Characterisation of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques

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Introduction

Eastern Africa, comprising Kenya, Tanzania, Uganda, Ethiopia, Eritrea, Djibouti, Somalia and Sudan has over 200 million head of livestock of which cattle account for 32% (Herlocker 1999). The majority of these cattle (95%) are indigenous (Rege 1994), with an estimated 60% found in the arid and semi-arid uncultivatable zones that comprise 70% of eastern Africa (Winrock International 1992).

Indigenous cattle form the backbone of relevant and sustainable livestock production in most of eastern Africa because when compared with their exotic counterparts, they are better adapted to survive and reproduce under the region's harsh environments. These cattle often possess valuable traits such as disease tolerance/resistance, high fertility, good maternal qualities, longevity, and adaptability to harsh conditions and poor-quality feeds—all of which are qualities that form the basis for low-input, sustainable agriculture. Indigenous cattle are vital to subsistence and economic development in eastern Africa, contributing 50% of the total food production from livestock (Jahnke 1982). They sustain the employment and income of millions of East Africans, two-thirds of whom are rural-based. They also provide transport, much of the draft used in cultivation of crops and large component of the manure essential to agriculture. In addition, they play an important role in African culture as they are used for gifts, dowry and cultural rituals. Some of their products are also medicinal.

There is general concern that the genetic variation within East African cattle is disappearing through breed substitution, indiscriminate crossbreeding and the absence of breed development programmes. Other contributing factors include increasing human population that has led to intensified settlement in pastoral areas—thereby reducing the land available for livestock grazing and famine and civil conflict, which have severely affected localised populations and accelerated admixtures and interbreeding among breeds. This situation has been worsened by neglect, livestock disease epidemics and livestock rustling. Any reduction in the diversity of genetic resources narrows the scope to respond to changes in the environment, disease challenges or demand patterns. The gradual disappearance of indigenous breeds that are able to survive in extreme environments undermines food and livelihood security of the poor and the capacity of people to survive in marginal areas. Immediate steps must therefore be taken to conserve these cattle.

An important strategy in the conservation and utilisation of animal genetic resources is the dissemination of information on these resources. However, the indigenous East African cattle breeds are not well classified or are defined with very limited information available regarding the number of breeds, size of population of each breed and amount of genetic variation. Their current classification is based on available historical and anthropological evidences, as well as phenotypic data, which can be influenced by the environment (Rege 1992). Such information is subjective and inaccurate, making implementation of rational and effective conservation and utilisation strategies difficult. Genetic characterisation of these cattle breeds based on DNA studies is therefore necessary as it is more reliable, since it is based on precise genotypic information.

Genetic uniqueness of populations is measured by the relative genetic distances of such populations from each other. Molecular genetics offers a wide range of markers and techniques that allow detection of variation or polymorphism among individuals in a population for specific regions of the DNA and hence an understanding of the genetic basis of biodiversity. Microsatellites are currently the markers of choice for the detection of genetic diversity in livestock due to their abundance, ubiquitous distribution, polymorphic nature, and suitability for amplification by polymerase chain reaction (PCR) (Bruford and Wayne 1993).

Genetic characterisation of 7 indigenous East African cattle breeds using 18 microsatellite DNA markers

Summary

A total of 18 autosomal microsatellite markers were used to genetically characterise seven cattle populations indigenous to East Africa. These included two Sanga (Abigar and Danakil), one East African *Bos taurus* (Sheko) from Ethiopia, one 'zebu × Sanga' intermediate from Eritrea (Arado), two *B. indicus* (zebu) from Kenya (Kenya Boran and Kavirondo Zebu) and one zebu from Tanzania (Kilimanjaro Zebu). Three breeds (one *B. indicus*–Sahiwal, and two *B. Taurus*–N'Dama and Friesian) were included in the study to serve as reference breeds.

Materials and methods

Blood samples were collected from 35–40 unrelated animals of each of the above breeds. DNA isolation and amplification was performed as described by Okomo (1997). Genotyping was performed on an ABI Prism™ 377 automated DNA sequencer (Applied Biosystems l998). Genescan™ analysis software (Version 3.1) was used to determine the molecular lengths of the resultant DNA fragments, using the 3rd Order Least Squares method. The resultant data were then imported into the Genotyper™ (Applied Biosystems l998) analysis software (Version 2.0) for exact sizing of the alleles at each of the microsatellite loci.

Allele frequencies and observed heterozygosity (H_0, number of heterozygote animals) were calculated manually. Standard genetic distance (D_S) (Nei 1972; Nei et al. 1983) and UPGMA (unweighted pair-group method with arithmetic mean) dendrogram (Sneath and Sokal 1973) with bootstrapping of 1000 replications were calculated by the DISPAN program (1993). Tests for deviation from Hardy-Weinberg equilibrium (HWE) were done using the GENEPOP (Version 2) program (Raymond and Rousset 1995). A multivariate statistical analysis (principal component analysis) was performed according to the procedure described by Cavalli-Sforza et al. (1994).

Relative frequencies of specific alleles (characteristic of *B. taurus* as represented by the N'Dama and the Friesian or *B. indicus* as represented by the Sahiwal) at four diagnostic *loci* ILST36, ILST28, TGLA122 and TGLA227 were used to determine the level of indicine and taurine introgression in East African breeds.

Results

Genetic diversity

The analysed microsatellite *loci* were highly polymorphic, with a total of 208 different alleles observed across the 18 autosomal *loci*. Within-breed diversity was high in all breeds with observed heterozygosities ranging from 0.511±0.214 (N'Dama) to 0.660±0.128 (Friesian) (Table l). There was significant variation in allele numbers and frequencies among the breeds. The mean number of alleles observed per breed ranged from 4.3 in the N'Dama to 7.7 in the Kenya Boran. No significant difference was found between the number of alleles in the two Sanga breeds (Danakil and Abigar) and the African Zebu. All the populations were at Hardy-Weinberg equilibrium except the Kenyan Boran, where allele frequencies of 8 of 18 *loci* deviated from HWE.

Table 1. *Mean heterozygosity, mean number of alleles and number of* loc*i showing deviations from HWE (Hardy-Weinburg equilibrium).*

Breed	Mean observed heterozygosity, H_o (s.e.)	Mean number of alleles	Number of *loci* showing deviations from HWE
N'Dama	0.511 (0.214)	4.3	2
Sahiwal	0.555 (0.211)	6.4	1
Danakil	0.657 (0.224)	6.9	1
Kilimanjaro Zebu	0.620 (0.199)	6.8	1
Friesian	0.660 (0.128)	6.6	1
Kavirondo Zebu	0.617 (0.146)	6.6	4
Arado	0.641 (0.175)	6.9	0
Abigar	0.614 (0.184)	6.9	2
Sheko	0.655 (0.219)	7.2	3
Kenya Boran	0.620 (0.168)	7.7	8

Genetic composition

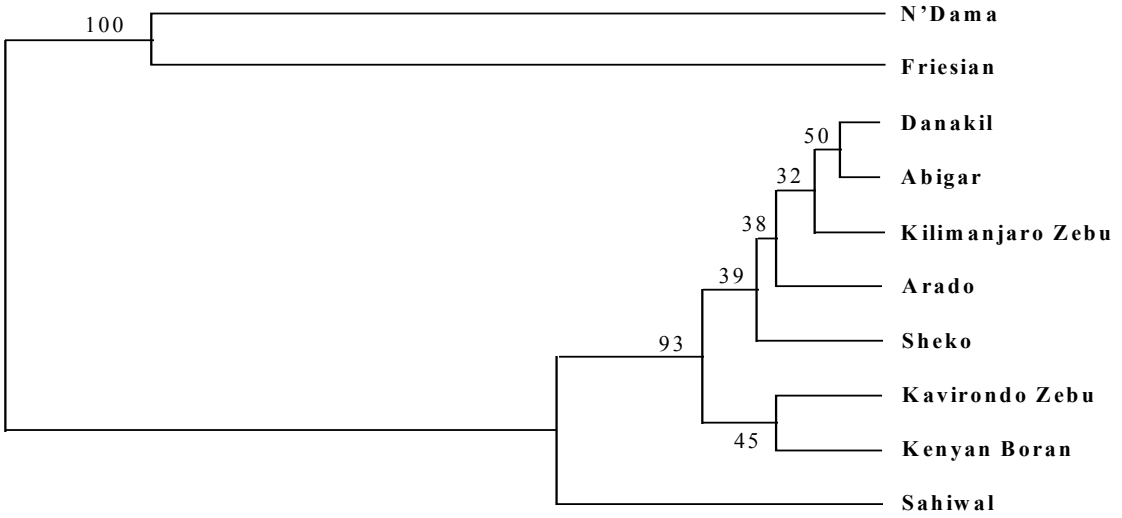
An allele-specific analysis suggested taurine influence in the East African Zebu breeds, zebu introgression in the East African taurine, and a higher proportion of zebu background relative to taurine in the East African Sanga breeds. The results indicated that all East African cattle studied, while genetically separate, contained both indicine and taurine backgrounds (Table 2). Their unique genetic identity calls for their conservation and utilisation.

Table 2. *Mean frequencies of indicine- and taurine-specific alleles.*

		Mean frequencies (s.e.)	
Class	Breed	Indicine alleles	Taurine alleles
B. indicus	Kavirondo Zebu	0.56 (0.33)	0.32 (0.20)
	Kenya Boran	0.32 (0.28)	0.67 (0.37)
	Kilimanjaro Zebu	0.74 (0.26)	0.29 (0.14)
B. taurus	Sheko	0.55 (0.24)	0.38 (0.21)
Sanga	Abigar	0.56 (0.10)	0.32 (0.20)
	Danakil	0.66 (0.09)	0.41 (0.09)
Zebu × Sanga	Arado	0.66 (0.14)	0.22 (0.08)

Genetic distance

Standard genetic distances (D_S) estimated between breed pairs ranged from 0.023±0.009 (Danakil and Abigar) to 0.868±0.200 (N'Dama and Sahiwal). Average genetic distance between the East African breeds was small (0.173±0.185).



Note: Numbers indicate bootstrap values in percentage (1000 replicates)

Figure 1. ***Unrooted UPGMA (unweighted pair-group method with arithmetic mean) tree built up based on Nei's standard genetic distances.***

An UPGMA tree built up from D_S genetic distances (Figure 1) revealed that the Friesian and N'Dama breeds (*B. taurus* from Europe and Africa, respectively) were clustered together but were clearly separate populations, while the Sahiwal breed (*B. indicus*) native to Asia was more closely related to the East African breeds than to the N'Dama and Friesian. No evidence of clear genetic distinction between the East African humped (zebu and sanga) and the humpless (Sheko) breeds was revealed (Figure 2).

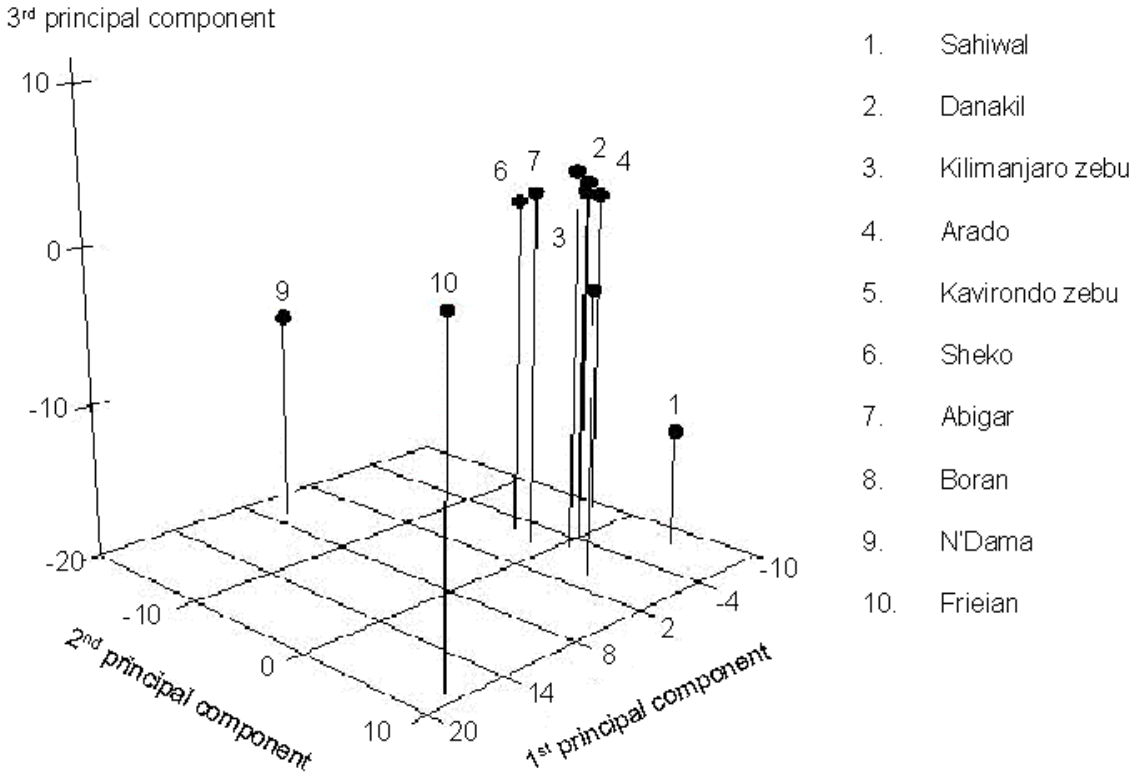


Figure 2. ***Principal components (PC) graph of the first three PC axes: 10 populations, 18 loci, 208 alleles.***

A principle components graph of the multivariate statistical analysis (Figure 2) shows a clear separation of the East African breeds from pure *B. indicus* (Sahiwal) as well as from Friesian and N'Dama (pure *B. taurus* from Europe and Africa, respectively). All the different classes of indigenous East African cattle studied showed close genetic relationship. Again, no clear genetic distinction was evident between the humped breeds (zebu and sanga) and the humpless taurine breed (Sheko), and, therefore, the current classification of these breeds based on morphology is inappropriate.

Discussion

The East African breeds studied showed a considerable amount of within-breed variation based on allele numbers and heterozygosity values, suggesting that the populations studied were fairly outbred. Only the allele frequencies in the Kenyan Boran showed a significant deviation from the HWE. Artificial selection is the major genetic influence on modern domestic cattle populations and it is known that the Kenyan Boran has undergone intensive artificial selection since the 1920s (Maule 1990).

The terms ‘zebu-specific’ and ‘taurine-specific’ alleles used in this case study to denote alleles of indicine and taurine origin are not completely satisfactory given the small number of reference breeds examined. Nevertheless, an analysis of these 18 autosomal diagnostic alleles produced interesting results and suggested that the East African Zebu breeds in this study are not genetically pure *B. indicus*, as they all exhibit a significant proportion of taurine backgrounds. The taurine alleles are possibly the result of recent crossbreeding (with European breeds) to improve production, though there is no reason to dismiss the possibility that the presence of taurine alleles in the East African Zebu breeds may be the result of historical selection and crossbreeding with indigenous East African taurine populations. Results also showed strong zebu introgression in the Sheko, the only cattle breed in eastern Africa currently classified as taurine (Rege et al. 1996). As expected, the sanga sample contained both taurine and indicine backgrounds. The zebu × sanga intermediate (the Arado) exhibited very strong indicine influence.

Based on genetic distance estimates and phylogenetic tree analysis, the indigenous East African cattle studied were very closely related. These indigenous breeds were found to form a relatively homogenous and genetically unique group of populations that was distinct from the pure *B. indicus* and *B. taurus* breeds, but more closely related to the *B. indicus* than to the *B. Taurus.*. The unique genetic background of East African cattle and their known adaptation to local environmental conditions support the need for their conservation and utilisation in local farming systems.

Gaps in knowledge

Information regarding number of breeds and their population sizes, as well as the amount of genetic variation of indigenous African cattle is an important prerequisite in their conservation and utilisation. The status of livestock breeds in the developed countries of Europe and North America is better known and documented, while relatively little is known about animal diversity in Africa and other developing regions of the world. Yet, it is in these latter regions where many of the more unusual and best-adapted animals are found today. It is also where breeds are in greatest danger of genetic erosion. Unfortunately, the lack of data from regions containing the greatest diversity gives an incomplete and distorted picture of the status and trends of domestic animals breeds worldwide. By all accounts, however, the rate of breed extinction has accelerated dramatically over the past 100 years. When a breed becomes extinct, the already narrow genetic base shrinks irreversibly.

In general, proper definition and classification of indigenous African cattle breeds is deficient. Breeds are defined on the basis of subjective data and information obtained from local communities. Reliance on these criteria as the basis for classification for utilisation and/or conservation is in most cases, quite misleading. In addition, historical and archaeological evidences are not always accurate, as they often rely on subjective judgements.

Another constraint that hinders the study of indigenous African cattle is the complexity and uncertainty of their history and origin. Several attempts have been made to trace the origins and evolution of indigenous African cattle using archaeological, anthropological and historical evidence (Epstein 1971), but uncertainties still remain in many aspects (Epstein and Mason 1984; Payne and Hodges 1997). Despite recent evidence for possible presence of an African centre for domestication (Bradley et al. 1996), the common view in the literature is that African cattle originated from three major phases of introduction, with successive migrations from Asia, through the Nile Valley in Egypt or via the Horn of Africa (Epstein

1957). Subsequent migrations led to dense populations of cattle in the East African highlands, around present day Ethiopia and neighbouring areas (Payne 1970). It is considered that the first introduction was that of the humpless taurine Hamitic Longhorn (*B. taurus*) and that it arrived in the Nile delta around 6000 BC, while the second introduction, that of the taurine shorthorns (*B. taurus*), supposedly occurred about 2750–2500 BC (Epstein 1971). The introduction of the humped zebu (*B. indicus*) cattle was in the third phase and is believed to have occurred in two waves: the first in about 1500 BC and the second wave associated with Arab invasion of Africa in about 699 AD. However, Marshall (1989) provides evidence suggesting existence of zebu cattle in Africa as early as 2000–1788 BC.

Historical and archaeological evidence may reveal much about the original type of a breed or strain but it is molecular genetic evidence that is factual and precise. The gene frequencies and subsequent genetic distances estimated through DNA-based genetic characterisation, play an important role in helping to define the classification, as well the evolutionary process and history of indigenous African cattle more precisely.

The International Livestock Research Institute (ILRI), based in Kenya and Ethiopia, under the Food and Agricultural Organization of the United Nations (FAO) global strategy on biodiversity conservation, has initiated research to co-ordinate and develop African animal genetic resources. The institute aims to: identify, monitor and characterise domestic animal diversity; use and develop animal genetic resources to promote productivity and sustainability in agriculture worldwide; to manage genetic resources to assure long-term availability; train and involve people in management and use of animal genetic resources; and to communicate to the world community the importance of diversity in domestic animals and their wild relatives.

Recommendations

To obtain accurate information on genetic variability between cattle breeds, an average sample size of 40 animals per breed and 20 microsatellite markers is recommended for breed genetic characterisation (Hanotte 2000). The most important information produced in this phase is the amount of between-breed genetic diversity (genetic distance) as was done in this case study. The second phase depends on the results of analysis of the sets of breeds in the first phase and entails the choice of breeds for conservation, utilisation or development. Low genetic distances among a set indicates little between-breed diversity and close relationship between the breeds in the set, while the reverse is true for high genetic distances. In cases where the breeds are not closely related the choice of breeds, say for conservation would be simple, depending on the method to be employed and economic feasibility. The situation is, however, different where a set of breeds shows close genetic relationship, as was the case in this study.

With a closely related set of breeds, the choice between to conserve or not is difficult, yet it is not cost-effective to conserve them all. Therefore, detailed genetic evaluation and comparisons of these breeds is needed to determine if more than one member of the group should be conserved. The candidate breed/s for conservation, therefore, represent all the other closely related breeds. The final decision on choice of breeds for conservation must take into account any available information on productivity traits of economic value, specific adaptive features (tolerance to heat, low quality feeds, disease etc.), presence of unique genes or phenotypes, local or regional importance of a breed in production systems and availability of resources and infrastructure in the region where the breed is located.

Once decisions on which breeds to conserve have been made, the most practical and economically feasible method recommended for conservation of livestock genetic diversity in eastern Africa and the rest of the developing world is *in-situ* conservation of live animals. The inter-dependence between people and domestic animals is key to the future conservation and use of animal genetic resources, particularly in developing countries. Both *in-situ* and on-farm conservation and use of animal breeds must play an increasingly important role in the future of genetic resource conservation. *In-situ* conservation enables animal populations to continue to adapt, evolve and be selected for use in their natural environments. This method conserves not only the livestock, but also the traditional systems of which they are a part. Unlike cryogenic techniques, which require technology, equipment, knowledge and training for collection and storage, *in-situ* conservation can be carried out at any level, in any country and with the skills and resources already available. *In-situ* livestock conservation programmes are currently administered by national governments, by non-governmental organisations, by co-operative groups of farmers and by individuals.

The seven breeds analysed in this case study represent only a subset of East African cattle breeds. Therefore, the information obtained here regarding the actual amount of genetic diversity of these cattle is incomplete. The choice of breeds for conservation, if made from such a small subset, would be biased. Such a choice should be based on the analysis of a more representative set of breeds. On-going work on African cattle genetic characterisation at ILRI aims to genetically characterise approximately 150 cattle populations sampled throughout Africa. On completion of this study, information on genetic diversity of the entire African cattle population will be more or less intact, and hence, more reliable choices can be made on which breeds to conserve in different parts of the continent.

Discussion questions

Question 1.
Loss of biodiversity may have a wide range of potential effects on both natural and managed ecosystems and the human populations that depend on them. Discuss.

Question 2.
Gathering and dissemination of biodiversity information is important in science, education and culture as well as in the exploitation, conservation and management of biotic resources including animal genetic resources. Discuss ways in which this information can be best sought, managed and made available to all concerned.

Question 3.
Conservation/sustainable development of animal genetic resources (AnGR) requires a shift towards a broad focus on many 'adaptive' breeds that survive well in the low external input agriculture typical of developing countries. Farmers in these countries practise agriculture that allows livestock to adapt to changes, whether to evolving pests, diseases, climate change or human intervention. However, government policies and commercial pressures push farmers to replace their own varieties with high-input, higher-yielding varieties of livestock breeds. Discuss the pros and cons of these situations.

Question 4.
Discuss how to best manage and utilise indigenous animal genetic resources for maximum economic, scientific and cultural benefit to mankind.

Question 5.

a) Outline the objective criteria on which the choice of livestock breeds for conservation should be based.

b) From the results of this case study, which breed/s would you target for conservation, if all were at risk of extinction? Give reasons to support your answer.

Question 6.

Do you consider the number of breeds analysed in this case study as sufficient to represent the genetic diversity within the entire East African indigenous cattle population? Discuss giving relevant examples and recommendations.

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