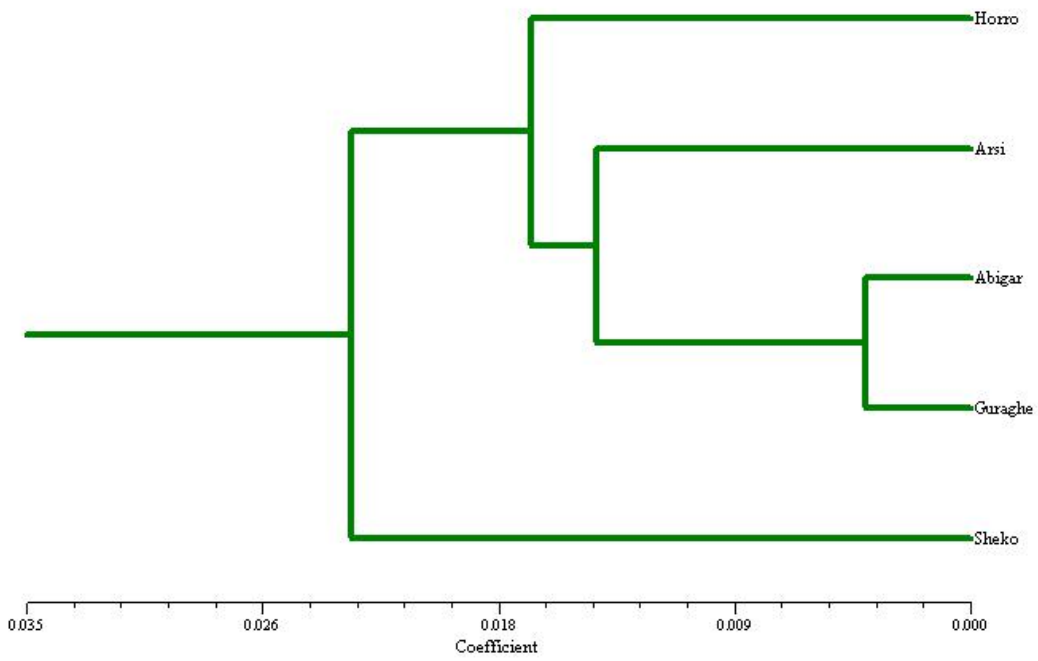


Figure 11. Dendrogram generated by the UPGMA method

6. Discussion

The results of this study indicated that RAPD can be used for detecting DNA polymorphism in cattle and establishing the relationships within and among different breeds. The primers gave distinctly reproducible band patterns in the entire breeds studied. However, primers varied in the extent of information they generated. The mean number of amplified loci per primer was 11, which is slightly higher than the mean number (10.23) of amplified loci per primer using RAPD marker in Uruguayan Creole cattle as reported by Rincon *et al*., (2000) even though the primers used in the two studies are not the same. Nei and Kumar (2000) also reported that in most

experimental studies, about ten or fewer bands can be scored in RAPD marker for each individual when a primer of about ten nucleotides is used.

The size of bands, as comparatively measured using 100 bp DNA ladder, ranges from 300 to 1000 bp. Such band size range is supported by different reports. Rincon *et al*., (2000) observed bands that vary from 300- 2500 bp in Uruguayan Creole cattle and 300-2000 bp in Brazilian native bovine breeds (Serreno *et al*., 2004) using RAPD marker. However, Nei and Kumar (2000) reported that DNA fragments shorter than 400 bp or longer than 2000 bp usually do not show up on the gel.

The average value of percent polymorphic loci (60.61 %) is high when it is compared with percentage of polymorphic allozyme loci in vertebrates (23 %) and mammals (19 %) as reported by Parker *et al*. (1998). As far as the number of polymorphic loci per primer (5-10) is concerned, it is in the ranges of polymorphism that can be detected under optimal conditions which varies from 1 to 10 polymorphic loci per gel (Parker *et al*., 1998). Nei and Kumar (2000) also explained that the existence of two or more alleles with substantial relative frequencies in a population (usually more than 1 %) is called genetic polymorphism.

Within each breed, out of 20 polymorphic loci, calculations showed that the highest proportion (85 %) is observed in Sheko, 80 % in Horro and Guraghe highland, 75 % in Arsi and the least (70 %) in Abigar. Estimation of allelic frequency (Appendix I) showed that the marker alleles in all of the polymorphic loci were in relatively low frequency, which agrees with concept reported by Lynch and Milligan (1994) explained that to avoid bias in parameter estimation, the marker alleles for most of loci amplified by RAPD primer should be in relatively low frequency and a

need for pruning of loci with low frequency null alleles. Unbiased estimates of population-genetic parameters can be achieved with RAPDs provided the analysis is restricted to markers that are not too common and they recommended the restriction of analysis to bands whose observed frequency is less than 1-(3/N), i.e. markers whose incidence is less than 0.70 for N= 10, 0.90 for N= 50, etc (Lynch and Milligan, 1994).

The gene diversity with in population was estimated based on the mean allelic frequency of the twenty polymorphic loci. Lynch and Milligan (1994), Nei (1972), Nei (1973) and Nei and Chesser (1983) reported that with the estimates of the allelic frequency in hand, it is possible to estimate the gene diversity (hetrozygosity) within population. The gene diversity values in this study ranged from 0.25 (0.15) to 0.35 (0.20) and is higher than the range of values reported by Serreno *et al*. (2004) in Brazilian native bovine breeds using RAPD markers amplified by 22 reproducible primers. More or less similar values of hetrozygosity varying from 0.202 in the Patagonian Creole cattle to 0.356 in the Argentinean Creole cattle breed was reported by Liron *et al*. (2002) using five loci related to milk production.

However, the value in all the breeds were found to be lower than hetrozygosity values (0.40 – 0.46) reported by Sisay (1996) using seven polymorphic blood proteins in seven indigenous Ethiopian cattle breeds including those breeds included in this study. MacHugh *et al*. (1997) also reported higher average hetrozygosity (0.643 ± 0.045) in taurine and zebu (*B. tarus* and *B. indicus*) cattle using 36 micro satellite loci.

The relative lower hetrozygosity value obtained in this study possibly be associated with less number of primers and less number of individuals used and the dominance property of the RAPD

markers. Lynch and Milligan (1994) reported that because of the dominance property of RAPDs, gene frequency estimates for such loci are necessarily less than those obtained with co dominant markers such as isozymes and RFLPs. The down ward estimate and less accuracy may be associated with the rather relatively higher sampling variance of the gene frequency in the case of RAPDs (Lynch and Milligan, 1994), and they suggested that increase in sampling efforts (2-10 times more individuals) needs to be applied to a RAPD locus in order to achieve a genetic parameter as accurate as would be acquired in a locus with co dominant markers.

Though there is no significant difference in gene diversity among breeds, the lowest value was obtained for Arsi (0.25) while the highest (0.35) in Sheko followed by Horro (0.32), Guraghe highland (0.31) and Abigar (0.27). However, Sisay (1996) reported that the highest for Abigar (0.4611) followed by Sheko (0.4246), Arsi (0.4187) and Horro (0.4028). The relative highest diversity value observed in Sheko may be due to the distinctness of the breed as it was classified in to humpless shorthorn while the rest four breeds belong to humped cattle type. More over, the highest gene diversity value for this breed may be associated with the relatively longer period of introduction (during the 2nd millennium B.C.) of this breed (humpless cattle) in to Ethiopia and hence have resulted from long term natural selection for adaptation to harsh environmental condition as the Sheko cattle breeding area is hot and humid. In addition there are various livestock diseases in the area including trypanosomiasis, although trypanosomiasis can not be ignored in the habitat of Horro, Abigar and Guraghe highland.

As recently reported by ILRI- EARO joint research project, among the four breeds (Sheko, Horro, Abigar and Guraghe highland), Sheko showed relatively better level of trypanotolerance characteristics (ILRI, 2004). That means, the variability observed in this breed may indicate that

the specific adaptation of the breed to the tsetse infesting area and heterozygote advantages may have contributed towards ability to live and produce in this environment.

The average F_{ST} and pairwise F_{ST} comparison between breeds showed significant subdivision of the breeds studied. This heterogeneous genetic pattern seems to be a major characteristic of indigenous cattle breeds (Liron *et al.,* 2002) and could be the consequence of geographic isolation and/or with a low degree of gene flow among them, and adapted to a wide range of environments. However, insignificant pairwise F_{ST} comparison for genetic differentiation between Guraghe highland and Abigar was unexpected because in previous reports (DAGRIS, 2006; Epstein, 1971) these two breeds were classified in to zebu and sanga type respectively. But the insignificant subdivision between Guraghe highland and Arsi may be accepted as both breeds belongs to zebu type and they are geographically closer populations. The average F_{ST} (0.1186) value obtained in this study was more or less similar to other reports. MacHugh *et al*. (1998) reported F_{ST} (0.112) value in seven European cattle breeds assessed by microsatellite markers. Liron *et al*. (2002) also reported an average significant F_{ST} value of 0.115 across Creole cattle populations using five loci related to milk production.

When data are available from more than one population, it is usually of interest to evaluate the degree to which the total gene diversity partitions in to its within and between population components (Lynch and Milligan, 1994). In addition to its implication to conservation, partitioning the genetic variation in to its components has significant impact in the future breeding plan. According to present study, the inter breed difference accounted 11.86 % of the total variation ($p < 0.00$) (Table 5).

Because of the fact that lack of comparative studies in Ethiopian indigenous cattle specifically, the result obtained in this study was compared with results obtained from similar studies elsewhere. MacHugh *et al*. (1998) reported about 11 % of the proportion of genetic variation in seven samples of European cattle breed was due to inter population subdivision which is very similar to 11.86 % obtained in this study. The remaining 88.4 % was due to intra breed variation. In other instance relatively higher (14.92 %) of inter breed differences was reported by Paiva *et al*. (2005) in Brazilian hair sheep breeds. However, extremely lower (0.23 %) genetic variability among six (five indigenous to Ethiopia and one exotic) chicken ecotypes tested for 10 microsatellite loci was reported by Tadelle (2003).

Another important parameter included in this study was genetic distance between breeds. The smallest distance observed between Guraghe highland and Abigar (0.0039) would be unexpected if the previous morphological based classification of these two breeds as zebu and sanga (DAGRIS, 2006; Epstein, 1971) respectively was accepted. The highest distance (0.0354) obtained between Guraghe highland and Sheko followed by Arsi and Sheko (0.0233), however, was expected since Guraghe highland and Arsi previously were classified as humped (zebu) while Sheko as humpless (taurine) cattle (Epstein, 1971; Tesfaye *et al*., 1994; Albero and Haile-Mariam, 1982). The relatively higher genetic (0.0503) distance between Arsi and Sheko was supported by Sisaye (1996).

The Sheko breed consistently had the largest distance with all other breeds, the maximum figure being that involving Guraghe highland (0.0354) and the minimum was that involving Abigar (0.0139). The smallest distance observed between Abigar and Sheko possibly be associated with the cross- breeding history of Abigar. In other words, many authors including Epstein (1971),

Epstein and Masson (1984) and Rege *et al*. (2001) reported that sanga in general and Abigar in particular were the result of cross-breeding between zebu and taurine cattle in Ethiopia. DAGRIS (2006) also reported that Sheko breed is affecting by gradual inter breeding with Abigar.

Another interesting result obtained in this study was the characteristic of Horro breed. As discussed above Horro was classified in to sanga-zebu (zenga) type because it was believed to have originated from zebu and sanga, and sanga inturn was obtained from zebu and taurine cattle (Alberro and Haile-Mariam, 1982; Tesfaye *et al*., 1994). That means Horro was a result of combination of breeds and hence is a breed with intermediate characteristics. This attribute was supported by the present study that Horro being involved an average genetic distance of the population which was about 0.017.

Figure 11 showed dendrogram generated by UPGMA based on Nei's (1978) genetic distance. Examination of this tree revealed a remarkable degree of breed structure. In other words the tree assorted the breeds more or less based on their genetic origin. It was clearly observed that the Sheko breed formed a distinct branch on its own. The second branch contains zebu proper (Arsi and Guraghe highland) and breeds with zebu blood (Horro and Abigar). This can strongly be justified by their genetic origin in which Sheko is taurine (humpless) type of cattle where as the rest are zebu proper and breeds with zebu blood.

Within the second cluster, the smallest genetic divergence shown between Guraghe highland and Abigar again was difficult to explain. However, Horro still was found on intermediate divergence between Sheko (taurine), and zebu and sanga together. This can be again explained by the history

of origin in which, Horro was resulted from zebu and sanga, the latter believed to have taurine blood.

The insignificant correlation between genetic and geographic distances indicated that the genetic distance does not depend on the geographic distance between breeds. In other words the geographic distance did not contribute to the existing genetic divergence between or among the breeds studied. This low positive correlation could also be a good indicator that there was no more gene flow between or among closely located breeds than the distantly located ones as even they (the distant ones) were favored by such geographic factor. Livestock movement, in strict sense gene flow, is highly influenced by human migration due to their strong association. Cattle keepers can traverse long distance (temporarily or permanently) and cross borders along with their cattle in search of more conducive environment for themselves and their cattle too. During such kind of movements there could be higher possibility of exchanging genetic material (gene flow) from population to population than the unconducive environment (s) in their vicinity. But in the case of plant, unlike animals, so many other natural factors can be involved in their distribution and hence gene flow more likely between or among geographically related populations than geographically distant populations.

7. Conclusions

The RAPD markers used in this study were found to be moderately polymorphic and were found to be useful for genetic characterization of the five indigenous cattle breeds studied.

Moderate level of gene diversity was obtained in this study when compared with previous reports obtained from the same kind of markers studied in indigenous cattle of some other countries. But the gene diversity level was found to be low when compared with other co dominant markers such as microsatellites and when the gene diversity of indigenous cattle in general was concerned.

Among the breeds studied, Sheko had the highest percent polymorphic loci and gene diversity.

All the breeds except Guraghe highland and Abigar, and Guraghe highland and Arsi were differentiated significantly and can be considered as distinct genetic entities.

As Horro breed was found on average deviation (distance) from other breeds both cattle type, this result agreed with previous reports that Horro was developed from these two, zebu and sanga (humped zebu x humpless taurine) major cattle groups in Ethiopia.

The genetic relationships obtained in this study more or less accorded with the previous historical and archeological based classification.

The genetic distance did not show significant correlation with geographic distance.

8. Recommendations

Characterization of indigenous Ethiopian cattle breeds and/ or types is highly recommended using more markers and large number of samples (both breeds and individuals within breed) coverage.

The relation between the relatively high-level gene diversity and present trypanotolerance attributes of Sheko breed should be studied.

Although using RAPDS for genetic variation was promising, it was not clear to what extent the variation found in these marker loci is related to genetic variation in traits of interest. Therefore it is recommended to combine the genetic distance estimates together with information on morphological traits in order to determine the future importance of the breeds.

Breed conservation is recommended considering their importance from socio-economic point of view and as the farming of many local breeds supports local economies in marginal areas.

10. References

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Appendix I. Ethiopian cattle breeds

Breed/strain name	Common name/synonym	Local name
Adwa		
Aliab Dinka		
Ambo		
Anuak	Abigar	
Arado		
Arsi	Arusi	Arusi
Bale		
Barka	Begait	
Danakil	Adal, Adali, Afar, Kereyu, Keriyu	
Ethiopian Boran	Borana	
Fogera		Wogera
Goffa	Goffa Dwarf	Goffa Dwarf
Guraghe		
Hammer		
Harar		
Horro		
Jem-Jem	Black highland cattle	
Jijiga		
Kuri	Baharie, Bare, Borrie, Boundouma, Dongole, Kouburi, Buduma, White Lake Chad, Boenca, Boyenca	
Murle		
Mursi		
Ogaden Zebu	Lowland Zebu	
Raya-Azebu	Lowland zebu	
Red Fulani	M'Bororo, Red Bororo, Wodabe, Fellata, Abori, Bodadi, Brahaza.	
Sheko	Shewa-Ghimira, Goda, Mitzan	
Smada		
Somali Boran	Awi	

Source: DAGRIS 2006

Appendix II. Allele (band) frequency for the total population at each polymorphic locus.

Allele \ Locus	OPC16-1	OPC16-2	OPC16-3	OPC16-4	OPC10-1	OPC10-2	OPC10-3	OPC10-4	OPC10-5	OPC10-6	OPB15-1
Allele 0	0.8474	0.9781	0.8976	0.9329	0.8552	0.8379	0.9131	0.8598	0.7039	0.8074	0.7530
Allele 1	0.1526	0.0219	0.1024	0.0671	0.1448	0.1621	0.0869	0.1402	0.2961	0.1926	0.2470

Allele \ Locus	OPB15-2	OPB15-3	OPB15-4	OPB15-5	OPB15-6	OPB15-7	OPB15-8	OPB15-9	OPB15-10
Allele 0	0.8769	0.9118	0.9435	0.8760	0.3610	0.6739	0.8983	0.9428	0.9464
Allele 1	0.1231	0.0882	0.0565	0.1240	0.6390	0.3261	0.1017	0.0572	0.0536