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# Genetic variability of five indigenous Ethiopian cattle breeds using RAPD markers

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**Genetic diversity is the basis for present day diversified living systems and future genetic improvement needs. This diversity should be properly utilized, improved and conserved. Conservation and improvement strategies ought to be based on proper genetic characterization in association with phenotypic characterization. The objective of this work was to assess between and within breed genetic variability of five indigenous Ethiopian cattle breeds (Horro, Sheko, Arsi, Abigar and Guraghe highland) using RAPD markers. The Guraghe highland and Arsi breeds fall under Zebu breed group, the Sheko come from Humpless Shorthorn, the Abigar from the Sanga group and the Horro from Zenga group. Genetic relationships were estimated using three primers which produced 33 loci. The gene diversity obtained in this study was found to be moderate and Analysis of Molecular Variance revealed that within breed genetic variation is much higher than that between breeds. Except few cases, genetic differentiation was highly significant. The smallest genetic divergence was between Guraghe highland and Abigar followed by Guraghe highland and Arsi. Sheko, historically considered to be humpless shorthorn, formed a distinct cluster whereas the remaining breeds formed another cluster. The RAPD markers were found to be useful to distinguish the breeds studied, but they failed to differentiate between Guraghe Highland and Abigar as they did the two zebu breeds -Guraghe Highland and Arsi. The Pearson's correlation between genetic and geographic distances (r = 0.22) was found to be statistically insignificant (P > 0.05).**

**Key words:** cattle, breed, genetic characterization, RAPD, Ethiopia.

## INTRODUCTION

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. The schematic paintings and engravings of pastoral scenes showing herdsmen and humpless longhorn cattle at Genda-Biftou, Sourre, in Harar, Ethiopia indicate that the raising of cattle has a more recent history than early domestications in Asia might suggest, and indicate that cattle may have penetrated to the Ethiopian region by the middle of the second millennium B.C (Epstein, 1971).

Ethiopian cattle population is estimated about 40.6 million and ranked first in Africa (FAOSTAT, 2002). The genetic diversity among indigenous cattle of Ethiopia is also considered to be very high based on the number of recognized breeds (DAGRIS). Cattle production in Ethiopia is an integral part of almost all farming systems in the highlands, and the major occupation in the lowlands. The role of livestock in general and cattle in particular in the national economy is more significant than what the official production figures would suggest when their contributions for farm traction, farm fertilization and fuel (through manure) are considered (Mekonen, 1992).

Despite the significant contribution of livestock to the

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**Figure 1.** Horro cattle. Courtesy of DAGRIS (2006).



**Figure 2.** Sheko cattle. Courtesy of Workneh Ayalew (2006).

country, little attention has been given to identify, characterize and conserve the diversity in indigenous livestock types. Ethiopian cattle genetic diversity is currently under threat mainly due to extensive planned as well as indiscriminate cross breeding, and to some extent interbreeding among the local populations. Increasing human migration, trade, cultural and social interactions exacerbate interbreeding between adjacent indigenous breeds. Well meant genetic improvement programs, commercialization and subsidies favor indiscriminate crossbreeding. Loss of genetic diversity increases the risk of difficulties in subsistence for the millions of livestock keepers who depend on these resources to secure their livelihoods.

For the development of appropriate breeding strategies and sustainable use of the genetic diversity, it is essential to characterize the phenotype and genotype of the various cattle breed types. Some African indigenous cattle breeds have been lost before they were characterrized and their unique attributes recognized (Rege, 1992).

The current classification of the Ethiopian indigenous

cattle breeds is primarily based on some morphological attributes such as body size, horn shape and size, coat color and hump size and their geographical distribution. However, the usefulness of phenotypic traits to study the genetic variation between populations is very limited (Meghen et al., 1994). In the absence of proper genetic identification, the risk of loss of surviving genetic diversity is high. Unlike morphological traits, molecular markers which are assumed to be neutral to the selection forces are appropriate in the study of genetic relationship between breeds (Parker et al., 1998). Classification using molecular markers provides a large unbiased basis for the estimates of average breed similarities and/or differences.

To date alternative molecular markers are available to characterize cattle breeds, and the Random Amplified Polymorphic DNA (RAPD) method is one of them with relatively low cost, efficient and easy to apply widely. The number of loci that can be examined is essentially unlimited unlike other methods (Lynch and Milligan, 1994). However, RAPD analysis presents some practical problems including lack of reproducibility, thus requiring stringent protocols to be set and optimized, and it follows co-dominant inheritance. To lay the ground for further studies, this study was designed with the specific objective of assessing between and within breed genetic variability among five indigenous Ethiopian cattle breeds using RAPD marker.

**MATERIAL AND METHODS**

**Description of the breeds**

**Horro:** This breed was developed through interbreeding of various Abyssinian Highland Zebu and Nilotic Sanga, particularly the Abigar (Figure 1). 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 Horro in to Sanga-Zebu (Zenga) type. 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. Although the total number of population is not yet recorded, DAGRIS (2006) reported that the breed is not at risk of genetic erosion.

**Sheko breed:** The Sheko cattle are believed to be the last remnants of the original humpless shorthorn taurine cattle in eastern Africa (Figure 2). They were first reported in 1929 from south-western Ethiopia, and later in 1982. At present some of the Sheko cattle manifest small humps that they inherited from zebu introgression. The breed is now considered endangered by gradual interbreeding with local Zebu and Sanga (Abigar) and its population size is estimated to be 31,000 (Rege, 1999). 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 in size 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

**Figure 3.** Arsi cattle. Courtesy of DAGRIS (2006.)



**Figure 5.** Guraghe highland Courtesy of DAGRIS (2006).



**Figure 4.** Abigar breed. Courtesy of DAGRIS (2006).

Africa (Alberro and Haile-Mariam, 1982; Tesfaye et al., 1994).

**Arsi breed:** It is descended from the recent introductions of zebu into Africa from West Asia, and 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 high-lands of Ethiopia especially in Arsi, Shewa and Bale administrative regions (Figure 3). Their number is estimated over 2,012,000 (Rege, 1999). They are small, short and compact. Red, with a black muzzle, is the predominant color although many animals are black, light grey or white with black spots. It is classified in to zebu cattle type (Epstein, 1971; Alberro and Haile-Mariam, 1982; Tesfaye et al., 1994).

**Abigar breed:** It is classified in to Sanga type that evolved in Ethiopia from early migrations and spread across south western Ethiopia into southern Sudan (Figure 4). 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 grey coat colors are also found (Alberro and Haile-Mariam, 1982). Their population size is estimated to be 548, 600 (Rege, 1999) and not at risk.

**Guraghe highland:** 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 short-horned zebu inhabits the higher altitude, wetter agricultural areas, and hence they are also referred to as Ethiopian Highland Zebu (DAGRIS, 2006) (Figure 5). The Guraghe highland is one of this on the wet central and south western highlands of Ethiopia, mainly with the Guraghe and Hadiya people (Rege et al., 2001; DAGRIS, 2006). They are small-sized, usually with red, chestnut or roan coat color, 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).

### Sampling of animals

The sample animals for the Sheko, Abigar, Horro and Guraghe Highland breeds were selected from the pure-breeding experimen-tal cattle herd at Tollaye cattle breeding station of the International Livestock Research Institute (ILRI) and the Ethiopian Agricultural Organization (EARO) in western Ethiopia. Those from the Arsi breed were selected from the Gobe cattle breeding and improve-ment ranch in south eastern Ethiopia. Ten mature unrelated cows were randomly selected and blood sample drawn from jugular vein.

### DNA extraction and amplification

Genomic DNA was extracted from blood according to the procedure described by Bruford et al. (1992), with slight modification of reducing the amount of proteinase K to 25 µl. A total of 7 primers (OPB07, OPB11, OPB14, OPB15, OPC04, OPC16 and OPC10 of Opera Technology Inc.) were screened using representative sam-ples of the breeds. Finally three primers (OPB15, OPC16 and OPC10) were selected and used for amplification. Amplification reaction was performed using a mixture having final concentration of 1 X PCR buffer 0.1 mM of each dNTP, 3.5 mM $MgCl_2$, 10 ng/µl primer, 0.6 UI *Taq*, 8.98 µl ddH$_2$O and 2.5 µl template DNA in a final volume of 20 µl. DNA amplification was performed in a thermal cycler, T3PCR Biometra (Biometra™), programmed as preheating 94$^o$C for 3 min followed by 45 cycles consisting of 94$^o$C for 1 min,

**Table 1.** Pair-wise ($F_{ST}$) (below diagonal) and genetic distances (above diagonal).

| Breed | Horro | Sheko | Arsi | Abigar | Guraghe highland |
|-------|-------|-------|------|--------|------------------|
| Horro |  | 0.0193 | 0.0194 | 0.0125 | 0.0169 |
| Sheko | 0.1385 |  | 0.0233 | 0.0139 | 0.0354 |
| Arsi | 0.1515 | 0.1516 |  | 0.0146 | 0.0132 |
| Abigar | 0.1424 | 0.1094 | 0.1196 |  | 0.0039 |
| Guraghe Highland | 0.1280 | 0.1644 | 0.0716 | -0.0156 |  |

37°C for 1 min, 72°C for 2 min and a final extension at 72°C for 10 min. PCR products were separated by agarose gel (1.5%, w/v) electrophoresis. 1 X TBE electrophoresis buffer was used to cook and run the gel. The gel was stained with ethidium bromide (1.75 µg/ml) for 30 min and rinsed with distilled water for about 20 min to remove excess ethidium bromide. Picture was taken using cannon power shot G5 camera connected with BidocAnalyzer (BidocAnalyz™) and computer.

### Data scoring and analysis

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. Genetic variability was calculated according to Nei (1987) using the ARLQUIN 3.01 software package (Excoffier, 2006). Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) and population pair-wise comparison for genetic differentiation and their significance were tested using ARLQUIN version 3.01 (Excoffier, 2006). Population pair-wise genetic distance matrix was calculated using POPGENE version 1.31 (Yeh et al., 1999) based on Nei's (1978) genetic distance. Dendrogram was constructed using Unweighted Pair Group Method using Arithmetic Averages (UPGMA). NTSYS software version 2.1 was used to construct the tree (Sneath and Sokal, 1973).

### RESULTS AND DISCUSSION

The gene diversity value for each breed (standard deviation in bracket) varied 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) for Horro, Guraghe Highland and Abigar, respectively. These values are higher than the range of values reported by Serreno et al. (2004) for Brazilian native bovine breeds using RAPD markers amplified by 22 reproducible primers. More or less similar values of heterozygosity varying from 0.202 in the Patagonian Creole cattle to 0.356 in the Argentinean Creole cattle breed were 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 heterozygosity values of 0.40 to 0.46 reported by Sisay (1996) using seven polymorphic blood proteins in seven indigenous Ethiopian cattle breeds including those breeds included in this study.

In general, the gene diversity observed in the breeds studied is moderate and the relatively higher diversity

value observed in Sheko may be due to the divergent breed group of the breed when the other four breeds share some Zebu type background. Moreover, the Sheko is believed to have a different and longer evolutionary history (Epstein, 1971), perhaps associated with a different pattern of natural selection for adaptation under harsh environmental conditions of the warm and humid climate of southwestern Ethiopia and continual exposure for Trypanosomosis. The latter factor however influences the Horro, Abigar and Guraghe Highland, albeit at much less prevalence. Lemecha et al. (2006) reported that the only surviving indigenous taurine type breed in Ethiopia- the Sheko- exhibited better trypanotolerant attributes than the other three breeds (Abigar, Horro and Guraghe).

Pair-wise $F_{ST}$ comparison between breeds showed significant differences between the breeds studied (Table 1). 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 a low degree of gene flow among them, and when adapted to a wide range of environments. However, insignificant pair-wise $F_{ST}$ comparison for genetic differentiation between Guraghe Highland and Abigar was unexpected because in previous reports (Epstein, 1971; DAGRIS, 2006) these two breeds were classified into Zebu and Sanga breed groups, respectively. Nevertheless it is likely that these two breeds share a closer evolutionary history as they occupy nearly adjacent habitats. The similarity between the Guraghe Highland and Arsi may be explained by their being classified as Zebu type and inhabit adjacent habitats. The average $F_{ST}$ (0.1186) and $G_{ST}$ (0.1229) value obtained in this study were more or less similar to those of other reports. MacHugh et al. (1998) reported $F_{ST}$ of 0.112 and $G_{ST}$ of 0.104 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.

The smallest distance observed between Guraghe Highland and Abigar (0.0039) is inconsistent with the genetic diversity implied by the distinct breed identities of the two breeds as explained by Epstein (1971) and DAGRIS (2006). 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 are known as Zebu type while the
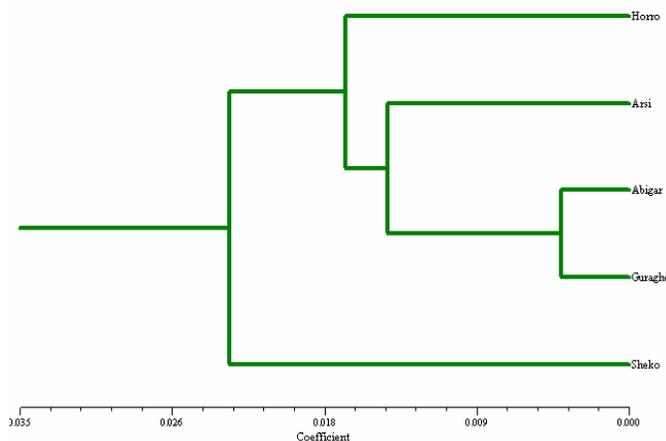
**Figure 6.** Dendrogram generated by the UPGMA method for Nei (1978) genetic distance using NTSYS program.

Sheko is taurine Humpless Shorthorn type (Epstein, 1971; Alberro and Haile-Mariam, 1982; Tesfaye et al., 1994). 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 is possibly associated with their common taurine background (Abigar is a Sanga being a cross of Zebu and taurine) and geographical proximity of their natural habitats. 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 possible interbreeding between the Sheko and Abigar around the common border of their habitats.

Another interesting result obtained in this study was the characteristic of Horro breed, which showed intermediate value in this measure of genetic diversity, as is also its evolutionary origin. Its average genetic distance from the other breeds was 0.017 which is almost equal to the population mean of 0.0172. This is also apparent from the pair-wise $F_{ST}$ values with the other breeds (Table 1). The Horro is a Zenga type breed produced from a cross of Sanga and Zebu breed types with intermediate phenotypic values (Alberro and Haile -Mariam, 1982; Tesfaye et al., 1994).

Figure 6 shows the classification tree or dendrogram generated by UPGMA based on Nei's (1978) genetic distance. Examination of this tree revealed a remarkable classification of the breeds that matches the pattern of $F_{ST}$ values in Table 1. The Sheko breed formed a distinct branch separate from the others. The second main branch includes all the other groups, presumably because of their common Zebu background. This classifica-

tion is consistent with the evolutionary histories of the breeds discussed above.

The Pearson's correlation between genetic and geographic distances (r = 0.22) was found to be statistically insignificant (P > 0.05). This indicates that the genetic distance does not necessarily depend on the geographic distance between breeds, although the breeds under investigation are known to interbreed across common borders. Unlike plants, livestock movement, in strict sense gene flow, is highly influenced by human migration due to the strong and long-term association of humans with livestock. Historically pastoral cattle keepers traverse long distances across borders along with their cattle in search of more conducive environments for themselves and their cattle. During these movements there could be possibility for exchange of breeding animals (genetic material) to allow gene flow from one population to another. This contributes to reduction in genetic divergence.

## Conclusion

All the breeds except Guraghe highland and Abigar, and Guraghe highland and Arsi were differentiated significantly. The observation that the Horro breed had average deviation (distance) from the other breeds was consistent with previous reports that Horro was developed from these two, the humped zebu and humpless taurine. The genetic relationships obtained in this study more or less accorded with the previous historical and archeological based classification.

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