Genetic diversity in indigenous cattle for East Africa—using RAPDs

Paul S. Gwakisa (2002)

Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3258, Morogoro, Tanzania

Introduction

Background

The primary aim of studying genetic diversity is to understand the extent of differentiation of populations within species. Population-specific genetic markers (alleles) can be generated using a range of methods available for detection of polymorphic *loci.* Polymorphic genetic markers are extremely useful for a number of applications, such as measurement of the amount of genetic diversity in species, discrimination between individuals, strains or species, identification of markers linked to economically useful traits as well as analyses of animal kinship relationship and behavioural and population ecology.

In the past decade there have been tremendous efforts globally to study genetic diversity in livestock species. This, in part, reflects a measurable indicator of an output to the Food and Agriculture Organization (FAO) global programme that addresses the needs of both development and conservation of animal genetic resources in the different parts of the world. The efforts also reflect global concern that a surprisingly narrow range of animal species is used for agriculture (sheep, goats, cattle, pigs, buffaloes, chickens and others that have local importance to certain areas) and this pool is threatened by genetic dilution. Shrinkage of the animal genetic resource pool is, to a great extent, a result of the radical shift of livestock development from subsistence to commercial farming systems. The world is witnessing increased specialisation of animal production objectives and competitive pressures arising from the specialisation. In sub-Saharan Africa, where the role of the livestock sector in gross agricultural output is increasing, specialisation has resulted in breed replacement, where indigenous zebu cattle are silently upgraded via artificial insemination with semen of *Bos taurus* breeds to achieve productivity gains. Although indigenous zebu cattle account for as much as 90% of the total cattle populations in African countries (Teale et al. 1993), crossbreeding with taurine breeds constitutes a significant threat to the zebu genetic resource. African zebu cattle are of particular importance because of their adaptation to a wide range of environmental conditions, such as heat, drought and tropical diseases. It is important to know the diversity and relationship between the African breeds and strains of livestock. Moreover, it is important because, quite often, the same breed may be known by different names or two breeds may be known by the same name, based on geographical locations of such populations (Gwakisa et al. 1994). The need to curb the threats of dilution and extinction of the African animal genetic resources, by strategic development and conservation, has never been more justifiable and is now, with the available technology, timely. Efficient strategies for conservation require sets of genetic markers, which characterise distinct populations (Kemp and Teale 1994).

Rationale for molecular genetic approach

Traditional methods used to study individual genetic variability of animals and populations employed polymorphism in protein markers. However, these techniques lack the power to resolve differences between closely related breeds, since a great deal of genetic variation remains undetectable by using protein markers. Moreover, the genotype frequencies estimated from protein markers may be influenced by natural selection among alleles, making it difficult to interpret interpopulation comparisons. With recent developments in molecular genetics, it has been solidly established that a measure of relative genetic diversity in animal populations can be attained through description of nucleotide sequence differences and similarities in the deoxyribonucleic acid (DNA) of animals in such populations. Analysis of DNA has several significant advantages over protein markers for the study of molecular population genetics and systematics. First, the genotype rather than the phenotype is assayed. Second, the expression of DNA markers is not influenced by development or by environmental factors. Third, one or more sequence(s) appropriate to a problem can be selected on the basis of evolutionary rate or mode of inheritance. Fourth, the methods are, for most part, general to any type of DNA and, fifth, DNA can be prepared from small amounts of tissues and is relatively stable, even in non-cryogenetically stored tissues. The last attribute means that genetic information on rare or endangered species can be obtained without destructive sampling and that it is possible to analyse DNA from extinct populations or species. More recently, molecular data from DNA markers have received particular attention in the study of population variability because of their possible use in determining the chronology of evolutionary events. This is because DNA markers are much less subject to natural selection than are phenotypic traits.

The problem

Molecular techniques in the study of animal genetic diversity

A variety of different molecular techniques are being used in various laboratories for the study of inter- and intra-specific genetic variation at the DNA level. The most widely used techniques are restriction fragment length polymorphisms of nuclear DNA and mitochondrial DNA, minisatellites, microsatellites, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism and sequencing of mitochondrial DNA. These techniques differ in the type of data they generate, in the way that they resolve genetic variations and in the taxonomic levels at which they may be most appropriate. As a rich selection of molecular techniques is available, choice of method to use for genetic diversity is quite often dictated by the power of the method to generate reproducible polymorphism that can either be tracked in a Mendelian fashion or can segregate a phenotypic trait in a predictable manner. However, aspects such as lack of specialised equipment, high running costs and lack of technical competence continue to impede many laboratories in research institutions and universities in sub-Saharan Africa. Many researchers in Africa would benefit if laboratory technology development was paralleled by (1) simplification in technology and ultimately reduction in expense and (2) increase in analytical power per unit effort. The discovery of the polymerase chain reaction (PCR)-based RAPD technique has made a substantial contribution as a simple technique, which is affordable to a broad community working in molecular genetics with limited resources.

Principle of RAPD analyses

The PCR-based RAPD technique is an attractive complement to conventional DNA fingerprinting. RAPD analysis is conceptually simple. Nanogram amounts of total genomic DNA are subjected to PCR using short synthetic oligonucleotides of random sequence. The amplification protocol differs from the standard PCR conditions in that only a single random oligonucleotide primer is employed and no prior knowledge of the genome subjected to analysis is required. When the primer is short (e.g. 10-mer), there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation. The technique essentially scans a genome for these small inverted repeats and amplifies intervening DNA segments of variable length. The profile of amplification products depends on the template-primer combination and is reproducible for any given combination. The amplification products are resolved on agarose gels and polymorphisms serve as dominant genetic markers inherited in a Mendelian fashion (Williams et al. 1990; Rothuizen and van Wolferen 1994). Amplification of non-nuclear RAPD markers is negligible because of the relatively small non-nuclear genome sizes. Two modifications of detection of RAPD markers have been described as DNA amplification fingerprinting (DAF) and arbitrarily primed polymerase chain reaction (AP-PCR). DAF uses short random primers of 5–8 base pairs (bp) and visualises a relatively greater number of amplification products by polyacrylamide gel electrophoresis and silver staining (Caetano-Anolles et al. 1991). AP-PCR uses slightly longer primers (such as universal M13) and amplification products are radioactively labelled and are also resolved by polyacrylamide gel electrophoresis (Welsh and McClelland 1990). Standard RAPD analysis is performed according to the original method (Williams et al. 1990) using short oligonucleotide primers of random sequence which can either be locally synthesised or are commercially available (Operon Technologies Inc., Alameda, California, USA). Only high-molecular weight, i.e. non-degraded, DNA should be subjected to RAPD analyses. Amplified products can be resolved by gel electrophoresis on 1–2% agarose gels.

How to generate RAPD data

By employing different oligonucleotide primers, molecular characters that are characteristic of individuals can be generated.. For any given primer, RAPD amplification products can be broadly classified into two groups: variable (polymorphic) and constant (non-polymorphic). For instance, consider a RAPD analysis of several individuals within a breed and several breeds within a given species. Constant fragments characteristic for the species may be identified, as well as fragments which are polymorphic between breeds within the species. Both types of product can be exploited for establishing relationships. In this example, constant fragments operationally identify members of a certain species exclusively, if the fragment is a unique polymorphism in a comparison of several breeds (species-specific marker). Similarly, fragments polymorphic at the breed level will operationally identify individuals of the breed if the fragment is constantly detected among the individuals (breed-specific marker). RAPD fragments, polymorphic among individuals of one breed, or belonging to one pedigree or one sex have also been obtained with different primers (Kemp and Teale 1994; Rothuizen and van Wolferen 1994; Gwakisa et al. 1994). Thus, RAPD products that serve as molecular genetic markers at different levels can be generated.

Advantages and disadvantages of RAPD markers

The advantages of RAPDs are that: there is no requirement for sequence information for the design of primers or probes; the procedure is quick, simple and can be automated; and a very small amount of DNA (e.g. 10 ng per reaction) is required. Different RAPD primers produce

different banding patterns because the polymorphisms generated by any primer are due to differences in spacing between primer binding sites and point mutations. RAPD has been successfully used in genetic studies of many animal, plant and microorganism species (Michelmore et al. 1991; Chapco et al. 1992; Kantanen et al. 1995) including African goat populations (Chalya et al. 1997), East African Zebu cattle (Gwakisa et al. 1994, 1997; Teale et al. 1995) and Tanzanian local sheep ecotypes (Stephen et al. 2000).

Disadvantages of RAPDs are many. First, resultant fingerprint patterns can be complex; thus, they require skilful scoring. Second, the method is very sensitive to reaction conditions, DNA quality and PCR temperature profiles. It is absolutely critical to maintain strictly constant PCR reaction conditions in order to achieve reproducible results. Third, the markers are dominant; thus, heterozygotes cannot be detected. Fourth, in the absence of pedigree material, the identity of individual bands in the multi-band profiles is not known and there can be uncertainty in assigning markers to specific *loci.* This makes RAPDs difficult to use in interpopulation or interspecific comparisons. Fifth, single bands on the gel can sometimes be comprised of several co-migrating amplification products. This makes it difficult to distinguish many of the polymorphisms apparent from PCR artifacts. Moreover, RAPD amplification occurs only at low, non-specific annealing temperatures. It is therefore likely that, depending on the surrounding DNA structures, a certain site is amplified in DNA from one animal but not from another (Rothuizen and van Wolferen 1994).

In the present case study, the potential application of RAPD fingerprinting in the study of genetic diversity in East African Zebu cattle are discussed.

Source of the material used

The results of two independent studies using RAPDs to study genetic diversity in East African Zebu cattle are described here.

Study I (Gwakisa et al. 1994)

Objective: Characterisation of zebu cattle populations in Tanzania using random amplified polymorphic DNA markers.

Representative animals of three cattle breeds in Tanzania (Mpwapwa, Tanganyika Shorthorned Zebu (TSZ) and Boran) were sampled from several herds. Random samples from 30 animals of both sexes were obtained from each breed.

Study II (Gwakisa et al. 1997)

Objective: Studies of genetic diversity in some indigenous cattle breeds of East Africa.

The cattle breeds and their respective locations in parenthesis were Boran (Kongwa, Central Tanzania), Maasai Zebu (Kongwa, central Tanzania), Iringa Red (Iringa, southern Highlands of Tanzania), Dwarf Chagga Zebu (Moshi, Kilimanjaro, northern Tanzania), Tarime (also called Sukuma, Tabora and Shinyanga; western Tanzania) and Ankole Zebu (Tabora, western Tanzania). Also included were Boran and Sahiwal breeds from Kenya and West African N'Dama breeds sampled from the International Livestock Research Institute (ILRI) in Kenya. Twenty cattle (10 of each sex) were sampled from each breed.

Sampling protocol, DNA extraction and analysis

The sampling strategy for each breed avoided selection of more than 2–3 animals from herds that grazed together or exchanged breeding bulls. Blood was collected and used to prepare DNA using published procedures. In essence, the blood cells were lysed using hypotonic solutions and non-ionic detergents and the nuclei were pelleted through centrifugation. The DNA was purified by treatment with protein denaturants and proteinases and then these were removed using organic solvents and high concentrations of sodium chloride. The DNA was concentrated by precipitating it with ethanol and then re-suspending it in water (Sambrook et al. 1989). Samples were adjusted to a concentration of 20 ng/µl before PCR amplifications which were performed on single-animal DNA and on breed-specific DNA pools. The breed-specific DNA pools were each made by mixing equal quantities of DNA from 15 individuals of the same breed at a 1:3 male to female ratio.

Random amplified polymorphic DNA–Polymerase chain reaction (RAPD–PCR)

The RAPD–PCR was performed on a Programmable Thermal Controller (MJ Research Inc., Watertown, MA, USA). Each 10 µl reaction mix comprised: 20 ng template DNA, 6 pmol primer, dNTPs (deoxynucleoside triphosphates) each at 50µM, 0.5 units Taq DNA polymerase (Promega Biotec, Madison, Wl, USA), 10mM Tris pH 8.3, 50µM KCI, 0.001% gelatin, 0.025% Tween-20, 0.25% NP 40 and 1.5mM $MgCl_2$. Temperature cycling comprised 40 cycles of 10 s at 95°C, 5 s at 35°C and 1 min at 72°C. PCR products were electrophoresed at 150 V in 2% agarose gels containing ethidium bromide. Phi × 174 RF DNA digested with HaeIII (Gibco-Bethesda Research Laboratories Paisley, Renfrewshire, UK) was used as a molecular size marker. RAPD fingerprints were visualised by ultraviolet (UV) illumination and documented by photography.

Primers

A total of 141 arbitrary oligonucleotide primers were used singly for amplification of the breed-specific DNA pools. The primers, which discriminated between the DNA pools, were investigated further on single DNA samples to determine the proportion of animals carrying the pool-specific fingerprints. All the 141 primers were 10 bp long with a GC content of 45–80%. Sequences for the primers that amplified apparent pool-specific products are shown below.

ILO 1065: 5' CCG GTG TGG G 3'

ILO 1127: 5' CCG CGC CGG T 3'

ILO 1204: 5' GAC GGC GCA A 3'

ILO 1212: 5' GCG GCC GTA A 3'

ILO 525: 5' CGG ACG TCG C 3'

ILO 526: 5’ GCC GTC CGA G 3'

Scoring of bands and statistical analysis

Only distinct, prominent bands were scored. Such bands represented RAPD fingerprints ranging from 0.31 to 1.1 kb in size. RAPD fingerprints were compared only on samples run in the same gel. Amplified DNA representing separate PCR products was scored and put in a data set for analysis of the following statistics.

- Frequency of polymorphic fingerprints in the different breeds, determined from the ratio of the number of animals carrying the fingerprint (n) to the total number of animals screened within a breed (N).
- Similarity index or allele (band) sharing (AS), calculated between breeds as follows:
- AS between animals a and b = (2Bab)/(Ba+Bb), where Bab is the number of fingerprint bands shared by animals a and b, Ba is the number of fingerprint bands in animal a and Bb is the number of fragment bands in animal b (Jeffreys and Morton 1987; Dunnington et al. 1990),
- Genetic distances between breeds.
- Mean average percentage difference (MAPD). This value was calculated using the following three formulae (Gilbert et al. 1990):

1) Percentage difference (PD) = $Nab/Na + Nb \times 100$

$$\text{2) Average percentage difference (APD)} = 1/C\sum_{i=1}^{C} PDi$$

$$\text{3) Mean average percentage difference (MAPD)} = 1/R\sum_{i=i}^{R} APDi$$

Where Nab is the number of fragments that differed between two individuals for a single primer; Na is the number of fragments resolved in individual a; Nb is the number of fragments resolved in individual b; C is the number of interbreed pair-wise comparisons; and R is the number of random primers used.

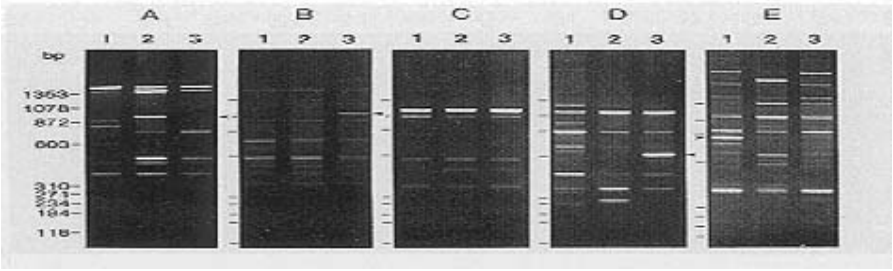
The MS Excel (version 5) and STATISTIX (version 4) analytical software were used to compute allele frequencies and mean values, respectively. The DISPAN computer programme (1993) was used to calculate genetic distances and phylogenetic relationships between breeds.

The findings (the story)

Study I.

Random primers generate breed-specific RAPD fingerprints

Results from Study I (Gwakisa et al. 1994) showed that, out of the 141 primers used to detect genetic differentiation in the three cattle breeds of Tanzania, only five primers, designated ILO 1065, ILO 1127, ILO 525, ILO 526 and ILO 1204 gave banding patterns which discriminated clearly between the breed-specific DNA pools (Plate 1). For example, primer ILO 1127 revealed a TSZ pool-restricted RAPD band at 0.97 kb, while ILO 1065 amplified a Boran pool-specific RAPD fragment of 1.13 kb. The five primers were used subsequently to amplify individual DNA samples to establish the proportion of animals showing pool-specific fingerprints. Each of the five primers produced a different banding pattern and a variable number of PCR products in the three breeds as shown in Table 1. Thus, the ILO 1127-primed RAPD was revealed in 61% of the TSZ breed but less than 6% of the Mpwapwa breed. This RAPD marker was not detected in the Boran breed. The ILO 1065-amplified fingerprint appeared with a frequency of 89% among Boran individuals but less than 30% in the other two breeds.



Note: On the left of each triplet are indicated Phi × DNA digested with HaeIII as molecular size markers. Apparent pool-specific RAPD fingerprints are indicated by arrows.

Plate 1. RAPD fingerprints of breed-specific DNA pools of Mpwapwa (1), TSZ (2) and Boran (3) breeds detected with five different primers: ILO 1127 (A), ILO 1065 (B), ILO 526 (C), ILO 525 (D) and ILO 1204 (E

Table 1. Number and frequency of RAPD bands per primer in different breeds (n = 30).

Primers	ILO 1127	ILO 1065	ILO 525	ILO 1204	ILO 526
Number of bands (average)					
Mpwapwa	8–11 (9.5)	7–12 (9.5)	5–7 (6)	8–10 (9)	3–7(4.4)
TSZ	5–7 (6)	7–10 (8.6)	5–8 (6.5)	8–12 (10)	4–6(5)
Boran	7–11 (8.7)	7–12 (10)	5–8 (6.5)	8–10 (9)	3–7(5)
Frequency of apparent breed – specific bands					
Mpwapwa	0.06	0	0.29	nd	nd
TSZ	0.61	0.3	0.82	nd	nd
Boran	0	0.89	0.34	nd	nd

nd = not determined; TSZ = Tanganyika Shorthorned Zebu

Allele sharing within and between breeds

Allele sharing was calculated using RAPD fingerprints obtained with individual animal DNA rather than pooled DNA. Fifteen pair-wise comparisons were made for each primer. Data presented in Table 2 suggest considerable homogeneity of individuals within the breeds. The average allele sharing was 0.79 ± 0.02 within the Mpwapwa breed, 0.73 ± 0.02 within the TSZ breed and 0.74 ± 0.05 within the Boran breed. The inter-breed allele sharing was lower than the intra-breed allele sharing. Average values calculated on the banding patterns revealed with four singly used primers were 0.58 ± 0.17 between Mpwapwa and TSZ breeds, 0.59 ± 0.22 between Mpwapwa and Boran breeds, and 0.59 ± 0.24 between TSZ and Boran breeds (Table 3).

Table 2. Allele sharing within the three breeds.

Primers	Mpwapwa *n* = 30	TSZ *n* = 30	Boran *n* = 30
ILO 1065	0.80	0.86	0.88
ILO 525	0.77	0.73	0.76
ILO 1204	0.82	0.61	0.58
Average for three primers	0.79 ± 0.02	0.73 ± 0.02	0.74 ± 0.05

TSZ = Tanganyika Shorthorned Zebu

Table 3. Allele sharing (AS) and mean average percentage difference (MAPD) between breeds (means calculated based on three primers; ILO 1127, ILO 525 and ILO 1240).

Primers	Difference between breeds		
	Mpwapwa–TSZ	Mpwapwa–Boran	TSZ–Boran
AS	0.58±0.17	0.59±0.22	0.59±0.24
MAPD	25.47±9.05	35.47±13.77	27.30±12.90

TSZ= Tanganyika Shorthorn zebu

Mean average percentage difference between breeds

Inter-breed dissimilarities were calculated on RAPD fingerprints obtained with three primers. In each case, 15 inter-breed pair-wise comparisons of individual animals were made. The data in Table 3 show that these values varied for each primer and for each paired breed comparison. The inter-breed divergence was narrower between Mpwapwa and TSZ breeds (25.47 ± 9.05%) than between the other paired breed comparisons.

Study II

In Study II (Gwakisa et al. 1997), additional breeds were included for the purpose of studying genetic diversity in the selected African cattle breeds. As data in Table 4 show, apparent genetic differences were demonstrated between cattle breeds. This is exemplified by the diversity of RAPD fragment sizes and their frequencies in the breeds. While a few fingerprints, e.g. ILO 525.210 bp, ILO 525.280 bp and ILO 525.350 bp, were restricted uniquely to one or two breeds, the majority of the fingerprints were shared, to different extents, by all breeds. Presuming that each of the random primers amplifies a different *locus* and each of the RAPD

fingerprints shown in Table 4 represents a separate allele, the three primers have amplified a total of seventeen alleles, each potentially informative to differentiate between the seven cattle breeds. Primer ILO 525 can be considered to be the most informative, having amplified the highest number of alleles (n = 11) in comparison with the other two primers (5 alleles by primer ILO 1204 and one allele by ILO 1212). The Ankole breed carried the highest number of alleles (15/17) followed by the Chagga Zebu (12/17). The lowest number of alleles was detected in the N'Dama breed (4/17). The mean number of alleles per *locus* at RAPD *loci* is shown in Table 5.

Table 4. Frequency of RAPD markers generated with three random primers in eight cattle breeds.

		Breeds						
Primer	Marker, bp	Maasai	KeBoran	TzBoran	Chagga	Iringa Red	Ankole	N'Dama
ILO 525	200	0.47	0.736	0.684	0	0.389	0.437	0
	210	0	0	0	0	0	0.187	0
	230	0.117	0	0.05	0.352	0.222	0.312	0
	280	0	0	0	0	0	0.125	1
	300	0.941	1	1	0.235	0.889	0.75	0
	325	0	0	0.211	0.764	0.277	0.063	0
	350	0	0	0	0	0.444	0.625	0
	400	0.117	0.158	0.421	0.235	0	0.5	0
	460	0.412	0.684	0.842	0.647	0.889	0.25	1
	500	0.529	0	0.474	0.352	0.111	0.625	0
	650	0	0	0	0	0	0	0
ILO 1204	235	0.705	0.158	0	0.588	0	0.214	0
	250	0.176	0.578	0.631	0.176	0.222	0.428	0.571
	271	0.47	0	0	0.647	0.222	0	0
	310	0	0.176	0.105	0.117	0.056	0.312	0
	603	0.058	0.105	0.157	0.235	0	0.375	0
ILO 1212	400	0	0.667	0.764	0.5	0.6	0.4	0.4

A similarity index was calculated for each breed pair to compare the extent of similarity/dissimilarity between the breeds. Based on a cumulative analysis for the 3 primers, the similarity indices (see Table 6) between the breeds, were as high as 68.9% between the Tanzanian Boran and Sahiwal (n = 73) and 67.4% between the Tanzanian Boran–Kenyan Boran (n = 36). Lowest similarity indices were indicated between the Maasai Zebu–N'Dama (23%; n = 15) and Kenyan Boran–N'Dama (26.7%; n = 38).

Genetic distances between the breeds based on RAPD polymorphism are indicated in Table 7 and Figure 1. In most cases, the distances conform to the expected relationships between these breeds by the indication that the zebu breeds all show close relationships. The closest breeds are the Kenyan Boran and Tanzanian Boran. The N'Dama breed, as expected, is most distant from the zebu breeds.

KeBoran = Kenya Boran; TzBoran = Tanzanian Boran

Table 5. Mean number of alleles in six breeds of cattle calculated for three RAPD loci.

Cattle breed	Mean number of alleles in three *loci*
Maasai Zebu	5.0 ± 1.4
Iringa Red	5.0 ± 2.8
Tanzanian Boran	5.0 ± 2.8
Kenya Boran	4.0 ± 0
Sahiwal	3.0 ± 2.8
N'Dama	1.5 ± 0.7

Table 6. Percentage similarity between cattle breeds based on RAPD data.

	N'Dama	Maasai	KeBoran	TzBoran	Ankole	Chagga	Iringa
Sahiwal	27.9	68.5	60.8	68.9	35.9	49.8	61.4
N'Dama		23.3	26.7	27.1	39.5	37	29.8
Maasai			58.3	54.5	47.2	52.6	60.2
KeBoran				67.4	34.1	40	51.6
TzBoran					39.6	43.3	59.8
Ankole						37.9	46.1
Chagga							59.9

KeBoran = Kenya Boran; TzBoran = Tanzanian Boran

Table 7. Standard genetic distances based on (Nei 1972) estimated between six cattle breeds based on three RAPD loci.

	TzBoran	Maasai Zebu	Sahiwal	N'Dama	KeBoran
Iringa Red	0.028	0.048	0.053	0.161	0.026
TzBoran		0.061	0.069	0.182	0.005
Maasai Zebu			0.059	0.194	0.047
Sahiwal				0.172	0.077
N'Dama					0.183

KeBoran = Kenya Boran; TzBoran = Tanzanian Boran

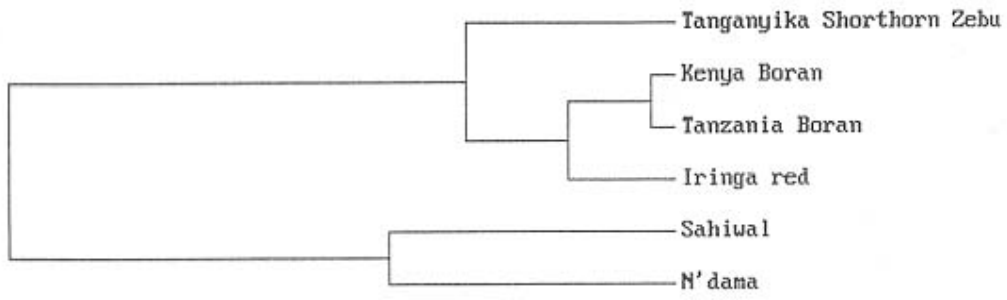


Figure 1. UPGMA (unweighted pair-group method using arithmetic averages) tree based on RAPD analysis of six African cattle breeds.

Implications and lessons learned

This study demonstrates that using short random primers of arbitrary nucleotide sequence, it is possible to identify reproducible DNA markers, characteristics of individual animals, populations or breeds of cattle. Several implications and lessons can be derived from this case study. First, RAPDs allow rapid differentiation of cattle breeds by looking at each breed as a 'pool' using a breed-specific DNA pool followed by a subsequent tracking of prominent fingerprints in individual animals. Pooled DNA analysis allowed the screening of a large number (n = 141) of random primers within a short period, comparison of the three breed pools on one gel in close proximity and rapid identification of 'average' pool-specific DNA fingerprints. The results of Study I showed that primer ILO 1127 amplified a RAPD fingerprint whose frequency is 61% in the TSZ breed. Since this fingerprint was obtainable in male and female TSZ animals, it is a potential marker for the sampled subpopulation of this breed. This fingerprint was not detected in the Boran breed, but appeared in 6% of the Mpwapwa breed. The observation that 39% of the TSZ animals did not show this marker

reflects genetic diversity within this breed, which consists of distinct populations within Tanzania (Gwakisa et al. 1994). It is probably possible to increase the frequency of such a RAPD marker by increasing the sample size from the animal population. Another primer, ILO 1065, revealed a RAPD fingerprint appearing in 89% of the Tanzanian Boran cattle. This RAPD was further shown to be male-associated and restricted to Boran, TSZ, Brahman and N'Dama breeds. The finding that this fingerprint was also obtainable with DNA of West African taurine N'Dama males seemingly weakened the notion that this was an indicine fingerprint. However, the conforming possibility of incursion of *B. indicus* genes into some N'Dama individuals (Sharp 1993) was later proven by the finding that the ILO 1065-primed RAPD fingerprint is actually a *B. indicus*-Y chromosome specific polymorphism. Possession of this polymorphism by all indicine males as well as N'Dama males, but not females of any breed, suggests the possible introgression of *B. indicus* Y alleles into the N'Dama (Teale et al. 1995). RAPD markers are therefore useful to study genetic differentiation of cattle populations. Moreover, RAPD polymorphism can be used to describe the nature and specificity of a sex-linked allele.

Second, the results of Study I and Study II together have shown the applicability of RAPD markers for statistical estimation of allele sharing and genetic divergence between breeds. Like other DNA polymorphisms, e.g. restriction fragment length polymorphism (RFLP) and microsatellites, RAPDs offer a potential alternative marker for genetic characterisation of populations (Ragot and Hoisington 1993; Gwakisa et al. 1997). Since each RAPD fingerprint may represent or be linked to a separate allele, any shared fingerprint may be contemplated as a product of the same allele. RAPD fingerprints that are not shared are, therefore, powerful tools for discrimination of phenotypically different populations or breeds. Results of both studies described above show that allele sharing is greater within breeds than between breeds. In this regard, the calculated allele sharing reflects the *B. indicus* ancestry shared by the African cattle breeds, whereas the mean average percentage difference provides a measure of genetic divergence between the breeds.

Third, the case study cited here shows that measurable genetic differences can be generated using RAPD markers in order to study phylogenetic relationships and genetic diversity between breeds. Estimation of the latter can be based partly on overall number of alleles per *locus* in the breeds. As expected with DNA *loci*, while most of the RAPD primers amplified fragments that were detected in all the breeds, some primers amplified polymorphisms that were not detected in all breeds. This finding is important in relation to improvement of knowledge of the histories of the indigenous African cattle breeds chosen for this case study. The pattern of allele sharing, genetic differences and phylogeny demonstrated with RAPDs is a good reflection of fixed diversity within each breed. With the exception of the Kenyan and Tanzanian Boran, Sahiwal and N'Dama, the diversity in the remaining breeds (Study II) is most likely a result of natural selection, undergone by these breeds for generations. It is known that the Boran breeds in Kenya and Tanzania, as well as the N'Dama and Sahiwal have undergone strong artificial selection (Maule 1990) and therefore, as expected, these breeds showed less genetic diversity. This concept was particularly evident with the mean number of alleles per *locus* obtained with the RAPD analysis. The genetic distances established in Study II showed an interesting pattern of breed relationships. While the distances between the different zebu breeds were small in relation to those between zebu breeds and the distant N'Dama breed, the distances suggested that the zebu breeds might have intermingled, to different extents. Given that a great deal of crossing occurs among the various breeds, some of these zebu breeds may possess some taurine genes. This, however, was not evidenced, although with a suitable RAPD primer, it could be demonstrated.

In conclusion, the RAPD–PCR technology, in comparison with other methods used for breed characterisation, has several advantages. RAPD markers allow a cheap and quick fingerprinting technique. In contrast to protein polymorphisms, which normally examine a single *locus,* RAPD markers are multi-*locus*. However, comparison of the RAPD–PCR to other DNA-based methods, such as mitochondrial sequencing and microsatellites, still requires a reliable designation of the RAPD allelic patterns in order to allow exchange of such markers between laboratories.

Questions for discussion groups

- What are the unique features of the RAPD–PCR?
- How does the PCR for RAPDs differ from that of microsatellites?
- How is RAPD polymorphism generated?
- In comparison with other DNA based genetic markers, what are the strengths and weaknesses of RAPDs?
- Can RAPDs be used to follow segregation of a trait in (a) a pedigree (b) a randomly mating herd?
- Why did this case study start with 141 primers instead of just five primers to study genetic differentiation of the breeds?
- How would you use RAPD data in the Hardy-Weinberg equation, knowing that RAPDs are a dominant type of markers?

Gaps in knowledge

To date, there are only a few scientific publications on use of RAPDs in studies of genetic diversity of livestock. The technique, however, has been extensively used in genetic characterisation of microorganisms, insects, domestic pets and plants (Lipman et al. 1996; Rothuizen and van Wolferen 1994; King et al. 1993; Chapco et al. 1992). At Sokoine University of Agriculture, Tanzania, the technique has been extensively tested and used to differentiate local breeds of cattle in Tanzania (Gwakisa et al. 1994, 1997), goats strains in Tanzania (Chalya et al. 1997), sheep ecotypes in Tanzania (Stephen et al. 2000) and ecotypes of the scavenging local chickens of Tanzania (Gwakisa unpublished). However, the following remain to be the major weaknesses of the RAPD technique.

1. The need to strictly adhere to PCR conditions in order to maximise reproducibility of the banding patterns. When any change is introduced, e.g. water, buffer, enzyme batch or thermocycler, it is important to test the reproducibility of results. It should be emphasised that when a change in technique is introduced, it is worth running all samples again under the new conditions, as the effect of changing the technique will then affect all samples.
2. The issue of reproducibility is of much concern. It has been overstated that RAPDs are not reproducible from day to day, laboratory to laboratory and even within one experiment. However, practical evidence has shown that reproducibility can be controlled by first, working only with good quality DNA and second, by ensuring that adequate and optimal quantities of DNA and amplification reagents (dNTP, primer and enzyme) are used each time.
3. Complexity of the resultant fingerprint patterns and scoring technique are other weaknesses of the RAPD technique, especially to beginners. It is good practice not to

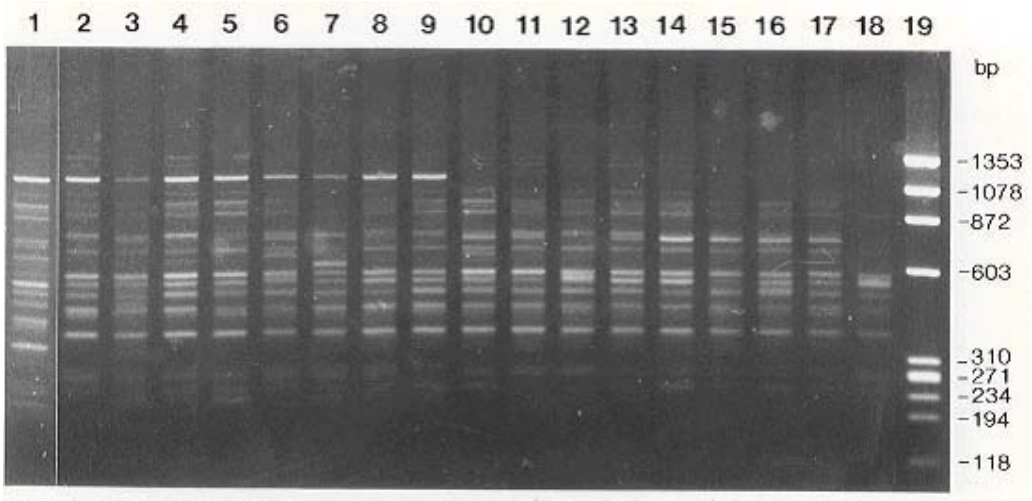
score those fragments on gels that are of extremely high and low sizes. Fragments that are resolved in the middle of the lanes are highly reproducible. If a standard sample is included in several gels and a DNA ladder (1 kb, Phi × 174 or other) is available, it is possible to score fingerprint patterns with greater confidence.

4. In order to optimise the RAPD technique for diversity studies of livestock, it will be necessary to set up a panel of standard alleles and their nomenclature. This will facilitate exchange between laboratories and provide a prudent approach to optimisation of the technique in a new laboratory, or when totally new conditions must be introduced.

The major strengths of the RAPD technique have been discussed above. Nevertheless, it is worth considering strengthening of the following aspects.

- RAPD–PCR is cheap and allows rapid screening of DNA pools for 'average population-specific fingerprints'. It is easy to screen hundreds of arbitrary 10-bp primers for fingerprint patterns that are favourable and moderately easy to score, and then to use these on the populations under study. In some cases one may wish to use arbitrary primers in pair-wise combinations. This increases the number of targeted genomic regions for amplification. Thus, just 20 RAPD primers can be used in 380 different combinations (Welsh and McClelland 1990).
- PCR products from RAPD analysis may be directly sequenced, or cloned and used as specific primers or probes thereafter. In work at ILRI (Teale et al. 1995), a RAPD polymorphism that can be used in a simple dot blot assay as a probe for RAPD–PCR products was developed. It provides a convenient, reliable and effective means of detecting introgression of zebu genes in *Bos taurus* cattle populations, like the trypanotolerant N'Dama cattle of West Africa.

Plate 2 illustrates breed differentiation with RAPD fingerprints. Pooled and individual data are presented for Mpwapwa and Maasai Zebu cattle; data also include a DNA ladder to facilitate scoring of the RAPD fingerprints.



Lane 1 represents a pool of 8 animals of Mpwapwa breed. Lanes 2–9 represent 8 individual Mpwapwa animals. Lane 10 represents a pool of 8 Maasai Zebu cattle. Lanes 11–18 represent 8 individual Maasai Zebu animals. Lane 19 shows a DNA ladder – Phi × 174.

Plates 3, 4 and 5 illustrate heterogeneity/types of Tanganyika Shorthorn Zebu (TSZ) cattle.

Plate 3. A Ugogo Zebu.

Plate 4. A Singida White.

Plate 5. A Maasai Zebu.

References

Caetano-Anolles G., Bassam B.J. and Gresshoff P.M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio/Technology* 9:553–557.

Chalya J.N., Gwakisa P.S. and Kifaro G.C. 1997. Genetic characterization of three strains of goats in Tanzania based on DNA profiles. *Proceedings of Tanzania Society for Animal Production* 24:261–274.

Chapco W., Ashton N.W., Martel R.K., Antonishyn N. and Crosby W.L. 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. *Genome* 35:569–574.

Dunnington E.A., Plotsky Y., Haberfeld A., Kirk T., Goldberg A., Lavi V., Cahaner A., Siegel P.B. and Hillel J. 1990. DNA fingerprints of chickens selected for high and low body weight for 31 generations. *Animal Genetics* 21:247–257.

Gilbert D.A., Lehman N., O'Brien S.J. and Wayne R.K. 1990. Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature* 344:764–766.

Gwakisa P.S., Kemp S.J. and Teale A.J. 1994. Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. *Animal Genetics* 25:89–94.

Gwakisa P. S., Barendse W. and Teale A. J 1997. Genetic diversity in indigenous cattle breeds of Tanzania. *Proceedings of Tanzania Society for Animal Production* 24: 242–252.

Jeffreys A.J. and Morton D.B. 1987. DNA fingerprints of dogs and cats. *Animal Genetics* 18:1–15.

Kantanen J., Vilkki J., Elo K. and Maki-Tanila A. 1995. Random amplified polymorphic DNA in cattle and sheep: Application for detecting genetic variation. *Animal Genetics* 26:315–320.

Kemp S.J. and Teale A.J. 1994. Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiates *Bos indicus* and *B. taurus*. *Animal Genetics* 25:83–88.

King I.P., Purdie K.A., Rezanoor H.N., Koebner R.M..D., Miller T.E., Reader S.M. and Nicholson P. 1993. Characterisation of *Thinopyrum bessarabicum* chromosome segments in wheat using random amplified polymorphic DNAs (RAPDs) and genomic *in situ* hybridization. *Theoretical and Applied Genetics* 86:895–900.

Lipman L.J.A., de Nijs A., Lam Theo J.G.M., Rost J.A., van Dijk L., Schukken Y.H. and Gaastra W. 1996. Genotyping by PCR, of *Staphylococcus aureus* strains isolated from mammary glands of cows. *Veterinary Microbiology* 48:51–55.

Maule J.P. 1990. The cattle of the tropics. In: Maule J.P. (ed), *The cattle of the tropics*. Red Wood Press, Melksham, Wiltshire, UK. pp. 11–22.

Michelmore R.W., Paran I. and Kesseli R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences* (USA) 88:9828–9832.

Nei M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.

Ragot M. and Hoisington D.A. 1993. Molecular markers for plant breeding: Comparisons of RFLP and RAPD genotyping costs. *Theoretical and Applied Genetics* 86:975–984.

Rothuizen J. and van Wolferen M. 1994. Randomly amplified DNA polymorphisms in dogs are reproducible and display Mendelian transmission. *Animal Genetics* 25:13–18.

Sambrook J., Fritsch E. F. and Maniatis T. 1989. Analysis and cloning of eukaryotic genomic DNA. Molecular cloning—A laboratory manual. Volume 2, pp. 9.14–9.23.

Sharp P.M. 1993. *Genetic characterization of cattle in West and Central Africa*. Annual Report 1: February 1992–February 1993.

Stephen J., Kifaro G.C., Wollny C.B.A. and Gwakisa P.S. 2000. Molecular genetic variation among five local sheep ecotypes in Tanzania. *Proceedings of Tanzania Society for Animal Production* 27:69–78.

Teale A.J., Gwakisa P., Maillard J.C. and Kemp S.J. 1993. Progress in molecular and genetic characterization of cattle populations, with emphasis on African breeds. In: Rowlands G.J. and Teale A.J. (eds), *Towards increased use of trypanotolerance: Current research and future directions. Proceedings of a workshop organised by ILRAD (International Laboratory for Research on Animal Diseases), ILRAD, Nairobi, Kenya; and ILCA*

(International Livestock Centre for Africa, Addis Ababa, Ethiopia) held at Nairobi, Kenya, 26–29 April 1993. 155 pp.

Teale A.J., Wambugu J., Gwakisa P.S., Stranzinger G., Bradley D. and Kemp S.J. 1995. A polymorphism in randomly amplified DNA that differentiates the Y-chromosomes of *Bos indicus* and *Bos taurus*. *Animal Genetics* 26:243–248.

Welsh J. and McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18:7213 –7218.

Williams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531– 6535.