summary
SUMMARY

The preliminary attempt to analyse the bubaline genome for allelic variation at micromolecular level using synthetic oligonucleotides has yielded informative data. With the present study, many of the earlier assumptions on evolutionary conservation of some repeat sequences in the vertebrate genome and the genetic similarity of the bubaline to bovine species have been proved to be incorrect. A probe based on trinucleotide repeat was found to be the most informative one for DNA fingerprinting of this species and a shorter version of this probe was useful for establishing breed affiliation. The offshoots of this study are as follows:

I) A detailed multilocus restriction fragment length polymorphism study on bubaline genome with a set of eight synthetic DNA probes and eighteen different enzymes gave rise to an informative probe/enzyme combination for conducting DNA fingerprinting. Dot blot hybridisations of buffalo genomic DNA revealed that, of the six evolutionarily conserved repeats used in the study, viz. GATA, GACA, GGAT, TTAGGG, TGG and (CA)n, GATA repeats are totally absent in the bubaline species.

II) A less polymorphic (GGAT) probe could detect a strong mutant band at high molecular weight when the DNA was digested with Mbol enzyme. This contradicts the earlier report that mutations are more common at hypervariable loci and that the novel bands are noticed more commonly at low molecular weight regions.

III) An eighteen base long probe OAT18, based on trinucleotide repeat (TGG) in combination with Hinfl enzyme can be used as an informative and reliable genetic marker for genome individualisation of this species.
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IV) Since the OAT18/Hinfl probe/enzyme combination has a very high paternity index \( (PI = 1.57 \times 10^3) \) in a heterogeneous population, it may be used in disputed parentage cases.

V) The analysis of pedigreed animals from dairy farms revealed that there should be regular "bull rotation" between farms to avoid the chances of inbreeding which might otherwise lead to deterioration of superior germplasm.

VI) The repeat locus (TGG) is somatically stable with a negligible rate of germline mutations (0.029 per gamete) and hence may be used as fingerprinting probe in combination with Hinfl enzyme.

VII) A comparatively less polymorphic OAT15/MboI probe/enzyme combination was found to be useful for breed affiliation studies in the bubaline genome.

VIII) Studies on genetic similarity of different breeds using TGG and GGAT repeats revealed that the semi-wild Toda is a greatly diversified breed as compared to other domestic breeds (Murrah, Surti and Mehsana). This native breed of South India may be treated as a separate gene pool for conservation without intermixing the population with other domestic breeds of the buffalo.

IX) The relative time of divergence between each breed revealed that the Toda diverged from Surti and Murrah breeds at a very early stage.

X) Genetic similarity between different species at GGAT repeat loci in Artiodactyles
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showed that both bubaline and bovine species shared a common ancestor for quite some time after the separation of caprine and ovine species as a single taxonomic unit.

XI) Genetic distances measured at three different repeat loci viz. CA, TGG and GGAT in bubaline, bovine, caprine, ovine, equine, swine and lagomorph species revealed the close proximity of buffalo to sheep genome and not to that of cattle.

XII) The SAHN clustering of genetic distance values placed equines as a genetically divergent out-group as compared to other members of the orders Artiodactyla and Lagomorpha.

The potential use of synthetic oligonucleotide probes as markers useful for breeding programs of buffalo has been discussed. The genetic analysis and the relative degree of genetic distance of this species will not be complete and precise until a database on a large number of individual animals from each breed population and species is developed, using greater number of independent genetic markers. The present report seems to be the first one on buffalo DNA fingerprinting and during the course of investigation it became clear that not all the repeat loci detect hypervariable loci though they may be expected to be hypervariable in a genome. Similarly, it also became clear that a repeat probe highly polymorphic in one species may be isomorphic in other species. This monomorphism or polymorphism is directly related to the organisational status of the repeats in the genome.

Finally, whether microsatellites are a good measure of evolutionary change between species is still debatable since the conservation of these repeats, their genome
organisation and mutation rate are highly variable across the taxa. Nevertheless, this study provides information about the overall genomic organisations of some of the repeat motifs in the bubaline species and their possible similarities or dissimilarities with other related species.