A bubaline-derived satellite DNA probe uncovers generic affinities of gaur with other bovids

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DNA typing using genome derived cloned probes may be conducted for ascertaining genetic affinities of closely related species. We analysed gaur *Bos gaurus*, cattle *Bos indicus*, buffalo *Bubalus bubalis*, sheep *Ovis aries* and goat *Capra hircus* DNA using buffalo derived cloned probe pDS5 carrying an array of *Bam*HI satellite fraction of 1378 base residues to uncover its genomic organization. Zoo-blot analysis showed that pDS5 does not cross hybridize with non-bovid animals and surprisingly with female gaur genomic DNA. The presence of pDS5 sequences in the gaur males suggests their possible location on the Y chromosome. Genotyping of pDS5 with *Bam*HI enzyme detected mostly monomorphic bands in the bubaline samples and polymorphic ones in cattle and gaur giving rise to clad specific pattern. Similar typing with *Rsa*I enzyme also revealed clad specific band pattern detecting more number of bands in buffalo and fewer in sheep, goat and gaur samples. Copy number variation was found to be prominent in cattle and gaur with *Rsa*I typing. Our data based on matched band profiles (MBP) suggest that gaur is genetically closer to cattle than buffalo contradicting the age-old notion held by some that gaur is a wild buffalo. The pDS5 clone has a potential for estimating the generic and genetic relationship amongst closely related bovid species.

1. Introduction

Despite several studies based either on morphology or molecular biology, the accurate clad positioning and phylogenetic relationship amongst the bovids is still incomplete (Tanaka *et al* 1995). In this context, DNA analysis is of great relevance for estimating the genetic and generic affinities between the desired species (Tanaka *et al* 1996; Georges *et al* 1990; Mattapallil and Ali 1998). Of the several bovids, gaur *Bos gaurus* is a highly endangered species. This has a peculiar physical appearance with anterior like a buffalo whereas the posterior part of the body resembling the cattle. In the Indian-subcontinent, this species is found in central (Madhya Pradesh) and north eastern regions of India and Himalayan terrains. In terms of systematics, gaur belongs to genus *Bos*, but in the routine parlance, this is referred to as wild buffalo. This creates confusion about its origin and genetic relatedness with cattle and buffalo. Till date, only limited cytogenetic studies have been conducted on this species (Winter *et al* 1984; Riggs *et al* 1997). In an attempt to uncover its generic affinities, we undertook analysis of DNA from gaur *B. gaurus*, cattle *Bos indicus*, buffalo *Bubalus bubalis*, goat *Capra hircus* and sheep *Ovis aries* using a bubaline derived cloned probe pDS5. This evolutionarily non-conserved probe represents a single array of *Bam*HI satellite fraction of 1378 base pairs. The importance of its differential organization in establishing genetic relationship amongst bovid's group of animals is discussed.

2. Materials and methods

2.1 *Genome derived cloned probe pDS5*

The origin and details of the development of bubaline derived cloned probe pDS5 have been reported elsewhere

Keywords. Comparative genome analysis; genotyping; biodiversity; endangered species; DNA probe; generic affinities

(Ali *et al* 1998). The sequences of the pDS5 have been deposited in the Gene Bank (Accession No. Y-07658).

2.2 *Isolation of DNA*

Approximately, 5 ml of gaur blood was drawn into a vaccutainer containing 200 µl of 0·5 M EDTA as an anticoagulant from two males and one female after darting them with a tranquilizer shot containing xylazine-ketamine mixture (1·2–1·4 ml per 1200–1400 kg body weight). The animals were then given 1–1·5 ml of Revivon antidote and left for recovery until they returned to their secure habitat. Blood samples of three known breeds of buffalo such as Surti, Murrah and Toda and of semi-domesticated animals from Assam, cattle, goat, sheep and human were used for DNA isolation following standard protocols (John and Ali 1997).

2.3 *Zoo-blot hybridization*

DNA samples from Toda and Murrah breeds of buffalo and the same from Assam, cattle and human in duplicate having two different concentrations were used for zoo-blot hybridization. Approximately, 300 and 150 ng of total genomic DNA from both the sexes in a 15 µl volume were electrophoresed briefly on a 1·5% agarose gel using 0·5 × TBE buffer for 20 min at 50 volts. As a positive control, approximately, 300 picogram of released insert from pDS5 was also included in the gel. The DNA samples trapped in the gel matrix were stained with ethidium bromide and photographed under UV. DNA transfer onto the membrane (Southern 1975), blot hybridization with labelled pDS5 and autoradiography (John and Ali 1997) were done following standard protocols.

2.4 *Southern blot analysis*

For Southern blot analysis, approximately 1–2 µg of total genomic DNA from both the sexes of different bovids were digested with *Bam*HI and *Rsa*I enzymes following supplier's specifications (Bangalore Genei, India). The samples were resolved on a 25 cm long 1·5% agarose gel in 0·5 × TBE buffer for 18 h at 50 constant volts. As a molecular size marker, $\phi \times 174$ and λ*Hin*dIII DNA were included in the gel. Southern hybridization and autoradiographies were done following established procedure (John and Ali 1997).

3. Results

3.1 *Zoo-blot hybridization of different genomic DNA with pDS5*

The pDS5 probe showed cross hybridization with cattle, buffalo and gaur male but not with human or gaur female genomic DNA (figure 1). Subsequent hybridization confirmed its presence in goat and sheep genome also although the signal intensity was found to be much reduced (data not shown). As expected, two different concentrations of the genomic DNA showed correspondingly varying signal intensities. However, maximum signal was seen in buffalo samples whereas the least was detected in gaur. The signal intensity obtained in the buffalo samples using about 300 ng of genomic DNA is comparable with that of control plasmid (figure 1C) where approximately 0·3 ng of released insert from pDS5 was used. This quantity of about 1·4 kb cloned DNA corresponds to approximately 2000 copies of the pDS5 in the bubaline genome assuming that the haploid genome of the same has about 3×10^9 base pairs. The signal intensity in the cattle and gaur (figure 1, slots 1–2 and 7–8) showed relatively fewer copies of pDS5 compared to buffalo. *Bam*HI digested buffalo DNA probed with pDS5 showed a strong 1·4 kb band as expected, in addition to several faint ones mostly in the high molecular weight regions. However, in gaur, only male samples showed hybridization, giving rise to smeary signal whereas the female was found to be negative (figure 2) corroborating the zoo-blot hybridization result. Two to three folds longer exposure of the autoradiogram did not help in detecting the signal in the female slot (data not shown).

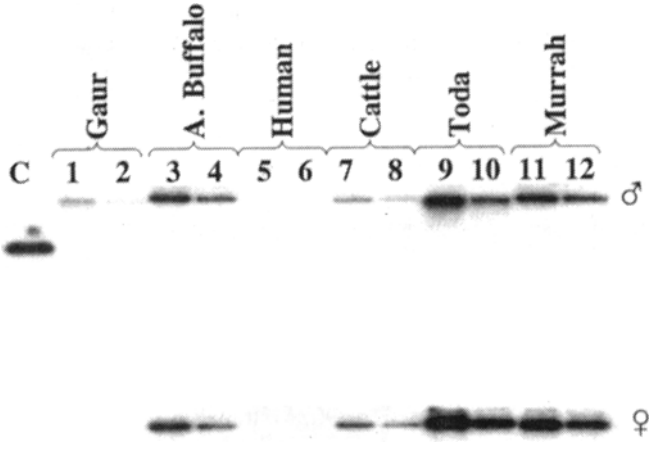

Figure 1. Zoo-blot analysis of genomic DNA from both the sexes of gaur, buffalo cattle and human with pDS5 clone. Slots 1, 3, 5, 7, 9 and 11 represent 300 ng of genomic DNA whereas the remaining ones contain 150 ng DNA. Slot C containing 0·3 ng represents 1·3 kb cloned fragment of pDS5. 'A. Buffalo' denotes samples collected from Assam. Hybridization signal detected in the slot C for which released insert of about 0·3 ng was used as positive control represents approximately 2×10^8 copies which is comparable with the signals seen in the slot containing 300 ng of buffalo genomic DNA. If a haploid genome of buffalo is taken to contain 2000 copies of pDS5 arrays, a total of 300 ng of the same will also have 2×10^8 copies. Note the absence of signals in human and gaur female DNA and strong signals in buffalo samples.

3.2 *The pDS5 uncovers monomorphic hybridization pattern in buffalo*

We used *Bam*HI, *Eco*RI and *Rsa*I enzymes for digestion of buffalo genomic DNA and for typing with pDS5. As mentioned earlier, *Bam*HI typing revealed a number of bands in addition to a strong 1·4 kb band whereas *Eco*RI showed several monomorphic bands of which a few were very prominent (data not shown). However, buffalo samples digested with *Rsa*I and probed with pDS5 showed more than 25 monomorphic bands (figure 3) with a prominent band of about 1·4 kb. Comparative *Rsa*I genotyping of bovids with pDS5 showed the presence of the 1·4 kb band in cattle and gaur (male) samples in addition to buffalo with varying signal intensity (figure 4). However, with respect to buffalo samples, the signal intensity of 1·4 kb band was comparable though not identical (figure 4, buffalo panel). Several other common and uncommon bands both in low as well as high molecular weight regions were also detected in these species. The pDS5 probe also detected a low level of polymorphism in cattle and gaur. Copy number variation of pDS5 was most prominent between the two samples of gaur male and cattle with respect to 1·4 and 1·1 kb bands (figure 4, gaur and cattle panel). In contrast to *Bam*HI, *Rsa*I typing with DNA samples from different breeds of buffalo showed multilocus monomorphic bands with discernible copy number variation. However, the number of bands so detected in the gaur samples were fewer and some additional bands not shared by buffalo or cattle were also detected in gaur males. Variation in the size and copy number of such satellite DNAs between different species are known and they reflect important evolutionary significance. Compared to buffalo in which about 29 monomorphic bands were seen, only 5 discernible bands were detected in gaur male.

3.3 *pDS5 uncovers genetic and generic affinities amongst bovids*

DNA typing of buffalo, cattle and gaur samples with *Rsa*I revealed more shared bands between cattle and gaur than gaur and buffalo (figure 4). Similar *Rsa*I typing with goat and sheep including DNA from cattle and buffalo showed the clear absence of 1·4 kb band in sheep and goat (figure 5). However, a strong 900 base pairs and a faint 1·6 kb band were detected in sheep. Interestingly, buffalo shared some monomorphic bands with sheep.

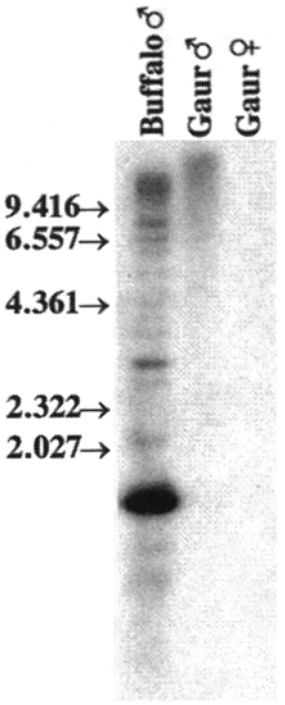

Figure 2. Genotyping of buffalo and gaur DNA with *Bam*HI enzyme and pDS5 probe. Note the 1·4 kb strong and several weak bands in the high molecular weight regions in buffalo and faint smeary signal in gaur male and the absence of signal in the female. Molecular size marker is given in kb.

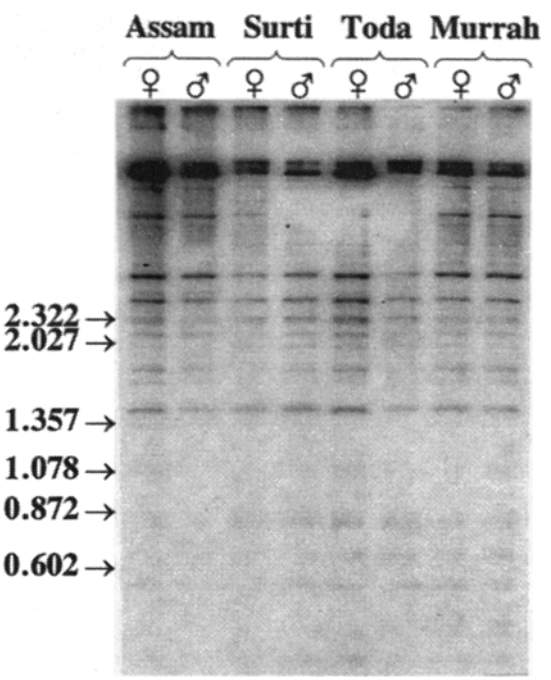

Figure 3. Genotyping of buffalo DNA from different breeds and samples collected from Assam with *Rsa*I enzyme and pDS5 probe. Note several monomorphic bands both in low and high molecular weight regions and two strong bands in the high molecular weight region. Molecular size marker is given in kb.

Cattle samples showed a total of 17 resolvable bands in the range of 300 base pairs to 9 kb. Male goat seems to be having fewer copies of pDS5 compared to other bovids.

4. Discussion

4.1 *Bubaline derived pDS5 sequences are not conserved evolutionarily*

Present zoo-blot hybridization (figure 1) and another independent one suggest that pDS5 sequences do not cross-hybridize with non-bovid samples and are not conserved evolutionarily. This was an attractive proposition for us to ascertain its genomic organization amongst the related bovids and to uncover the genetic and generic affinities of these animals. Detection of multiple bands of varying lengths amongst different bovids indicates sequence variation within the pDS5 repeats which is a common phenomenon of this type of satellite fraction (unpublished results). In buffalo samples, a reduced level of DNA polymorphism is seen that is confined to the higher molecular weight regions whereas in gaur males and cattle, the polymorphism is seen in the lower molecular weight regions. The distinct copy number variation uncovered by pDS5 in cattle and gaur and the less distinct variation in buffalo suggest differential and species specific organization of this repeat fraction amongst these animals. Interestingly, with *Rsa*I typing, neither a band was detected in gaur female nor was there any signal in zoo-blot hybridization indicating that this satellite fraction is either completely deleted or still undergoing differential progressive evolutionary deletion more in the females than in males. Alternatively, it may also be argued that pDS5 clone in gaur is related to the Y chromosome. As mentioned earlier, gaur is one of the most endangered species of Bovidae family and is distributed only in limited parts of India. Access to these animals in the wild poses logistic constraints and inability to procure more samples hampered our analysis on gaur females. Owing to this, our present inference regarding the absence of pDS5 due to deletion in the gaur female or its presence on the Y chromosome of gaur male remains inconclusive.

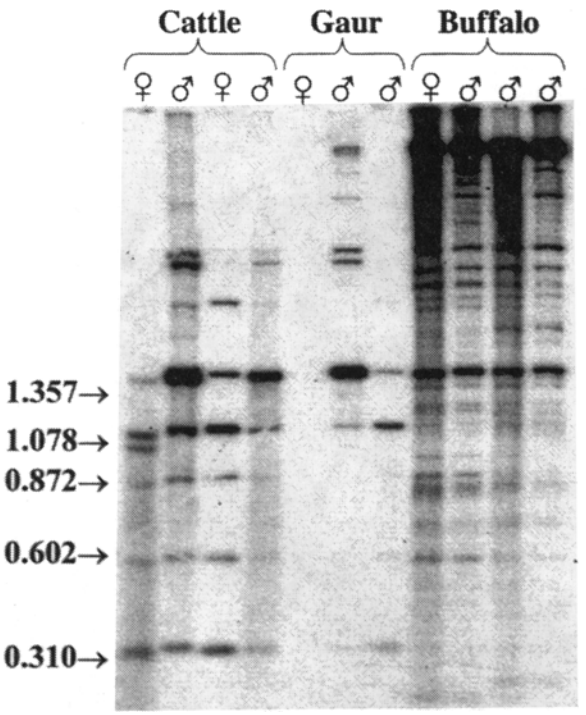

Figure 4. Comparative genotyping of cattle, gaur and buffalo DNA with *Rsa*I enzyme and pDS5 probe. Note a number of monomorphic bands in buffalo and 1·4 kb bands shared in all the three species showing distinct copy number variations in cattle and gaur. The other band of about 1100 base pairs also shows copy number variations in cattle and gaur. Molecular size marker is given in kb.

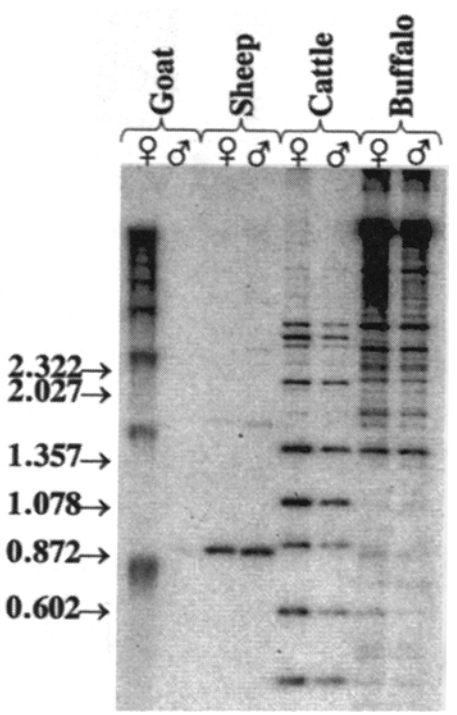

Figure 5. Comparative genotyping of goat, sheep, cattle and buffalo DNA with *Rsa*I enzyme and pDS5 probe. Note the species specific band profile with more number of monomorphic bands in buffalo and relatively reduced number in cattle compared with those in goat and sheep samples. Molecular size marker is given in kb.

4.2 *Generic affinities amongst bovids*

The bubaline derived pDS5 satellite showed mostly a monomorphic band pattern amongst different breeds of water buffalo but revealed entirely different restriction fragment length polymorphic (RFLP) pattern in goat, sheep, male gaur and cattle genomes suggesting that this satellite fraction underwent drastic alteration in these animals. The original *Bam*HI recognition site giving rise to a 1·4 kb band detectable in buffalo is lost due to this sequence alteration in other bovids (data not shown). Similar sequence alteration was evident from *Rsa*I genotyping. Probably, this has resulted in to an extensive deletion in the gaur female. This assumption is accredited by the band profile detected in the goat samples where putative sequence modulation has taken place in the goat male (figure 5). This is an analogous situation that we have observed earlier in *Tilapia* from two different geographical locations (John *et al* 1996). It may be noted that several monomorphic bands in sheep were shared by buffalo suggesting almost similar rate of evolution of these sequences in these two bovids. This view is in accordance with our earlier observation showing the close relationship between buffalo and sheep than buffalo and cattle using a set of short synthetic oligonucelotide probes (John and Ali 1997). Taken together the entire band profile, this study clearly demonstrates the genetic closeness of gaur to the cattle rather than to water buffalo at the molecular level. Thus, it may be more appropriate to refer gaur as wild cattle rather than wild buffalo.

Acknowledgments

A major part of this work was supported by a grant (No. SP/SO/D-51/93) from the Department of Science and Technology, New Delhi to SA and a core grant from the Department of Biotechnology, New Delhi to NII. Authors are thankful to Khem Singh Negi for technical assistance.

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MS received 23 March 1999; accepted 24 May 1999

Corresponding editor: SEYED E HASNAIN