Improving our knowledge of tropical indigenous animal genetic resources

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To better manage, use and conserve animal genetic resources (AnGR), we must understand the nature and distribution of both the phenotypic and genetic diversity that they posses. This module makes a case for and highlights issues and methods that underpin improved understanding of the diversity in AnGR as a basis for designing conservation and sustainable utilisation of the diversity (the subject of Module 3). The module emphasises the need to undertake phenotypic and genetic characterisation of indigenous breeds in order to improve our understanding. It also points out the role that modern technologies and indigenous knowledge may play in this process. Within the module there are links [blue] to the web resources and [burgundy] to case studies and other components that help illustrate the issues discussed. Various photographs referred to in the text are included in the appendix at the end of this document.

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1 What are animal genetic resources?

1.1 *Genetic diversity, species, breeds, strains and populations*

Diversity in general usage refers to the range of differences among some set of entities. Biological diversity thus refers to variety within the living world—the biosphere. The term *biodiversity* is commonly used to describe the number, variety and variability of living organisms. Diversity can only be measured if some quantitative value can be ascribed to it and these values compared. To do this, biodiversity is divided into its constituent elements, i.e. *genes*, *species* and *ecosystems* which correspond to three fundamental and hierarchically related levels of biological organisation.

The most common usage of the word biodiversity is as a synonym of *species diversity* or species richness. This is perhaps because the living world is most widely considered in terms of species (see photo set 1). Thus, discussion of global biodiversity is typically presented in terms of global numbers of species in different taxonomic groups. Estimates for the total number of species currently existing on earth vary from 5 million to nearly 100 million. A conservative working estimate suggests there might be around 12.5 million. Of these, only an estimated 1.7 million have been described to date. In terms of species number alone, life on earth appears to consist essentially of insects and micro-organisms!

Using species as the level at which to consider diversity does have disadvantages. Species cannot be recognised and enumerated by systematists with total precision. Moreover, the concept of what a species is differs considerably between groups of organisms (see photo set 2). Worse still, enumeration of the number of species alone, if not supported by data on the diversity within species, may be misleading. That is, a count of the number of species only provides a partial picture of biological diversity. Implicit within the term *species* is the concept of degree or extent of variation; that is, organisms that differ widely from each other in some respect by definition contribute more to overall diversity than those which are very similar. The more different one species is from another, the greater its contribution to the overall measure of global biodiversity.

Genetic diversity represents the heritable variation within and between *populations* of organisms. The *populations* may be entire species or a specific collection of individuals within a species such as a breed, strain, line, herd/flock etc. The diversity ultimately resides in the variations in the sequence of the four base pairs which, as components of nucleic acids, constitute the genetic code. New genetic variation arises in individuals by gene and chromosome mutations and, in organisms with sexual reproduction, is spread through the population by recombination.

The genetic diversity—the pool of genetic variation—in an interbreeding population is acted upon by selection, be it natural or artificial. Differential survival results in changes of the frequency of genes within the population and this constitutes population evolution. The significance of genetic variation is thus clear: it enables both natural evolutionary change and artificial selective breeding to occur.

The term *animal genetic resources* (AnGR) is used to include all animal species, breeds and strains that are of economic, scientific and cultural interest to humankind in terms of food and agricultural production for the present or the future. Another equivalent term increasingly used is *farm animal genetic resources*. There are more than 40 species of animals (Table 1) that have been domesticated (or semi-domesticated) during the past 10 to 12 thousand years which contribute directly (through animal products used for food and fibre) and indirectly (through functions and products such as draft power, manure, transport, store of wealth etc.) [Mammalian species-the evolutionary relationships]. Common species include cattle, sheep,

goats, pigs, chickens, horses, buffalo, but many other domesticated animals such as camels, donkeys, elephants, reindeer, rabbits etc. are important to different cultures and regions of the world (see Module 1, Section 4).

Table 1. List of animal species used for food and agriculture

Widespread species		Localised species (only some are domesticated)	
Species	No. of breeds		
Pig	350	Banteng	Bamboo Rat
Goat	320	Mithan	Red Deer
Sheep	850	Yak	Mouse Deer
Cattle	815	Gaur	Muntjac
Buffalo	70	Tamaraw	Water Deer
Horse	350	Kouprey	Duiker
Donkey/Ass	70	Anoas	Lizards
Dromedary	50	Rabbits	Green Iguana
Bactrian Camel	6	Agouti	Black Iguana
Llama	2	Capybara	Elephants
Alpaka	2	Coypu	Bees
Guanaco [a]	None	Giant Rat	Snails
Vicuna [a]	None	Grasscutter	Crocodiles
Chicken	>300	Hutia	Silkworm
Turkey	>30	Mara	Mink
Duck	>65	Paca	Fox
Muscovy Duck	None	Vizcacha	Nutria
Domestic goose	>60	Chinchillas	Guinea Pig
Guinea Fowl	10 varieties	Pacarana	
Japanese Quail	>6	Springhare	
Pigeon	150	Rock Cavy	
Pheasant	None	Salt-Desert Cavy	
Partridge	None	Solomon Islands Rodents	
Ostrich	4 races	Giant New Guinea Rat	
Cassowary	?	Porcupines	
Nandu [a]	?	Kiore	
Emu	?	Soft-Furred Rat	
Peafowl [a]	None	Giant Squirrels	
Mute Swan [a]	?	Squirrels	
Cormorant [a]	?	Colour Rat	
Little Egret [a]	?	Spiny Rat	

[a] Not domesticated.

Domestic animal diversity (DAD) refers to the genetic variation or diversity existing among the species, breeds, strains and individuals for all animal species which have been

domesticated to meet human needs for food and agricultural production, and their immediate wild relatives.

The concept of a *breed*, in which all members have a pedigree tracing their ancestry, was developed primarily in Western Europe during the 18th century. Today, in the developed world breeds are recognised as distinct intra-specific groups, the members of which share particular characteristics that distinguish them from other such groups, and formal organisations usually exist for each breed or breed group. In its strictest sense, a breed designates a closed population—mating pairs are drawn only from within the population and relationships among individuals are documented. Members of a breed have developed under the same selection pressures and share common ancestry. However, breeds are rather dynamic and, in many instances, are not completely closed populations. Changes in breeding objectives also affect breed characteristics over time (see photographs below).
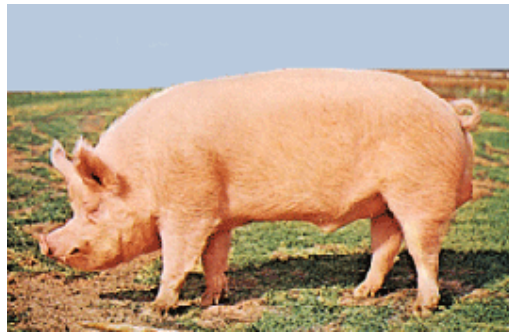
Friesian bull

Holstein bull

Daweizi Pig

Yorkshire (Large White) boar

Photograph 1: *Differences possible in livestock breeds and species over time and space.*

The term breed as a formal designation often has little meaning outside areas of Western influence, where pedigree recording is often non-existent. However, even under these circumstances, there exist strains or geographically separated interbreeding populations, or populations separated by cultural or community 'boundaries' or differential preferences for specific animal attributes. In any case, breed as a concept is rather complex in the context of developing countries. In developing countries, examples of breeds that could not fit the Western world definitions of the same include the East African shorthorn zebu, which is

found in most parts of East Africa (see different breeds/strains of East African Zebu cattle). Others include the Yellow cattle found all the way from South China, Lao, Thailand to Malaysia, the Djallonké sheep of West Africa [Breed information]; and the Grass-cutter also of West Africa [CS 1.32 by Mensah and Okeyo] [Breed Information].

Livestock populations developed in different ecological or geographical areas will become genetically distinct as a result of genetic drift and differential selection pressures, provided they have also been reproductively isolated from other populations developed under different conditions. Thus the indigenous livestock from different regions of the world should probably be assumed *a priori* to represent different 'breeds'. It seems clear that populations with different adaptive characteristics or possessing unique physiological characteristics should be recognised as different breeds. This distinction should be drawn even if the populations are shown to be relatively closely related based upon measures of genetic distance [Chinese pig breeds].

Within a breed there may be differentiation between populations due to differences in selection objectives. The best example in temperate breeds is the Holstein–Friesian. The Holstein of the USA is a bigger animal producing much more milk, but with less butter fat and protein contents than Friesian strains previously found in Europe which, in the middle of the last century, were developed into smaller dual purpose (meat and milk) animals. The [Friesian strain in New Zealand] is also, as it has been selected to produce milk from pasture-based production systems. Examples in Africa include the Boran/Borana (see Kenya Boran bull and Ethiopian Borana cow) strains in Kenya and Ethiopia and the strains of Djallonké sheep in several countries in West and Central Africa [Djallonké sheep]. Increasingly, the move is to recognise strains found in different countries as distinct breeds (see Breed information); [DAD-IS]; [DAGRIS]. This is principally a response to the Convention on Biological Diversity which emphasises national ownership of genetic resources.

The World Watch List for Domestic Animal Diversity [WWL-DAD] prepared by the Food and Agriculture Organization of the United Nations (FAO) in 1993, and which has since been revised two times (1995 and 2000), has defined a breed as: either a homogenous, sub-specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a homogenous group for which geographical separation from phenotypically similar groups has led to general acceptance of its separate identity.

1.2   *Phenotypic and molecular characterisation contribute to breed classification*

Clearly, the definition of a breed is, to say the least, a bit 'woolly'. More challenges creep in when degree and time of reproductive isolation between populations cannot be clearly determined. When breed identity is documented through pedigree records, one can presumably document the time of genetic isolation and thereby place some boundaries on likely distinctiveness between candidate breeds. However, a relatively small proportion of the world's livestock is listed in herd books. When potential 'breeds' are physiologically similar and have overlapping and, often, large ranges, we should probably then utilise measures of genetic relatedness to help sort out breed distinctions. Thus, if we have basically similar animals across a wide area (for example, fat-rumped sheep in Africa or the so called 'Small East African Zebu' [CS 1.10 by Okomo-Adhiambo]; [Yak ILRI] or the South East Asian Yellow cattle with little phenotypic variation among populations and little reproductive isolation between adjacent populations, estimates of genetic distances among populations at the extremes of the range may be very helpful in assigning estimates of genetic uniqueness and, more importantly, in assigning conservation priorities relative to other populations. Where herd books exist that appear to document genetic uniqueness among breeds, measures

of genetic distance can supplement this information in situations where breeds appear otherwise quite similar.

Thus, we work at two levels. First, there are the populations that are clearly distinct and unique based on adaptations and physiology. Here, there are degrees of 'distinctiveness', but either a group of knowledgeable breeders and scientists or a good discriminate analysis should be able to isolate the distinct genes and determine the extent of shared genes.

Next, are the populations that are not so easily discriminated and whose genetic uniqueness must be determined. It is at this level that 'breed' distinctions become a combination of genetic and cultural distinctions (Ankole-cattle strains) of the various strains of Ankole strains of cattle . Since, in these cases, we now have very imperfect information regarding genetic relationships and breed characteristics, including production and reproductive traits as well as adaptive attributes, we would do well to recognise the cultural definition as potentially valid until we have better information. This is basically the principle underlying ongoing AnGR research work in many laboratories/institutions worldwide, including ILRI. That is, to both recognise breeds when the owners claim that they are distinct and to proceed to attempt to acquire objective measures of genetic relatedness. In making these distinctions among breeds, and especially when several apparently similar breeds are found in the same area, a population can be accorded tentative breed identity when groups of farmers in the area can be identified who:

- claim to be raising animals of a distinct type and possibly have common breeding objectives [CS 1.36 by Sartika and Noor]

- can reliably recognise that type (e.g the Inyambu type of Ankole cattle compared to other Ankole cattle types)

- exchange germplasm only with other breeders dedicated to holding animals of the same 'type' (see photograph below).

The type of Ankole cattle that both Northern Tanzanian and Ugandan Bahima pastoralists prefer to keep

Photograph 2: *Deep Red Ankole type*

- indicate that such breeding programmes (formal or informal) have been going on for many generations (e.g the Red Maasai type of East African Fat tail sheep vs other strains of the same sheep type).

2   Breed characterisation, including on-farm surveys

Characterisation means the distillation of all knowledge which contributes to the reliable prediction of genetic performance of an animal genetic resource in a defined environment and provides a basis for distinguishing between different AnGR and for assessing available diversity. Characterisation thus includes a clear definition of the genetic attributes of an animal genetic resource and the environments to which it is adapted or known to be partially or not adapted to at all. It should include the population size of the animal genetic resource, its physical description, adaptations, uses, prevalent breeding systems, population trends, predominant production systems, description of environment in which it is predominantly found, indications of performance levels (milk, meat, growth, reproduction, egg, fibre, traction etc.), genetic parameters of the performance traits and information on genetic distinctiveness of the animal genetic resource and its evolutionary relationship with other genetic resources in the species [Oromia ILRI and FAO/ILRI Zimbawe reports]; [Yak-ILRI].

Sources of phenotypic data include: published and unpublished or grey literature; short-term on-farm (rapid) surveys; short- to long-term on-farm studies; and on-station studies, including laboratory analyses of samples collected on-farm or on-station. The on-farm or on-station samples may include use of biochemical or molecular (e.g. DNA) techniques to quantify genetic diversity, determine distinctiveness of breeds and/or measure genetic distances among populations (see Section 3, of this module) [CS 1.10 by Okomo-Adhiambo]; [CS 1.11 by Gwakisa]; [CS 1.38 by Ofelia]. It must be noted that molecular characterisation on its own is not adequate. Characterisation must therefore be presented and undertaken in a broader context of utilisation.

A thorough review of, and synthesis of data from, the (conventional and grey) literature should be a first step in any breed characterisation work. Not only does this provide an indication of performance under specific environmental conditions, but it also can be a useful source of anecdotal information and is invaluable for the formulation of hypotheses that can be tested in subsequent more detailed characterisation studies. It is also possible to identify, from the literature, multiple trials which have the same basic design and experimental protocol and which therefore qualify for meta-analysis. This is particularly useful in breed characterisation: it provides opportunity to include characterisation data which may not have been published simply because they did not meet the expectations of the researcher (e.g. they were 'not significant').

In the developed world, livestock recording schemes provide a continuous source of data for monitoring trends in the industry, including improved understanding of breeds and the production system. Unfortunately, such structures are not available in most developing countries. Here, designed, rapid, on-farm surveys can be useful for collecting basic (macro-level) information on production systems, population statistics of breeds, physical (or descriptive) characteristics and performance levels—milk production, fertility, mortality, longevity, growth, meat production etc. [Oromia ILRI]. While rapid surveys can provide indicative figures (see Module 4, Section 7), reliable compilation of data on production systems and particularly on phenotypic characteristics of a breed can only be obtained from more detailed on-farm studies. Such studies may involve whole flock or village as basic experimental units and require collection of data over relatively long periods of time, i.e. monitoring. On-farm studies can be used to collect such information as lactation length, parturition interval, growth rates, off takes or, if done over sufficiently long periods, estimates of herd/flock structures and population trends essential for assessing rates of decline and identifying causes of such declines [Indian case – National Bureau of Animal Genetic Resources-NBAGR].

Physical description of a breed should focus on characters which, in the view of keepers of the breed and local experts, facilitate identification of animals as being members of the breed or strain. These should include coat colour (common and/or special colours and colour combinations); horn shape and size; and presence or absence of hair/wool, hump (including relative size), tail type, dewlap and other specific visible characteristics . Physical or morphological characteristics can be particularly useful in the classification of populations/strains/breeds within a species (see photos of Indian cattle breeds) .FARM-Africa (1996) reported work in which various measurements—both qualitative (e.g. presence or absence of beard, wattles, ruff; and ear form, horn orientation, coat colour etc.) and quantitative (e.g. height, body length, chest girth, body weight, ear length etc.)—were subjected to multivariate analyses to classify heterogeneous, previously unclassified indigenous Ethiopian goat populations into taxonomically distinct, relatively similar entities or groups. This approach is recommended as a first step in the classification of heterogeneous previously uncharacterised populations. [FARM-Africa ILRI Goat Survey].

As part of surveys, farmers should be interviewed to determine the extent of 'indigenous' knowledge (see Section 5, this module) on common diseases, whether or not the breed is thought to be tolerant of/resistant to some diseases and what evidence (indicators) farmers have to support such claims [Ndama]. Farmers should also be asked to indicate whether they believe the breed has any other adaptive characteristics (e.g. tolerance of excessive heat, humidity etc.). In addition, farmers should be asked to rank current uses of the breed (e.g. traction, meat, milk, fibre etc.) and to indicate any outstanding characteristics of the breed (e.g. exceptional prolificacy, growth rate etc.)

It is also possible to design on-farm studies to estimate genetic parameters (see Section 3, this module) of certain traits and to provide an indication of specific adaptive attributes, e.g. heat tolerance or disease resistance. Compared to on-station characterisation, on-farm studies are less *precise*. However, they have the advantage of providing *accurate* indications of performance levels as measured under farm conditions in which the animals (are expected to) live and produce.

The advantage of on-station breed characterisation (and evaluation) is that the controlled experimental conditions ensure a high precision. Special adaptive attributes, which are difficult to measure at field level, are also generally best studied on-station. As has been stated, the high precision to which on-station studies can be undertaken make them appealing for breed evaluation despite the fact that they are less accurate as indicators of performance in farmers' flocks/herds. Indeed, in the presence of genotype × environment interaction, conclusions drawn from on-station characterisation could be misleading.

The objective of obtaining *population estimates* is to assess current population size for planning and to determine population trends to establish whether or not populations of certain breeds are declining. When declining trends are detected, investigations into possible reasons for decline can be initiated to identify appropriate corrective actions.

Current sources of livestock population statistics in developing countries include periodic national livestock censuses; occasional estimates by relevant government ministries; estimates by national scientists; and estimates by such agencies as FAO, NGOs etc. It is also possible to obtain indicative population figures and relative distribution of different livestock species from aerial photographs with or without application of GIS (geographic information systems). The major shortcoming of most of these methods is that they tend to provide only species level statistics and not breed/strain level information. Thus, the general situation is that available statistics are on numbers of cattle, sheep, goats, pigs, poultry etc. in a country but hardly any information on the composition of these species. Even where such statistics are

broken down into breeds, their accuracy is doubtful as the data are usually collected and compiled by people who might not be experts in breed identification, hence may not accurately differentiate between the different breeds. Such data are not useful for monitoring the status of within-species diversity.

To obtain breed level statistics, it is not practical to count animals over a whole country or region. A sampling scheme can be developed whereby total counts are obtained on randomly chosen sample areas [ILRI-SDP]. However, on the basis of known distributions of animals in the particular area of a country, sampling can be stratified according to breed and animal density. Similarly, the size and number of blocks, quadrants or strip transects to be used can be determined by several factors, including the heterogeneity of the area in terms of breeds/types [FAO/ILRI Zimbabwe Report]. The purpose of these surveys is to quantify the proportionate composition of animal populations by breeds and species and thus to estimate total population size of each breed. Where national animal population statistics are available for species, such as cattle, sheep and goats, estimated proportionate compositions (by region within country) can be used to partition the national or regional figures into breeds/types. Key statistics to be obtained during such a survey are number of herds/flocks in the sample area, their sizes and breed composition by species. In some countries, species level animal statistics at (administrative) regional level may already be accurate enough so that the main task is to partition these into breeds/strains.

Physical counts necessitating visits to localities in which these breeds are found can be combined with collection of data on factors which characterise the production systems. Longitudinal surveys can be undertaken to provide a time series of population figures over years [Oromia and FAO/ILRI Zimbabwe report].

Data on the physical environment can be obtained from local government stations, where available. These include climatic data (rainfall amount and distribution, monthly temperature figures, humidity etc.), vegetation and incidences of diseases. Where these are not already available, collection of such data can be in-built into the characterisation protocol. Indeed, even where facilities already exist for collection of these data, additional steps should be taken to ensure that an acceptable level of reliability is achieved. Participatory exercises such as focus group discussions, farmer workshops and phenotypic rankings by farmers are quite illuminating and therefore should be undertaken, in addition to the conventional questionnaire-based surveys (Wurzinger et al. 2005).

Detailed description of the production system should include a statement on the management system (sedentary, transhumant, nomadic etc.), housing, feeding practices and nutritive value of feeds by seasons.

3   What do their genes contribute?

Decisions about which breeds to be conserved should be based on *objective criteria*, but also consider both current utility and the need to maintain maximum genetic diversity in the gene pool of the species (Simianer et al. 2003). The latter can be achieved by conserving that subset of all breeds in a species that shows the most genetic differentiation among them, including those that contain unique alleles or allele combinations, while at the same time meeting the production needs of the farmers. Genetic analysis could facilitate identification of genetic duplicates and/or separation of breeds on the basis of genetic distinctiveness. Pair-wise genetic distances estimated among all the breeds/strains/populations of a species, and the single phylogeny constructed from these distances that best represent all the relationships among the breeds will aid objective and rational decision making in the choice of breeds for

conservation and breed improvement, including evaluation studies to determine comparative genetic merit.

3.1 How important are breeds/strains within species?

The process of domestication of animals involved a selection of only some 40 out of the estimated 40 thousand or so species of vertebrates. These represent the ancestors of the domestic species available today. The selected species accompanied human populations across the earth into a variety of new environments, gradually evolving to adapt to a wide range of environmental conditions.

The next stage in the evolution of domestic animal breeding was the development of controlled mating and human selection of preferred animal types. Breeds began to be formally recognised in Europe in the 18th century. Superior animals were identified and then herd registers and herd books were created for them (see Section 1.1, this module).

Although no compelling quantitative figures are available, it is estimated that 50% of the total genetic variation among domestic AnGR is at species level and the remaining 50% is accounted for by variation among breeds within species. There is no estimate of the extent of genetic diversity within breeds due to variation among strains. This is likely to vary considerably given the wide range in the number of strains available for the different breeds of all species. From a standpoint of utilisation and conservation, breeds and possibly strains are generally more important than species. It is the differentiation of species into breeds that has allowed existence of livestock production in many of the unique/special environments of the world (see also Module 1, Section 5). Moreover, livestock species (e.g. cattle, sheep, goats, pigs etc.) are not likely to become extinct, but certain breeds are. For example, the swamp buffalo breeds that used to be important for providing draft power in Thailand, Lao and many of the South-East Asian countries have declined and are now increasingly threatened with extinction due to increased mechanisation of paddy rice cultivation. Losing a particular breed of a species adapted to live in an environment in which no other breed of that species can live could have serious implications for human food and livelihood security [CS 1.1 by Mpofu and Rege]; [CS 1.24 by Dempfle and Jaitner]; [CS 1.37 by Kharel *et al*]. Also important is the fact that it is the differentiation of species into breeds that has produced a wide range of populations, each serving a specific set of purposes milk, meat, traction, pack, eggs, wool etc. for society. In the recent past, crossbreeding programmes where breed total replacement was not the goal have, however, created rather than reduced genetic variation (Madalena 2005). Such new variations have been exploited to varying extents. There are examples where positive impacts have been realised from breed combinations [Sunandini-Kerala]; [Boer goats] and [Dorper sheep]; [Mafriwal cattle].

3.2 Measuring diversity within breeds

As descriptive measures of genetic and environmental variation, it is more convenient to use what are called *genetic parameters* or, strictly speaking, *phenotypic*, *genetic* and *environmental* parameters, which are all ratios of variances and covariances. Genetic diversity has been defined above as the *heritable* variation within and between populations. *Heritability*, a quantitative measure of heredity, is thus an important parameter not only in understanding genetic diversity in a population but also in utilising that diversity.

- *Heritability* is defined as the ratio of (additive) genetic variance to the phenotypic variance and is an indicator of the proportion of the observable variation in a trait in the parental generation which will be passed to the offspring generation. Other important parameters in this context are *repeatability*, *phenotypic*, *genetic* and *environmental correlations* (see Module 4 section 5.1).

- *Repeatability* is defined as the ratio of the variance due to animal effects (both genetic and non-genetic) to the total phenotypic (observable) variance. It can also be considered as the fraction of the difference from the mean, which is expected, in another record of the same animal. Repeatability is an important measure in that it indicates the reliability of an existing record as an indicator of possible future records on the same individual.

- *Phenotypic correlation* measures observable association between two traits and is calculated as the ratio of the phenotypic covariance to the product of the phenotypic standard deviations of the two traits.

- *Genetic correlation* is a quantitative measure of the association, at the genetic level, between two traits. It arises from the fact that some genes affect more than one trait. It is estimated as the ratio of the genetic covariance between two traits to the product of the genetic standard deviations of the two traits.

- *Environmental correlation.* Just as there are two possible causes (environmental and genetic) of differences between individuals in expressed phenotype of one trait, there are also two causes of correlation between two traits or characters. *Environmental correlation* measures the association between traits due to environmental factors and is calculated in a similar manner as the genetic correlation but using environmental covariance and standard deviations.

These parameters are very important in animal breeding [CS 1.6 by Mpofu and Rege]; [CS 1.9 by Aboagye]. Indeed, they underpin both the understanding and utilisation of genetic diversity in populations [Manual exercises - Quantitative characters]; [Manual exercises - Genetic gain].

3.3   Measuring genetic diversity within and between breeds/strains

The field of molecular biology, particularly the application of molecular markers to study genetic diversity, has evolved very rapidly since the mid-1960s. The dominance of protein electrophoretic approaches to population genetics and evolutionary biology was, in the late 1970s, replaced by DNA analysis, primarily through the use of restriction enzymes, and in the 1980s by mitochondrial DNA analyses and DNA fingerprinting approaches [CS 1.38 by Ofelia]. More recently, the introduction of PCR-mediated (polymerase chain reaction-mediated) DNA genotyping/sequencing has provided the first rapid and easy access to the ultimate genetic data.

The various state-of-the-art analytical (statistical) methodologies available for assessment of genetic diversity using molecular data are described in (Module 4 Section 7). Although DNA-based technologies are now the methods of choice, it would be a mistake to conclude that DNA markers provide the ultimate solution. Several alternative assays, such as protein/allozyme polymorphisms, remain tremendously useful, especially in developing countries, because of their utility, ease, cost and amount of genetic information accessed or simplicity of data interpretation. The role or potential of these alternative approaches in animal genetic diversity studies should not be underplayed.

What biochemical or DNA-based molecular techniques are presently available?

a. *Protein polymorphisms:* Variation in proteins reflects changes in the genes that code for them. This has been widely used in studying genetic diversity (Hames and Rickwood 1990). The two approaches applied are protein electrophoresis and, to a small extent, protein immunology. The principle behind studies of electrophoretic mobility of enzymes (and other proteins) is that mobility across gels can be related to differences in allelic

groups responsible for amino acid changes in the protein. Such amino acid substitutions are, in turn, a direct consequence of gene mutations. Thus, we can quantify the amount of variation within and between populations by measuring frequencies of different variants in groups of individuals.

b. *Protein immunology* methods rely on the antigenic properties of proteins. When a protein from population 'A' is injected into a suitable host population 'B', this antigen elicits the production of antibodies with high specificity for antigenic sites on the injected protein. The difference in antigen-antibody re-activities in tests involving homologous versus heterologous antigens provides a measure of the genetic relationship, usually expressed as immunological distance (ID) units between these proteins for the two animal populations. Protein immunology methods are not used as routine procedures for diversity assessment. An example of its use is the blood group protein immunology studies by Baker and Manwell (1991) which demonstrated close genetic relationships among breeds of European *Bos taurus* cattle and their genetic separation from the humped *B. indicus* cattle of Asia and Africa.

c. *Restriction fragment length polymorphisms* (RFLP) analyses involve cutting double-stranded DNA with one or more restriction endonucleases, enzymes that cut DNA at sites containing specific base sequences, i.e. restriction sites. The cutting process produces DNA fragments, the restriction fragments, which are then separated, according to molecular weight, by electrophoresis. Differences among individuals in 'digestion profiles' (the banding pattern on the gel) are generated by the presence or absence of restriction sites resulting from mutations (e.g. base substitutions, deletions/insertions and rearrangements within the restriction site). Avise (1994) described the analyses in detail. The resulting bands are then scored for individuals and these generate the 'frequency data' analysed for genetic diversity. The major limitation of nuclear DNA RFLPs as genetic markers is their low degree of heterozygosity. Most tend to be diallelic and hence not highly informative for diversity assessment. Another disadvantage of these markers is their lack of resolving power when dealing with closely related populations such as breeds or strains. This is because the polymorphisms are results of mutation events at the restriction sites; the mutation rates are extremely low ($10-7$ to $10-9$ per generation).

d. *Random amplified polymorphic DNA* (RAPDs) are considered the easiest group of DNA polymorphisms to detect and are based on the PCR amplification of random DNA segments with single, short (10 base) primers of arbitrary sequence (Williams et al. 1990). The resulting highly polymorphic pattern of bands is revealed by agarose electrophoresis with each random primer producing different pattern of bands. The bands are scored to generate data on individuals [CS 1.11 by Gwakisa]. In addition to its potential application in genetic distance estimation, RAPD can be used as a tool for mapping a trait (Michelmore et al. 1991). This can be important in searching for markers associated with quantitative characters (see Module 4, Section 5). The limitation of RAPD is the ambiguity of the resulting fingerprint patterns and the fact that heterozygotes cannot be distinguished from homozygotes due to its dominant inheritance mechanism. In addition, how the genetic variation observed is generated is not fully understood, making reconstruction of evolutionary histories from RAPD data difficult.

e. *Mitochondrial DNA* (mtDNA) is a highly conserved molecule whose genes are organised in a very compact manner, with some genes actually overlapping, e.g. in the bovine (Anderson et al. 1982). The only non-coding region, apart from small numbers of interspersed bases, is the D-loop or control region. Previous studies (e.g. Hausworth et al. 1984) indicated that the D-loop evolves at a higher rate than the rest of mtDNA. This has been used to support the argument that a sequence comparison of this region should be

most efficient at detecting differences between individuals at the mtDNA level (e.g. Cann et al. 1987). In addition, mtDNA has a high copy number per cell and a high mutation rate. Moreover, the fact that mtDNA is maternally inherited is of practical importance in the field: to ensure that only non-related mtDNA are sampled, one needs to only worry about the female side of the pedigree. Fortunately, information on the dam side is relatively easily available at field level and is usually reliable.

f. *Y-chromosome specific markers*. The Y-chromosome is a large linear molecule whose sequence is still largely unknown. Unlike the mitochondrial DNA, it is located in the nucleus and is paternally inherited. There are two major types of Y-chromosome in cattle. The typical *B. taurus* type is sub-metacentric and the *B. indicus* type is acrocentric (Kieffer and Cartwright 1968). Just like the mitochondrial DNA is useful in tracing female-mediated genetic relationships between populations, the markers on the Y-chromosome provide a means of studying male-mediated genetic introgression. For example, Hanotte et al. (1997) identified a polymorphic microsatellite marker in cattle. This locus has two alleles, one specific to taurine cattle and the other specific to indicine cattle. This specificity has been used to investigate the history of and genetic relationships among African cattle breeds (Hanotte et al. 2000).

g. *Microsatellites* are segments of genomic DNA which contain short tandem repeats of 2–6 bp nucleotides. They are now considered to be the markers of choice when trying to discriminate between closely related populations, e.g. breeds or strains (MacHugh et al. 1994). Microsatellite markers have several additional advantages which make them ideal for genetic characterisation. Microsatellite polymorphism refers to the differences in allele sizes due to variation in the number of repeats of base sequences that are detected by gel electrophoresis. These are scored on individual samples and provide the frequency data that are analysed to assess genetic diversity [CS 1.10 by Okomo-Adhiambo]. The advantages include: ease with which they can be identified and sequences of flanking regions determined as a prelude to primer design; the analysis procedure requires only a very small amount of DNA; microsatellite polymorphisms can be described numerically, facilitating computerised data handling and hence automation; and ease of sharing information on the relatively short primers between collaborating laboratories.

h. *Amplified fragment length polymorphisms* (AFLPs) (Vos et al. 1995) are based on the detection of restriction fragments by PCR amplification. Genomic DNA is restricted with two different restriction endonucleases and then a subset of these are amplified using a modified PCR and visualised using radioactivity, silver staining or fluorescent dyes for use with an automated sequencer. The main advantages of AFLPs for genetic diversity studies are that only small quantities of DNA are required because the technique is based on the PCR and the fingerprint traces are highly reproducible and consist of many markers, allowing for greater discernment between closely related individuals than other techniques including RAPDs and microsatellites. AFLPs are reliable informative multilocus probes and provide high levels of resolution that allows delineation of complex genetic structures (Powell et al. 1996).

i. *Single nucleotide polymorphisms* (SNPs) are DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered (Collins et al. 1997). For example a SNP might change the DNA sequence AAGGCTAA to ATGGCTAA. For a variation to be considered a SNP it must occur in at least 1% of the population. SNPs, which make up about 90% of all human genetic variation, occur every 100 to 300 bases along the 3 billion-base human genome (Taillon-Miller et al. 1998; Wang et al. 1998). Two of every three SNPs involve the replacement of cytosine (C) with thymine (T). SNPs can occur in both coding (gene) and non-coding regions of the genome.

SNPs offer several advantages over other types of DNA marker systems and are rapidly becoming the markers of choice for many applications in genome analysis due to their abundance (especially important in linkage disequilibrium based mapping approaches) and also because high throughput genotyping methods are being developed for their analysis. The additional advantage offered by this approach lies in the phylogenetic information gathered through sequence variation analysis that allows drawing inferences on allele and population history that cannot be gathered with any of the other marker systems available. SNPs are also evolutionarily stable (i.e. do not change much from generation to generation) making them easier to follow in population studies.

Module 4, Section 7 describes how data obtained from biochemical and DNA-level molecular genetic studies can be analysed to provide estimates of diversity, including relationships among breeds or strains, and within populations (e.g. measures of heterozygosity and extent of inbreeding).

3.4 Measuring the influence of the environment

Most of the economically important traits in livestock species are under the control of many genes (at many loci). Such traits are combined expressions of many different physiological systems, each contributing to the metric value additively or through interaction with other physiological mechanisms. If we take milk production as an example, the observable value is the overall expression of several 'macro-functions' such as appetite, feed intake, digestion efficiency, efficiency of utilisation of body reserves, udder function and volume, health status, ability to handle other environmental stresses etc. The list can be long. In addition, behind each macro-function, there are chains of enzymatic, hormonal and other biochemical reactions, each regulated by gene products. Thus, the number of genes involved in one trait is usually or likely to be very large. For such complex quantitative traits, the different genotypes cannot be distinguished on the basis of the phenotype (production record, measurement or appearance) of the individual. An important complicating factor is that environmental effects modify the expression of such characters and therefore contribute to the phenotypic variation among individuals. For example, the milk production by an individual is influenced by such factors as quality and quantity of feed, housing, effect of disease etc.

To the extent that environmental conditions are affected by climatic conditions, season becomes an important factor influencing animal performance. In the tropics, both quality and quantity of feeds and disease and parasite burdens can fluctuate considerably between seasons in response to differences in rainfall, temperature, humidity etc. These have important implications for housing and overall animal management and for herd/flock structures. In turn, management (housing, feeding, health care etc.) considerably influences the expression of quantitative traits.

To handle the complexity of these traits, quantitative genetic theory provides us with powerful tools (see Module 4, Section 4 and 5) for analysing quantitative variation to enable us to use the results in practical animal breeding. There are several analytical methods available. All of these are based on the fact that, no matter how complex the underlying causal mechanisms are for any trait, the expressed phenotype (P) can be attributed to two main sources, the genetic (G) and the environmental (E) components. (In complex models, these components are divided into sub-components [Manual exercises - Quantitative characters]; [Computer exercises - Prediction of breeding values] and interactions among components are also included.

While for a single trait in one environment, quantitative estimates of the causal (G and E) components, which are usually expressed in terms of variances, provide a good indication of

the contribution of the environment relative to the total phenotype, the situation is a bit more complicated for multiple traits and for one trait being evaluated in multiple environments. For quantitative estimates of a single trait in one environment, *environmental correlation* (Section 3.2, this module) provides a useful parameter, whereas for multiple traits and one trait being evaluated in multiple environments, the concept of genotype by environment interaction (G × E). Where G × E exist, the breed or genotype with the best performance in a given trait in one environment will not give the best performance in another environment, or the extent of superiority will differ between environments. Such differences provide a framework for quantitative analysis [CS 1.39 by Okeyo and Baker]. An instructive approach to the analysis of G × E is to treat records of the same trait taken in different environments as representing different traits and to proceed to estimate genetic correlations between these traits (see Falconer and Mackay 1996). Existence of G × E will be indicated if the genetic correlation is low (Section 5.3 Module 4).

What is the significance of G × E? It has important implications in the development of breeding programmes (Module 3, Section 3.4); [CS 1.39 by Okeyo and Baker]; (see Module 4, section 5.3). If selection is undertaken in good environments (feeding, health, housing or climatic stress), we need to know if the genetic improvement achieved will be exploited in a poor environment. Or should selection for adaptation to poor environments be made in similar environments? This is of direct relevance in stratified breeding systems where, for example, selection decisions are made on the basis of animal evaluations carried out on a few well-managed (commercial) farms or in extreme cases, where selection is based on evaluations carried out in different countries and under different production systems respectively (Ojango and Pollot 2002), [www.interbull.org].

On a more practical level, selection (performance tests) of breeding stock should be undertaken in environments that are similar to where their offspring are expected to be raised. More often, failures in realising the full genetic potential of exotic temperate breeds when they are exported to more stressful tropical environments or production systems is due to failure of the farmers and technical staff to fully recognise the importance of G × E, the most common of which is the continued use of high producing North American Holstein–Friesian bull semen to produce daughters in tropical farmers' herds where husbandry is generally inadequate. Many such examples exist in ill-designed (rather too sophisticated given the existing infrastructure) exotic breed-based livestock development programmes in the tropics.

4   What we can see and measure

As pointed out in Section 3, the phenotype is a result of both genotype and the environment. The animal phenotypes of interest can be divided into three main categories: 1) physical description or measurements; 2) performance characteristics; and 3) adaptation to the environment. Characterisation of animal genetic resources requires that data be collected on all these characteristics (see Section 2, this module).

Physical characteristics include such characteristics as presence or absence of horns, coat colour, body length, withers height, heart girth, tail length, tail type, presence or absence of hump, fur type (wool versus hair) etc. Some of these (e.g. presence or absence of horns) have simple Mendelian inheritance and have been studied extensively, at least in temperate livestock. Others such as withers height, heart girth and body length are obviously quantitative in nature. Physical characteristics are arguably the most commonly used criteria for breed or strain definitions. For this reason, attempts have been made to use these traits in classifying hitherto uncharacterised populations. One such example is the classification of

Ethiopian and Eritrean goat populations based on multivariate analyses of physical characteristics (see Section 2, this module).

Performance characteristics are the traits most familiar to animal breeders. In mainstream, 'western-type' animal production, they tend to be limited to such traits as milk yield and quality, meat characteristics (measures of growth and carcass quality), egg production and wool production (fleece yield and quality). They also include reproductive traits (age at first parturition, calving interval, prolificacy etc.). In traditional livestock production in the tropics, various species are also used for draft power and/or as pack animals [Yak]. Indeed, in these systems there is really no distinction between performance and adaptive traits. Thus, animals are expected to walk long distances in search of feed and water and to produce milk, pull a plough, produce offspring etc. Analyses at this level of complexity have generally been ignored in teaching of animal breeding in the tropics. Indeed, not much thought has gone into this area and sometimes the animals are unfairly condemned for under-performing, when indeed the whole picture has not been taken into account. As a result, breeding strategies for tropical low- and medium-input systems, which are generally livelihood-oriented, do not exist. Greater care should be taken to better design breeding strategies that best utilise breeds that would otherwise be unfairly condemned or ignored (see Sections 5 and 7, Module 1).

It is not good enough to only consider what may be important today, but rather a futuristic angle in which the potentials or importance of such genes in the predictable future are equally if not more important. Efforts should therefore be made to predict what genes would be important should the environment change in a given way or direction. Given that environment changes are predictable, with increasing degree of accuracy over time, livestock genotypes that thrive or perform best under environments that are similar to the future predictions however remotely would provide sources of candidate genes for conservation. In this regard, adaptive traits should be targeted. Traits such as trypanotolerance, helminth tolerance and the quantitative trait loci (QTL) associated with each of these are important in this respect

Adaptive characteristics include such traits as disease resistance [CS 1.19 by Yapi-Gnaore]; [CS 1.24 by Dempfle and Jaitner], cold tolerance [CS 1.37 by Mohan *et al*]; heat tolerance, salt tolerance goat of India ability to utilise low quality feeds, selective grazing [CS 1.1 by Mpofu and Rege] etc. Indigenous tropical livestock have, through millennia of exposure to the rigours of the tropical environment, evolved coping mechanisms. As has been alluded to above, the adaptive traits are probably the least studied in tropical livestock. Ironically, it is precisely because indigenous tropical breeds possess these characteristics that they need to be studied and conserved and genetically improved (selected or utilised in well structured crossbreeding programmes) [CS 1.36 by Sartika and Noor]; [CS 1.35 by Neopane and Prakash]. Admittedly, studying these complex traits is an expensive enterprise. Yet, without adequate characterisation of individual traits, including estimation of relevant genetic parameters, it is impossible to incorporate them into meaningful breeding programmes.

5   The contribution of indigenous knowledge

It is not through the keeping of animals *per se*, but rather the combination of rural peoples' knowledge of their environment and the way that they manage their livestock that maintains domestic animal diversity. This knowledge includes the recognition and evaluation of livestock characteristics and breeds or 'types'; the management of animal and plant genetic resources and how these interact in the production system; and ethnoveterinary knowledge. This rather extensive and complex knowledge system has not been adequately characterised and documented. Where documentation has been done, it has not been integrative enough to be applied in selection programmes although the indigenous knowledge on livestock from

livestock keepers, especially the pastoralist, can be complex and may be even more sophisticated than generally believed (Wurzinger et al. 2005). This is primarily because 'experts' often do not appreciate the value of this knowledge. This is a direct result of 'Western training'. Definition of comprehensive breeding objectives has been and would be impossible without inclusion of indigenous knowledge. Ignoring such wealth of knowledge could partly be the reason why livestock genetic improvement programmes that are solely based on Western designs and structures have generally failed in many developing tropical countries.

Livestock keepers have bred the trypanotolerant N'Dama cattle of West Africa [CS 1.24 by Dempfle and Jaitner] and the helminth resistant Red Maasai sheep of East Africa for centuries. Many similar examples exist in Asia, especially among the indigenous goat, pig, camel and buffalo breeders. The livelihood-oriented producers in these production systems understand the concept of risk avoidance by maintaining domestic animal diversity. They identify and select their animals for a wide variety of characteristics, such as drought tolerance, longevity, diseases resistance, ability to survive on low quality feeds etc. In addition, many smallholder and backyard livestock keepers can adapt quickly to changing circumstances as has been shown in shifting domestic animal production to urban and peri-urban environments.

Domestic animal diversity is ecologically and culturally embedded. Therefore, the knowledge of local people extends beyond the breeds themselves to the complex web of interactions between the animals and the environments in which they are kept, including the beliefs and cultures of the communities that keep them. For example, because of the need to make best use of the erratic and unpredictable rains and to avoid inter-community hostilities (rustling) and disease-prone areas, pastoral management systems, where indigenous breeds dominate, are flexible and dynamic. The flexibility and dynamism enables the people to respond quickly to changing conditions and complex systems of reciprocal favours and obligations. It also provides equity-sharing instruments that characterise the management systems which are often sanctioned by elaborate rituals and ceremonies. This knowledge system is crucial, not only in understanding the history and nature of existing diversity in animal populations, but also as a basis for developing strategies for its continued maintenance and sustainable exploitation (e.g. niche markets) in a way that accommodates the lifestyles, aspirations and livelihoods of the keepers [CS 1.32 by Mensah and Okeyo]. This is the only way that characterisation information can lead to formulation of sustainable utilisation and in turn, conservation of indigenous AnGR.

6 Intellectual property and rights

Intellectual property refers to creations of the mind such as inventions, all forms of literacy, artistic works, designs, including tradition-based creations, methodologies and the associated knowledge that are important for the management and sustainable use of genetic resources. The rights over such creations of the mind are what are known as intellectual property rights (IPRs).

The preservation, management and sustainable use of genetic resources and the associated knowledge and the equitable sharing of benefits of such resources and knowledge are some of the hottest IPR issues today (see World Intellectual Property Organization [WIPO].

The Convention on Biological Diversity (CBD) [CBD], a framework for action, in its articles, gave due rights to farmers, communities and governments over plant and animal genetic resources (e.g. livestock and poultry and strains). Under this Convention, the efforts of farmers and the community in the development of such resources, especially distinct livestock breeds and strains and products thereof, form an integral part of intellectual property. The

CBD recommended that legal frameworks and institutions be put in place in each contracting party (countries) and where such institutions already exist, they be strengthened so as to facilitate due protection, and promotion of such rights, including the development, optimal exploitation and equitable sharing of the benefits derived from such exploitations as a binding principle.

The Convention's interests are advanced, governed and implemented by Conference of Parties (COP) through meetings and negotiation fora, where procedural and substantive decisions take place. Access to the Convention's related information is done through what is referred to as '*The clearing-house*'.

The Convention also established and promoted institutional arrangements which provided mechanisms for further development of and for monitoring the implementation of the ideals of the CBD through meetings, programme review, capacity building and negotiations, thereby enhancing Trade related Aspects of Intellectual Property Rights (TRIPS) and other international institutional support for the same.

In the absence of country specific policies and legal frameworks, the noble benefits of the international agreements, including the CBD framework remain unrealisable and operationally toothless provisions. More important, is the need to create awareness among stakeholders as to their respective rights, especially the existing legal provisions and structures that are put in place to operationalise the important agreements and clauses.

Development of decision-support tools that would assist in the identification of policy constraints to the conservation and sustainable use of indigenous livestock and sharing these with all developing nations is an important step in ensuring that relevant policy and legal and regulatory frameworks are formulated and facilitated to address the constraints and improve opportunities for poor livestock keepers to derive increased benefits from their livestock.

All the countries, be it through regional or inter-country agreements and treaties have to formulate far-reaching rules/policies or enact laws, on management, transfers, benefit sharing and acquisition of germplasm. It is left to the respective individual and regional governments to make appropriate policies and legal frameworks to realise the noble CBD provisions. The extent to which issues such as 'prior informed consent' (PIC); 'mutually agreed terms' (MAT), Germplasm Acquisition Agreements (GAA) and Germplasm Transfer Agreements (GTA) are fully understood by the various stakeholders in the livestock industry is not clear. Similarly, the extent to which such provisions and clauses may currently and in future hinder or foster the sharing of livestock germplasm among the various stakeholders and countries in developing countries remains unclear and highly debatable. The extent to which the intellectual property related issues are legally supported would either impede or catalyse how the CBD provisions could both restrict and enhance benefit sharing, genetic improvement and wider utilisation of indigenous livestock germplasm within developing countries.

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9. Appendix

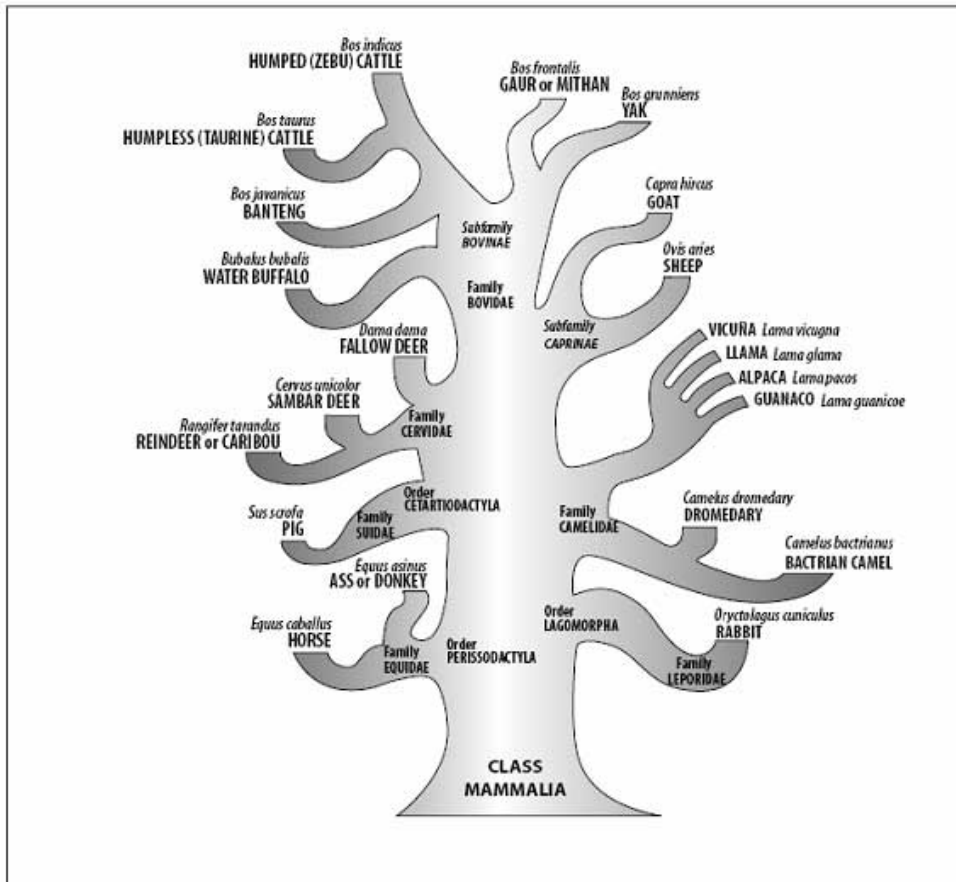Photo set1: Various animal species

Photos from biskitz4chez 2004, and A.M. Okeyo, ILRI

Photo set 2: Diversity between and within species

Photo from biskitz4chez 2004, and A.M.Okeyo, ILRI

Bos indicus
HUMPED (ZEBU) CATTLE

Bos frontalis
GAUR or MITHAN

Bos grunniens
YAK

Bos taurus
HUMPLESS (TAURINE) CATTLE

Bos javanicus
BANTENG

Capra hircus
GOAT

Bubalus bubalis
WATER BUFFALO

Ovis aries
SHEEP

Subfamily
BOVINAE

Family
BOVIDAE

Dama dama
FALLOW DEER

Subfamily
CAPRINAE

VICUÑA Lama vicugna
LLAMA Lama glama
ALPACA Lama pacos
GUANACO Lama guanicoe

Cervus unicolor
SAMBAR DEER

Rangifer tarandus
REINDEER or CARIBOU

Family
CERVIDAE

Order
CETARTIODACTYLA

Family
CAMELIDAE

Camelus dromedary
DROMEDARY

Sus scrofa
PIG

Family
SUIDAE

Camelus bactrianus
BACTRIAN CAMEL

Equus asinus
ASS or DONKEY

Equus caballus
HORSE

Order
LAGOMORPHA

Oryctolagus cuniculus
RABBIT

Family
EQUIDAE

Order
PERISSODACTYLA

Family
LEPORIDAE

CLASS
MAMMALIA

Evolutionary relationship of mammalian species used for food and agriculture (source: FAO WWL for domestic animal diversity, 3rd Edition, 2000. Edited by Beate D. Sherf)

Different breeds and strains of East African Zebu cattle

The Boran

The Taita/Taveta

The Kamba

The Duruma

The Oma-Boran

The Duruma

The Giriama

The Oma-Boran

The Giriama

Photos from J.Mwacharo 2000

Some Chinese pig breeds

Daweizi pig

Rongchang pig

Tongcheng pig

Hang pig

New Zealand Friesian cow in a pasture based production system

Courtesy of livestock improvement New Zealand

Kenya Boran bull

Boran bull photo taken by A.M.Okeyo
at ADC-Mutara, 1994

Ethiopian Borana cow

Various strains of Ankole cattle

Photos taken by Deo Ndumu,
National Animal Genetic
Resource Centre and Data Base
(NAGRC&DB), Uganda

Tanzania-Malagarasi

Uganda-Bahima2

DRC-Bashi

Uganda-Banyaruguru

Burundi-Inyaruguru

Tanzania-Enyambu

Burundi-Inyambu

Rwanda-Tutsi

Indian cattle breeds

Indian Sahiwal

Sunandini cattle

Sunandini cattle

Indian Sahiwal

Photo's courtesy of A.M.Okeyo,ILRI