MOLECULAR EVOLUTION OF AFRICAN, EUROPEAN AND ASIAN CATTLE.

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SUMMARY

The most common assumption about the history of cattle is that all modern breeds are the result of domestication events which took place in the middle East approximately 10,000 years BP. However, data from molecular genetic studies of thirteen African, European and Asian breeds do not support this view. The divergence between mitochondrial sequences of Asian and European/African origin imply a much more ancient separation between *Bos indicus* and *Bos taurus* with estimated lineage divergence times of 200,000-1,000,000 years BP. This suggests at least two independent domestication centres and is supported by microsatellite frequency data and also Y chromosome polymorphism. In addition the different tiers of genetic analysis, when considered together, illustrate the hybrid origins of African zebu breeds and the male-driven nature of the crossbreeding process.

INTRODUCTION

In this study, a combination of RFLP analysis and direct sequence comparisons in mitochondrial DNA (mtDNA) was employed to differentiate cattle breeds from three continents. This form of analysis provides high resolution information with good efficiency and data readily amenably to phylogenetic analysis. Consequently, robust and accurate genealogies can be reconstructed from which existing theories on bovine evolution may be evaluated. In addition, the variation in nuclear DNA present between and within breeds has been examined using highly polymorphic microsatellite markers. Some comparison between the population dynamics of maternal (mtDNA) versus paternal (Y chromosome) inheritance in the history of cattle breeds is also afforded by typing of a Y-specific RFLP.

MATERIALS AND METHODS

The breeds selected were Aberdeen Angus, Charolais, Friesian, Hereford, Jersey and Simmental from Europe, Hariana, Sahiwal and Tharparkar from Asia, Butana and Kenana from East Africa and, N'Dama and White Fulani from West Africa. Sampling of cattle and the subsequent isolation of DNA was carried out in four different locations. Whenever possible, pedigrees were consulted to ensure that the animals were unrelated.
The total numbers of animals sampled from each breed and the centres used were as follows:

Europe - University of Dublin			
Aberdeen Angus	33	Jersey	34
Charolais	36	Friesian	40
Hereford	34	Simmental	36
India - National Institute of Animal Genetics			
Tharparkar	10	Sahiwal	11
Hariana	10		
Africa - University of Ibadan, Nigeria; - National Dairy Research Institute, Sudan.			
N'Dama	19	White Fulani	24
Kenana	38	Butana	24

Three areas of genetic variation were investigated: that of the maternally inherited and swiftly evolving mitochondrial chromosome (mtDNA), that of highly polymorphic autosomal microsatellite markers, and that detected by a chromosome Y specific probe. mtDNA, because of

its clonal inheritance and high variation has been the subject of many productive intraspecific genetic studies (Avise et al. 1987). The methods employed here included restriction fragment length polymorphism studies and sequencing of the most variable region, the D loop. For the former, whole mtDNA molecules were isolated, restricted with seven different endonucleases and visualised after polyacrylamide gel electrophoresis by either silver staining or radioactive probing with whole mtDNA. D loop sequencing was achieved by polymerase chain reaction amplification of a 1.6 kb fragment containing the control region, using oligonucleotide primers from published sequence and whole DNA isolate as template.

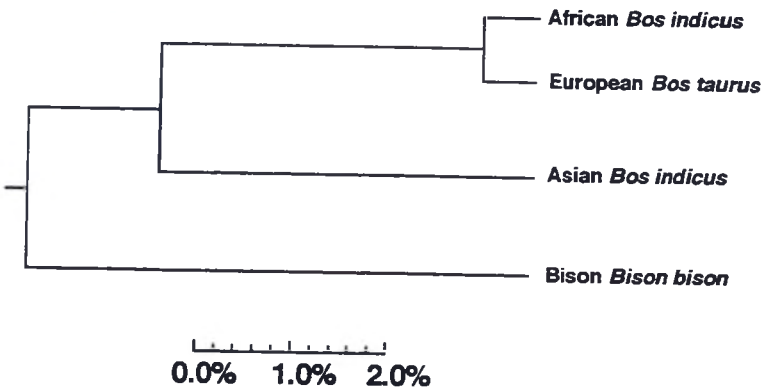
Nuclear DNA polymorphism described here used eight microsatellite markers: BOLADRBP1, BOLADRBP2, BOVGAP, BOVIRBP, BOVOCAM, BOVPRL, ETH152, ETH225, ILSTS001 (MacHugh et al., 1994). These loci show high variability in the length of short, PCR-amplified units of DNA. Polymorphism is due to the presence of tandem repeats of very short motifs which are known to readily vary in copy number (Weber and May, 1989). Y chromosomal inheritance was examined using Southern blotting and a DNA probe, btDYZ-1 described by Perret et al. (1990).

RESULTS

Mitochondrial chromosomes from the thirteen cattle breeds representing three continental varieties were assayed for genetic variation. Seven restriction endonucleases, each of which recognise six base pair sequences, were used in a RFLP study. These enzymes detected 20 polymorphisms which described 16 mitochondrial lineages. Two major mitochondrial clades were detected, one representing European and African or *Bos taurus* mitochondria, the other representing Asian zebu or *Bos indicus* populations. The lineages were found to differ at approximately 1.6% of sites surveyed.

Sequence analysis of the complete D-loop region (the most variable part of the mitochondrial genome) also supported the results of the RFLP study, *i.e.*, the presence of two major mtDNA lineages. Two animals, from each of the 13 breeds, revealed 24 different mtDNA haplotypes defined by 63 variable positions (Lotus et al., 1994). Phylogenetic trees relating the 24 lineages were constructed using both distance matrix (neighbor-joining) and maximum parsimony methods. The major lineages were found to differ at approximately 5% of sites sequenced. There was no other significant grouping of animals, except perhaps that the European cattle cluster together within the African radiation. The 380bp segment was also sequenced from two American bison which were then used to root the tree; a simplified version of which is shown in Figure 1.

FIGURE 1



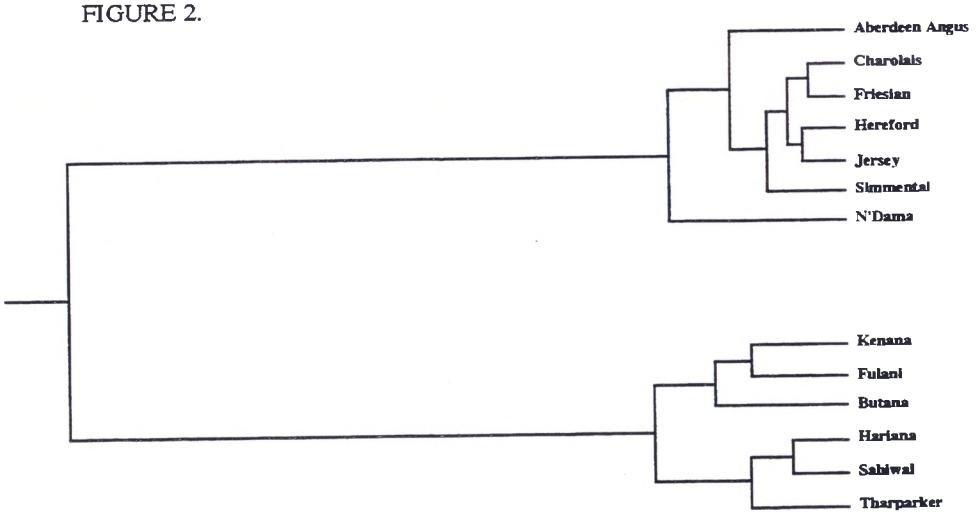
Genetic diversity levels were quite low compared with those detected in natural populations and could reflect the unusual population dynamics of domestic cattle populations. The extent of divergence between these two major lineages suggests (from a molecular clock estimation) that they diverged between 200,000 and 1 million years ago. This is clearly not consistent with the theory that *B.indicus* represent artificially selected *B.taurus*, but instead indicates that *B.indicus* and *B.taurus* were domesticated separately. Also, the presence in Africa, without exception, of only variants from the taurine lineage implies a crossbred history for the zebu of that continent. The East African animals have previously been considered as typical *indicus*.

Fifteen simple sequence microsatellite loci were assayed by PCR for length variation and then for polymorphism between panels of zebu and taurine animals. Eight of these were fully tested in DNA from 349 animals representing the 13 breeds of cattle involved in the study. Breed-specific allele frequencies were calculated for each locus and were lowest for all eight loci in the European groups, higher in the Indian groups and highest in the Africans.

Genetic distances were deduced and a dendogram constructed using the UPGMA method (figure 2). The main feature is also the gulf between *Bos taurus* and *Bos indicus*. However, unlike within the mtDNA phylogeny, the African zebu cattle group with the Asians whereas the taurine N'Dama form the first branch off the European clade. The other, shallower branching orders should not be regarded as robust given the limited number of loci utlised here.

The crossbred nature of the African cattle included, implied by the mtDNA results is clearly established by microsatellite data. Four of the loci tested possessed alleles which were predominant in the Asian breeds but absent in the Europeans. African zebu possessed, in a decreasing cline from the most zebu in the East, a mixture of *taurus* and *indicus* alleles. The Nigerian N'Dama population also, although predominantly taurine, showed some evidence of zebu crossbreeding.

FIGURE 2.



The Y-specific probe yielded distinct zebu and taurine types in 65 European and African cattle tested. There was a marked tendency for the *indicus* variant to introgress substantially into morphologically *taurus* populations where some crossbreeding had occurred. Of 30 Nigerian/

Gambian N'Dama tested 29 had a zebu Y type (Bradley et al., 1994).

DISCUSSION

The most widely accepted account of the domestication of the wild ox, the aurochs, places the primary events in the emerging civilisations of the middle East approximately 10,000 years BP (Epstein 1971). *Bos indicus* is given as having developed as a later variant from herded *Bos taurus* on the Eastern fringe of this area. However, such a timescale of divergence does not concur with the 200,000-1,000,000 years calculated from the mtDNA data described here. This represents the first strong molecular evidence for the separate domestication of the two cattle sub-species and accords with archaeologists who argue for the emergence of zebu from the wild Asian variant of aurochs.

A marked difference between the *taurus* and *indicus* populations is also a feature of the nuclear microsatellite and Y chromosome analyses. With each, distinct variants were found which were typical of each major cattle type. Four microsatellite markers gave alleles which were predominant in the Indian animals but totally absent in the European breeds. However, the typical, i.e. most frequent alleles from the former continent were present at low levels in the Asian populations. This imbalance may reflect an historical, minor gene flow from early taurine domesticants to the areas of zebu origin, a hypothesis which is strengthened by the presence of humpless cattle in the Far East e.g. Japan, Korea and China.

The actuality and shape of more recent gene flow has emerged from the African samples. Nuclear markers show a cline of zebu influence from East to West, with the predominantly *indicus* Kenana and Butana in the East, to the less pure White Fulani zebu of the West and the predominantly taurine N'Dama. That all African zebu examined were the products of historical crossbreeding with the indigenous humpless populations was evidenced by the exclusive presence of mtDNA of the taurine type. These have been introduced to the continent at several points in history but primarily during the Arab migrations post 669 AD. mtDNA lineages are especially susceptible to population bottlenecks, which may have been a significant feature of African history, and imported indicus mitotypes may have been thus lost. Also, zebu gene flow may have been primarily through male transmission. This is supported by the marked introgression of indicus Y-specific variant into morphologically taurine populations.

Interbreed genetic distances are not discernible from mtDNA data but the microsatellite data proved suitable for the construction of a breed relationship matrix and may have utility for addressing problems of genetic conservation. The levels of zebu/taurine contamination, the uniqueness of a given population, or its suitability for supplementing a closely related breed, all may be assessed.

Finally, the diversity of results from this study clearly illustrate the need to investigate intraspecific relationships through multiple lines of genetic analysis.

Avise et al., 1987. *Ann. Rev. Ecol. Syst.* 18:489-522.
Bradley et al. 1994. *Animal Genetics* 25:7-12.
Epstein, 1971. *The origin of the domestic animals of Africa. Africana Publishing Corporation. New York.*
Loftus et al., 1994. *Proc. Natl. Acad. Sci., U.S.A.* In press.
MacHugh et al. 1994. *Proc. Roy. Soc. Lond. Ser. B.* In press.
Perret et al., 1990. *Genomics* 6:482-490.
Weber and May, 1989., *Am. J. Hum. Genet.* 44:388-394.