

## Estimation of Genetic Divergence of Indian Zebu Cattle in Association with Molecular Genetic Approach and its Implication in Livestock Improvement: A Review

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### ABSTRACT

*Cattle are our most important livestock species because of their production and role in human culture. But the historic origin of the diverse phenotypes is not always clear. The domestication of taurine cattle initiated 10 000 years ago in the Near East from a wild aurochs (*Bos primigenius*) population followed by their dispersal through migration of agriculturalists to Europe. Population admixture analysis indicates a zebu gene flow in the Balkan and Italian Podolic cattle populations. Our analysis supports the previous report of gene flow between British and Irish primitive cattle populations and local aurochs. In addition, we show evidence of aurochs gene flow in the Iberian cattle populations indicating wide geographical distribution of the aurochs. We conclude that in addition to factors such as ancient human migrations, isolation by distance and cross-breeding, gene flow between domestic and wild-cattle populations also has shaped genomic composition of European cattle populations. Archeological data are now supplemented by analyses of modern and ancient samples of cattle with DNA markers of maternal, paternal, or autosomal inheritance. The most recent genetic data suggest that maternal lineages of taurine cattle originated in the Fertile Crescent with a possible contribution of South-European wild cattle populations, while zebu cattle originate from the Indus Valley. Subsequently, cattle accompanied human migrations, which led to the dispersal of domestic cattle of taurine, indicine or mixed origin over Asia, Africa, Europe and the New World. This has resulted in their adaptation to different environments and considerable variation in appearance and performance.*

**Key words:** Domestication, Aurochs cattle, zebu cattle, Domestic cattle

### INTRODUCTION

Genetics has the potential to provide a novel layer of information pertaining to the origins and relationships of domestic cattle. While it is important not to overstate the power of

archeological inference from genetic data, some previously widespread conjectures are inevitably contradicted with the addition of new informations.

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Conjectures regarding domesticated cattle that fall into this category include a single domestication event with the development of *Bos indicus* breeds from earlier *Bos taurus* domesticates<sup>1</sup>, the domestication of a third type of cattle in Africa having an intermediate morphology between the two taxa and the special status of the Jersey breed as a European type with some exotic influences. In reality, a wide-ranging survey of the genetic variation of modern cattle reveals that they all derive from either zebu or taurine progenitors or are hybrids of the two<sup>2</sup>. The quantitative divergence between *Bos indicus* and *Bos taurus* strongly supports a predomestic separation; that between African and European taurines also suggests genetic input from native aurochs populations on each continent<sup>2</sup>. Patterns of genetic variants assayed from paternally, maternally, and biparentally inherited genetic systems revealed that extensive hybridization of the two subspecies is part of the ancestry of Northern Indian, peripheral European, and almost all African cattle breeds<sup>3</sup>. In Africa, which is the most extensive hybrid zone, the sexual asymmetry of the process of zebu introgression into native taurine breeds is strikingly evident<sup>2</sup>.

#### **CATTLE GENETIC VARIATION**

The measurement and manipulation of genetic characteristics in cattle has a long history. Gross characteristics such as horn morphology and coat colorings have long been noted. The relatively trivial feature of coat coloration was a major preoccupation of the founders and improvers of modern breeds in the eighteenth century. The effects of centuries of artificial selection for horn size are evident in the contrasts between the giant horned Kuri and Ankole breeds of Africa and the short horned and even polled cattle of Europe<sup>2</sup>. Quantitative assessment of genetic relationships has its origins in the study of morphometrics, developed by Francis Galton in the late 19<sup>th</sup> century. Statistical analyses of various body or bone measurements are still used as indices of relationship. However, as with the use of craniometric assessment of human populations, such investigations are now

perceived as providing poor quantification of relationship. One set of distinctions that has systematic value is that which separates the two taxa of domestic cattle, *Bos indicus* and *Bos taurus*. *Bos indicus*, or zebu cattle, are distinguished from taurine cattle by the presence of a hump, large dewlap, skin anatomy, and physiological characteristics that involve adaptation to arid conditions and a particular suite of parasitic disease<sup>4</sup>. Two distinguishing features that are assayable archeologically are cranial morphology and the presence of bifid processes to the last thoracic vertebra. Animals with intermediate or hybrid ancestry can be detected by humps that are cervico-thoracic rather than thoracic, and that may be diminished in size<sup>2</sup>. Zebu cattle and their crossbreeds predominate in relatively arid regions such as the Indian subcontinent, the Near East, and most of Africa, whereas taurine cattle are native to North Africa, humid West Africa, and a vast swathe of Eurasia stretching from the Western European fringe to Japan<sup>5</sup>. More sophisticated assessment of genetically influenced characters has been a feature of the dramatic breed improvements of beef and dairy cattle for the last 30 years. The result has been the collation of data exhibiting marked genetic differences between breeds in production traits. However, the pattern of these traits between breeds is a poor indication of ancestral relationships (phylogeny) and reflects, rather, the remarkable effective efforts of scientific breeding. Two differences that are more ancient are the marked difference in beef quality between the tougher zebu beef and taurine meat, and the genetically determined ability to lactate without the proximity of a calf that is unique to European milk breeds. There is a common economic requirement with pedigree cattle for genetic tests based on blood protein and antigen polymorphisms to establish parentage. The development and application of suitable marker technology has contributed to more than 50 genetic systems in cattle that are detectable by serological or biochemical techniques. The detection of sequence differences in blood or milk proteins,

either through their immunological reactivity or their mobility in electrophoretic gels, has provided a level of information that is more fundamentally genetic than any that was previously available<sup>7</sup>. The frequencies of different alleles at a range of protein loci have been measured from a wide range of breeds, including European, African, and Asian populations. The variation in allele frequencies provides a measure of breed and population relationships that models ancestral origins more accurately than does the assessment of relationships at the morphological level<sup>8</sup>. The accuracy of genetic characterization at the phenotypic level is always compromised by environmental noise and the complexity of the underlying genetic systems<sup>9</sup>. In contrast, the hidden information revealed by biochemical genetic assessment may be sufficiently accurate to estimate the actual times of separation of progenitor populations under specific models of population genetic history. When a single ancestral population splits in two, the frequencies of the alleles represented within each will slowly change because of the processes of random genetic drift and, less often, those of selection. The extent to which two separated groups will differ is related to the time depth of the divergence event. However, a limit to the accuracy of protein-based genetic analysis exists. This limitation arises from the finite genetic variation that is accessible in blood and milk proteins as well as the complicating influence of selection.

#### **GENETIC DIVERGENCE AND THE DOMESTICATION PROCESS**

Comparisons between closely related groups may be complicated by local admixture; those involving distant and distinct groups may lead to valuable insights concerning the domestication process. A series of estimates of the genetic distance between European, Indian, and those elements of the African gene pool that can be securely identified as indigenous, have been derived using *mtDNA* and microsatellite variation<sup>10</sup>. These genetic distances in turn have been calibrated by a comparison of cattle with bison and the assumption of a minimum time depth of one

million years for the existence of their last common ancestor<sup>6</sup>. The different time depth for the divergence of *Bos indicus* and *Bos taurus* and the divergence of taurine African and European ancestors has been estimated. Estimates of divergence times such as those shown need to be treated with caution. Calibration of the molecular clock in both *mtDNA* and microsatellite evolution is notoriously difficult and is especially complicated in each case by variability in mutation rates<sup>10</sup>. However, the magnitude of estimates of the divergence between *Bos indicus* and *Bos taurus* are consistently of the order of hundreds of thousands of years BP and constitute a strong argument for the domestication, less than 10,000 years BP, of two biologically separate strains of aurochs. These data are simply not consistent with a view that all cattle developed from a single wild ancestral strain domesticated in the Near East (circa 9,000 BP), with an Eastern derivative strain later giving rise to zebu through breeding and selection. The biological distinction of the modern zebu strongly supports a separate domestic origin. Remains of the Asian variant of the wild ox, *Bos primigenius namadicus*, which are represented in early agricultural sites in Shar-i-Sokhta, Sistan, provide a putative progenitor for *Bos indicus*<sup>11</sup>. The site of Mehrgarh in Pakistan, through examination of a temporal series of faunal remains, has yielded evidence for cattle herding at least as early as 7,000 BP. This site is a strong candidate for a potential Eastern domestication site. That these cattle were of *Bos indicus* type is suggested by skull morphology and the finding of an early clay figurine with a pronounced hump. The biological divergence of African and European taurine cattle is less marked and potentially lies closer to the margins of error in these estimates. The difference between the microsatellite and mitochondria-derived figures may be an indication of inherent inaccuracy or perhaps reflects different sensitivities to phenomena such as admixture of early domesticates with aurochs<sup>12</sup>. Inferences about separate origins for the two

continental groups must be regarded as less secure than that concerning the two taxa. Nevertheless, the estimates point toward a surfeit of genetic diversity within *Bos taurus* and the possibility that all modern strains may not have originated from one primordial population<sup>11</sup>. In contrast to the Near East and, to a lesser extent, its Eastern margin, there are no descriptions of African archeological sites that reveal a temporal transition in faunal remains that is consistent with the domestication process<sup>13</sup>. The oldest African cattle remains found in an obvious domestic context are from Capeletti, Algeria. These date to 6,530 BP and occur together with ovine and caprine remains. The latter were not native to Africa and their appearance in the Sahara from 7,700 BP is indicative of livestock migration from the Near East<sup>10</sup>. However, some argument has been made that putative *Bos* remains from eastern Sahara sites in Nabta Playa and Bir Kiseiba, dating from up to 9,000 BP, may be domestic, given that the dry climate of that time was one in which cattle may have persisted only with human intervention. The fragmentary nature of the finds precludes a secure conclusion, but if they do represent domesticates, their early dating would support an independent origin from the near East<sup>2</sup>. The postulated excess of diversity within taurine cattle may therefore be attributed to biologically separate origins for African and European cattle. Indeed, some patterns in genetic variation suggest this<sup>14</sup>. However, this version of cattle origins is difficult to distinguish from alternatives such as the adoption of divergent local aurochsen by migrating early herders in either continent. It is expected that more comprehensive surveys currently underway of extant and ancient cattle samples from Europe, the Near East, and Africa will resolve this and other issues. Indeed, the first sequencing of domestic cattle and aurochsen *mtDNA* from archeological bone has been achieved, demonstrating a technology that promises much for the future<sup>15</sup>.

#### **RATIONALE FOR MOLECULAR GENETIC APPROACH USING RAPDs**

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<sup>16</sup>. 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<sup>17</sup>. 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 noncryogenetically 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 analyze 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<sup>17</sup>.

#### **PRINCIPLES 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, there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation<sup>17</sup>. 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<sup>18</sup>. 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 visualizes a relatively greater number of amplification products by polyacrylamide gel electrophoresis and silver staining<sup>19</sup>. AP-PCR uses slightly longer primers (such as universal M13) and amplification products are radioactively labelled and are also resolved by polyacrylamide gel electrophoresis<sup>20</sup>. Standard RAPD analysis is performed according to the original method<sup>21</sup> 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.

#### **GENERATION OF 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<sup>22</sup>. Constant fragments characteristic for the species may be identified, as well as fragments which are polymorphic between breeds within the species<sup>17</sup>. 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<sup>23,24</sup>. Thus, RAPD products that serve as molecular genetic markers at different levels can be generated.

#### **FUTURE CHALLENGES AND OPPORTUNITIES**

Till 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<sup>25,26,27</sup>. At Sokoine University of Agriculture, Tanzania, the technique has been extensively tested and used to differentiate local breeds of cattle in Tanzania<sup>28,29</sup>, goats strains in Tanzania<sup>30</sup>, sheep ecotypes in Tanzania<sup>31</sup> and ecotypes of the scavenging local chickens of Tanzania. However, the following remain to be the major weaknesses of the RAPD technique. 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 emphasized 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. The issue of reproducibility is of much concern<sup>34</sup>. 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. 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. 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. 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<sup>32</sup>. PCR products from RAPD analysis may be directly sequenced, or cloned and used as specific primers or probes

thereafter. At International Livestock Research Institute, a RAPD polymorphism was used in a simple dot blot assay as a probe for RAPD-PCR products<sup>33</sup>. 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.

## CONCLUSION

The breeds underwent different selection pressures due to climate, endemic parasites, illnesses, diet and criteria decided by man. Breed formation, probably, was associated with a loss of some genetic diversity in the initial stages, as well as with the concentration and eventual fixation of some specific traits<sup>35</sup>. The main evolutive source of modification between breeds was probably due to random drift<sup>36,37</sup>. However, animal breeding, carried out under unidirectional selection pressure, may involve both an increase in the frequency of favourable additive genes as well as break regulatory homeostatic mechanisms which were established during the process of natural selection of these populations<sup>38</sup>. It is equally important to ensure that the breeds selected for conservation include populations from the geographic areas representing the different domestication centers where we would expect to find large genetic diversity and genetically differentiated populations. Animal populations present at the geographic area of a center of domestication will also be expected to be very distinct from the ones found at other centers of domestication. Also, the understanding of the geographic pattern of livestock migration from a center of origin will allow the identification of populations present at the end of a migration route. It is expected that these populations will be genetically distinct from the populations present at the ends of other migration routes as a result of random genetic drift and the effect of local selection pressures. Importantly, knowledge of both the global diversity of the breeds and admixture events will be needed in order to be able to make sound priority decisions. Such a comprehensive approach would ensure

complete conservation of diversity. Both within and between breed diversity parameters are classically measured using molecular genetic markers. In both cases soundly-based priority decisions for conservation at the global level will require the availability of large datasets. The mean number of alleles (MNA), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity are the most commonly calculated population genetic parameters for assessing within breed diversity. For example, in a recent study, three distinct sets of microsatellite diversity cattle data were merged to provide for the first time within breed diversity ( $H_e$  and MNA) and admixture information combined for Europe, Africa, the Near East and South Asia. The geographic region with the highest diversity is found between the two likely Asian centers of cattle domestication in a broad geographic area corresponding to what are today Iran, Iraq and the Caucasian region. Global geographic analysis of admixture suggests that the region corresponds to a geographic area of around 50% admixture between taurine and indicine cattle. Genetic diversity and admixture information from more indigenous breeds are needed to confirm the results. If it is confirmed, this geographic area will undoubtedly represent a major livestock diversity hotspot, a priority region for a global plan for the conservation of the diversity of domestic cattle. The genetic diversity is fundamental for sustainable genetic improvement, facilitating the rapid adaptation to necessary and unpredicted change to the development of production systems, as it is not possible to objectively predict which traits may be necessary in the future<sup>39</sup>. The genetic improvement of animals is a continuous and complex process. Ever since the domestication of animals by man, he has always remained busy in improving his animals. In this pursuit many methods have been developed and tested. In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of marker techniques with variety of applications. Selection of markers for different applications

are influenced by several factors, viz. the degree of polymorphism skill or expertise available, possibility of automation, radioisotopes used, reproducibility of the technique, and finally the cost involved. Presently, the pace of development of molecular markers is tremendous, and the trend suggests that explosion in marker development will continue in the near future.

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