

Phylogenetic studies on interbreedable bovine species including yak and mithun

T K BISWAS¹, K P RAMESHA², JAYAKUMAR S³, M K GHOSH⁴ and S BANDYOPADHYAY⁵

National Research Centre on Yak, ICAR, Dirang, Arunachal Pradesh 790101 India

Received: 23 April 2009; Accepted: 20 September 2009

Key words: Bovine, Buffalo, Cattle, Mithun, Phylogeny, RAPD, Yak

Reproductive barrier is not well established among the bovine species yak, mithun, buffalo, zebu cattle, exotic cattle barring buffalo. Both the male and female offsprings are fertile only in zebu × exotic cattle cross while in other crosses male offsprings are always sterile. These species share some striking behavioural, morphological and karyotypic similarities as well as differences (Iannuzzi 2008).

The reconstruction of bovine phylogeny based on mitochondrion genome or on a single/ few nuclear gene sequences contains anomalies and is inconsistent (Chikuni *et al.* 1995, Janecek *et al.* 1996, Schreiber *et al.* 1999, Buntjer *et al.* 2002). On the other hand RAPD is a quick and effective method for producing species specific fingerprints (Stepniak *et al.* 2002, Sharma *et al.* 2004) as it represents the whole genome. RAPD based markers could be useful for parentage detection, calculating inbreeding coefficient and detection of meat adulteration in these closely related species.

Genomic DNA was isolated from blood samples (Sambrook *et al.* 1989) of a population of Indian yak from Arunachal Pradesh, mithun, zebu cattle (nondescript hilly cattle), exotic cattle (Jersey and Holstein Friesian) and buffalo (Murrah). Individual DNA samples from 150 yak as well as species-wise pooled samples from (6 male individual under each species) the 5 species were amplified by a set of 10 arbitrary primers were used. Reactions were carried out in 25 µl volumes containing 40–80 ng of genomic DNA, 30 ng of arbitrary primer, 2.5mM MgCl₂, 1 unit of Taq DNA polymerase, 0.2mM of each dNTPs and 2µl of 5% BSA under the cyclic conditions: at 94°C for 4 min followed by 40 cycles of 94°C for 60s, 40°C for 60s, 72°C for 90s and final extension at 72°C for 10 min. RAPD profiles were visualized, documented and edited using Syngene's Gel Documentation system after visualized on 1.5% agarose gel. These profiles were scored as matrices of zeros and ones corresponding to the presence and absences of a particular band (locus). Band sharing values (Jaccard similarity coefficients) were calculated for each primer separately. The average coefficients of all primers were used for multi-primer,

multi-species comparison. This was done by calculating mean average percentage.

Difference or MAPD (Gwakisa *et al.* 1994). A Neighbor Joining tree (Saitou and Nei 1987) of unrooted dendrogram showing phylogenetic relationship of the bovine species was constructed following cluster analysis.

Intra-and inter-species polymorphism: All the yaks genotyped were found monomorphic with respect to RAPD profiles. Although high degree of heterogeneity in morphology, production and reproduction traits were noticed in the studied population, extreme homogeneity at so many loci is due mainly to inbreeding rather than its recent origin. The traditional breeding followed at farmer's herds, small population size in isolated pockets and absence of gene flow between pockets, from bordering country China and from wild yaks. In Chinese yaks too the within breed genetic diversity is low (Yuping *et al.* 2004). On the other hand a considerable amount of polymorphism was recorded at species level. A total of 422 bands were amplified by all the ten primers in all the species out of which 117 bands were considered polymorphic (27.7%). Among the polymorphic bands some bands were species specific able to distinguish the species. About 8.5% of all the polymorphic bands were unique to yak, 7.69% to mithun, 5.12% to zebu cattle, 4.27 % to taurine cattle and 17.94 % to buffalo. Bhusan *et al.* (2008) also reported wide interspecies variation using RAPD technique. It could be inferred that the said primers are able to produce markers powerful enough for distinguishing different bovine species.

Phylogeny: Cluster analysis revealed that yak and mithun belong to same cluster, both the cattle species in a separate cluster and the buffalo found as an out group (Fig. 1). Based on molecular genetic data, earlier workers found buffalo in a distance cluster much diversified from the other bovine species (Janecek *et al.* 1996, Buntjer *et al.* 2002). Buffalo (chromosome number 50) differs karyotypically the most from the other species under study. Except buffalo other species are interbreedable with a varying degree of reproductive success. No authentic report on cattle and water buffalo hybrids is available but hybrid embryos up to

Present address: ^{1, 3, 5}Scientist, ^{2, 4}Senior Scientist.

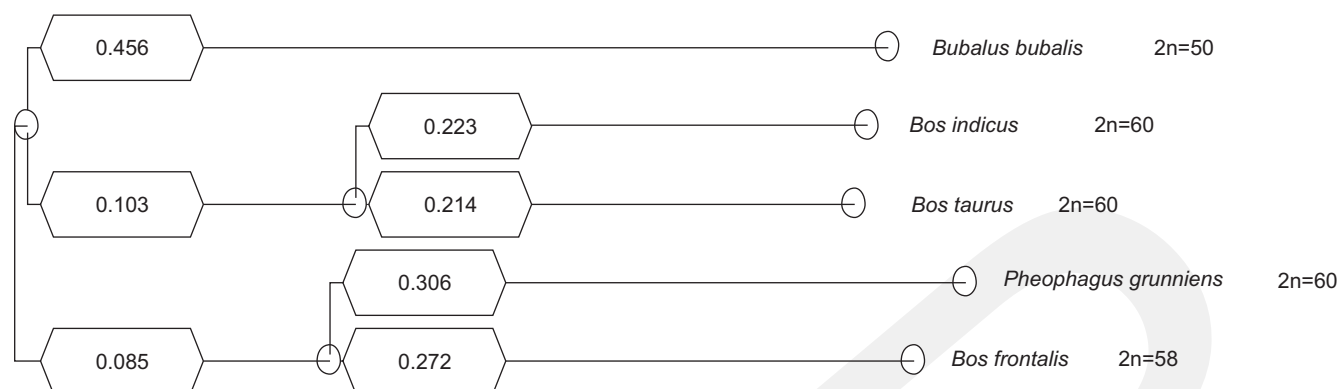


Fig 1. Dendrogram showing phylogenetic relations among the bovine species. Values on the paths indicate patristic distances to the individual species.

Table 1. Similarity coefficients and genetic distances (MAPD) among the bovine species

	<i>P. grunniens</i>	<i>B. frontalis</i>	<i>B. bubalis</i>	<i>B. indicus</i>	<i>B. taurus</i>
<i>P. grunniens</i>	1.00	0.58	0.84	0.71	0.73
<i>B. frontalis</i>	0.42	1.00	0.84	0.66	0.69
<i>B. bubalis</i>	0.16	0.17	1.00	0.81	0.76
<i>B. indicus</i>	0.29	0.34	0.19	1.00	0.44
<i>B. taurus</i>	0.27	0.31	0.24	0.56	1.00

Diagonal and below diagonal are similarity coefficients and above diagonal are coefficient of genetic distance (MAPD).

preimplantation stage can be produced *in vitro* albeit with developmental retardation (Patil and Totey 2003).

The genetic similarity and distance (MAPD) is presented in Table 1. Yak shares highest genetic similarity with mithun (42%) with respect to RAPD profiles followed by indicine cattle (29%), exotic cattle (27%), and buffalo (16%). All the species under study are said to possess the same fundamental number of chromosomal arms (=62). The genetic similarity between *indicus* (2n=60) and *taurus* (2n=60) cattle was higher than that between yak (2n=60) and mithun (2n=58). The former two species are without any reproductive barrier while the later 2 species are incompletely or semi interbreedable as the male offspring are always sterile. In comparison with yak, mithun was slightly closer to both the cattle species although mithun have 58 chromosomes while the other 3 have 60 chromosomes (Table 1). However, Buntjer *et al.* (2002) based on AFLP data reported that the gaur (which the mithun is descended from) in comparison with yak, was closer to the cattle species. In contrary to our findings, using RAPD Bhusan *et al.* (2008) reported that mithun is closer to buffalo than cattle although cattle and mithun shares a higher karyotypic and reproductive resemblance than mithun and buffalo.

SUMMARY

The RAPD analysis was applied to identify genetic markers able to distinguish yak, mithun, buffalo, zebu and

exotic cattle some of which are fully or partially interbreedable and having karyotypic similarities. Yak was monomorphic while there was considerable amount of between species molecular variability. A number of polymorphic loci (27.7%) were reported, some of which were species specific. With respect to RAPD profiles, yak shares highest genetic similarity with mithun (42%) followed by indicine cattle (29%), exotic cattle (27%), and buffalo (16%). Cluster analysis revealed that yak and mithun belong to same cluster, both the cattle species in a separate cluster and the buffalo found as an out group. RAPD based markers could be useful for parentage detection of species hybrids and possibly for detection of meat adulteration in these species.

REFERENCES

- Bhusan S, Sharma D and C Rajkhwa. 2008. Measurement of genetic relation of mithun (*Bos frontalis*) with other livestock species through RAPD markers. *Indian Journal of Animal Science* **78** (10): 1156–58.
- Buntjer J B, Otsen M, Nijman I J, Kuiper M T R and Lenstra J A. 2002. Phylogeny of bovine species based on AFLP fingerprinting. *Heredity* **88**: 46–51.
- Chikuni K, Mori Y, Tabata M, Monma M and Kosugiyama M. 1995. Molecular phylogeny based on κ -casein and cytochrome β -sequences in the mammalian suborder Ruminantia. *Journal of Molecular Evolution* **41**: 859–66.
- Iannuzzi L. 2008. *Eighteenth international colloquium of animal cytogenetics and gene mapping*. Endorsed by the International

- Chromosome and Genome society. June 08–10, 2008, Bucharest, Romania.
- Janecek L L, Honeycutt R L, Adkins R M and Davis S K. 1996. Mitochondrial gene sequences and the molecular systematics of the artiodactyl subfamily Bovinae. *Molecular Phylogenetics Evolution* **6**: 107–19.
- Patil S and Totey S. 2003. Developmental failure of hybrid embryos generated by *in vitro* fertilization of water buffalo (*Bubalus bubalis*) oocyte with bovine spermatozoa. *Molecular Reproduction and Development* **64** (3): 360–68.
- Saitou N and Nei M 1987. The neighbour joining method: a new method for reconstructing phylogenetic trees. *Molecular Biological Evolution* **4**: 406–25.
- Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning. A Laboratory Manual*. 2nd edn. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, USA.
- Schreiber A, Seibold I, Notzold G and Wink M. 1999. Cytochrome b gene haplotype characterize chromosomal lineage of anoa, the Sulawesi dwarf buffalo (Bovidae: *Babulus* sp.). *Journal of Heredity* **90**: 165–76.
- Sharma A K, Bhusan B, Kumar S, Kumar P, Sharma A and Kumar S. 2004. Molecular characterization of Rathi and Tharparkar indigenous cattle (*Bos indicus*) breeds by RAPD-PCR. *Asian-Australian Journal of Animal Science* **17**(9): 1204–9.
- Stepniak E, Zagalska M M and Switonski M. 2002. Use of RAPD technique in evolution studies of four species in the family Canidae. *Journal of Applied Genetics* **43**(4): 489–99.
- Yuping X, Shuang J, Kangyun D, Xiao Z, Zhihua C and Jincheng Z. 2004. Study on genetic variation in yak breeds (populations) by RAPD. *Proceedings of the Fourth International Congress on Yak*. pp. 149–53. Chengdu, P.R. China, 26–29 September 2004.