# G-banding homologies of a tandem fusion in Paralakhemundi (Swamp) and Crossbred (Murrah x Swamp) buffalo chromosome

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## ABSTRACT

Present cytogenetic study was conducted on Paralakhemundi and crossbred (MurrahXSwamp) buffaloes in Gajapati district of Orissa state. The whole blood culture technique was followed for morphological study of chromosome and for identification and characterization of individual chromosome conventional Giemsa stain solution (pH 6.8) and G-banding procedures were employed in this study. The diploid chromosome number obtained in G-banding procedure in Paralakhemundi and crossbreed buffaloes were 48 (24 pairs) and 49 (24 pairs+1), respectively, in both the sexes and both of them possessed 10 sub-metacentric chromosomes. The chromosomes of crossbred buffalo possessed a longer sub-metacentric autosome at 4$^{th}$ pair in both the sexes in comparison to Paralakhemundi. It is due to homologous translocation and the numerical polymorphism to a balanced tandem fusion between both members of chromosomes 4 and 9 of the Murrah karyotype. A break in the vicinity of the centromere of acrocentric chromosome 9 resulted in fusion of this chromosome to the short arm of the sub-metacentric chromosome 4, which probably broke in its telomeric regions. In morphological study comparison of mean relative percentage length of chromosome between Paralakhemundi and crossbred buffalo in the conventional Giemsa stained, G-banded metaphase chromosome showed that the Giemsa stained chromosomes were smaller than the G-banded chromosomes (not considering the sex chromosome). The mean relative percentage length varied from 1.187±0.002 to 7.231±0.004 in Giemsa stained and 1.211±0.002 to 9.817±0.004 in G-banded Paralakhemundi chromosome however; in crossbred buffaloes it varied from 1.107±0.002 to 11.714±0.005 in Giemsa stained and 1.295±0.003 to 12.510±0.004 in G-banded chromosome. The higher length in the upper side in crossbred (i.e. 11.714 and 12.51) was due to translocation. The centomeric index (%) and the arm ratio of first ten pairs of sub-metacentric chromosomes varied from 37.61 to 47.82 and 1.090 to 1.398 in Paralakhemundi and 34.93 to 48.28 and 1.081 to 1.498 in crossbred buffalo, respectively. The present findings on G-Banding profile could be important in physical gene mapping studies, which can be consider as one of the most important steps for molecular genetics improvement of the domestic animal.

**Key Words:** G-banding, relative length, tandem fusion, Paralakhemundi buffalo

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## INTRODUCTION

The cytogenetic studies with the advent of banding techniques of livestock species have given a new dimension to animal breeding particularly in unequivocal identification of chromosomes in a karyotype. The science of cytogenetics is developed for identification of individual chromosomes within and between different species and breeds of livestock. Chromosome morphology and gross anatomical or physiological functions of the animals were also confirmed by this study (Ford et al. 1980). Karyotype reveals the sex of an embryo at a very early stage, which is helpful in ETT. Further, chromosomal study can also help in analyzing the segregation of chromosomes though the crossbreds. Till date, 79 loci are mapped on buffalo, which are assigned to 16 autosomes and one sex chromosome 'X' (Iannuzzi et al. 1999). Karyotyping helps in the identification of correct position of loci on the chromosomes. In non-descript breeds of livestock it will equip us with information to design breeding programme and open the door for further molecular genetic characterization of breeds. Paralakhemundi is well-recognized buffalo breed of Gajapati district of Orissa state. It is a potential buffalo breed with distinct body features. Upgrading of this breed with Murrah has been taken up in its habitat. Studies on genetic structure, particularly the chromosome identification and characterization of various Indian buffaloes like riverine and swamp, utilizing the advanced banding techniques and molecular cytogenetic markers approach assume greater importance. Banding the chromosome, which is also helpful to study the evolution of karyotype of many species, can identify the reciprocal or non-reciprocal translocation. The indigenous buffaloes of Orissa have been classified as swamp buffalo possessing 2n=48 chromosome (Bidhar et al. 1986), whereas, Murrah possesses 2n=50 chromosome. Reports indicated that there is reduction in chromosome number of swamp buffalo owing to a reciprocal translocation involving two chromosome pairs. Keeping in view the above facts, the

present investigation was undertaken to study the chromosome compliment of Paralakhemundi and crossbred buffaloes by using conventional Giemsa staining and G-banding techniques to identify the homologous chromosomes and structural abnormalities.

## MATERIALS AND METHODS

Cytogenetic studies were conducted on Paralakhemundi and crossbred buffaloes. The crossbreds were produced by the local farmers of Paralakhemundi by crossing Murrah males with Paralakhemundi females. Whole blood culture technique developed by Ratnasabhapathy and Ganesh (1980) with little modification made by Barpujari and Bhende (1991) was employed. 10 ml of blood was collected from each of 20 Paralakhemundi (10 males and 10 females) and 18 crossbred (8 males and 10 females) buffaloes in heparinised syringe (5000I.U/10ml) with maximum aseptic precautions. The blood from the syringe was immediately transferred into 15ml sterilized centrifuge tube after discarding few drops of blood near flame to avoid contamination.

*Culturing of leucocytes and banding*

Sterile culture tubes of 30ml capacity were used for culture of leucocytes. To each tube 5 ml of culture media TC-199, 0.2 ml of mitotic agent Phytohaemagglutinin-M (Difco), 1.2 ml of autologous plasma and 0.1 ml of antibiotic solution containing (5000 I.U of Benzyl penicillin/ml) was added aseptically and then incubated at $38^0$C (body temperature of buffalo) for 72hrs. The culture tubes were shaked at hourly interval for the first 69 hours of incubation. Then 0.2ml (0.002mg) of colchicines was added to each tube and again incubated for three hours to help for the accumulation of cells at metaphase. After 72 hours of incubation the contents of each tube were transferred into graduated centrifuge tube and centrifuged at 1,800 for 8 minutes. The supernatant was removed and the cell bottom was treated with hypotonic solution (0.075M KCL) for 10

**Table 1.** Mean relative percentage length of Giemsa stained and G-banded chromosome in Paralakhemundi buffalo

| Chromosome | Giemsa stained chromosome | | G-banded chromosome | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 1st pair | 7.231±0.004 | 6.897±0.004 | 9.817±0.004 | 9.112±0.004 |
| 2nd pair | 7.082±0.004 | 6.711±0.004 | 7.332±0.004 | 6.957±0.004 |
| 3rd pair | 6.892±0.004 | 6.301±0.004 | 7.167±0.004 | 6.882±0.004 |
| 4th pair | 6.501±0.003 | 5.989±0.004 | 6.987±0.004 | 6.439±0.004 |
| 5th pair | 5.928±0.003 | 5.897±0.003 | 6.013±0.003 | 5.901±0.003 |
| 6th pair | 5.737±0.003 | 5.213±0.003 | 5.821±0.003 | 5.333±0.003 |
| 7th pair | 4.827±0.002 | 4.982±0.003 | 4.802±0.003 | 5.012±0.003 |
| 8th pair | 4.012±0.003 | 4.098±0.003 | 4.082±0.002 | 4.123±0.003 |
| 9th pair | 3.729±0.003 | 3.019±0.002 | 3.827±0.003 | 4.001±0.002 |
| 10th pair | 3.456±0.003 | 3.308±0.002 | 3.466±0.003 | 3.709±0.002 |
| 11th pair | 3.129±0.003 | 3.018±0.003 | 3.225±0.003 | 3.129±0.003 |
| 12th pair | 2.987±0.003 | 2.709±0.002 | 3.035±0.003 | 2.892±0.003 |
| 13th pair | 2.798±0.003 | 2.405±0.003 | 2.895±0.003 | 2.519±0.002 |
| 14th pair | 2.427±0.003 | 2.201±0.003 | 2.507±0.003 | 2.314±0.003 |
| 15th pair | 2.220±0.003 | 2.018±0.003 | 2.230±0.002 | 2.219±0.003 |
| 16th pair | 2.013±0.003 | 1.999±0.003 | 2.101±0.003 | 2.012±0.003 |
| 17th pair | 1.902±0.003 | 1.897±0.003 | 2.003±0.003 | 1.903±0.003 |
| 18th pair | 1.798±0.003 | 1.654±0.003 | 1.897±0.003 | 1.719±0.003 |
| 19th pair | 1.652±0.002 | 1.503±0.002 | 1.775±0.002 | 1.609±0.002 |
| 20th pair | 1.609±0.002 | 1.487±0.002 | 1.703±0.002 | 1.517±0.002 |
| 21st pair | 1.456±0.002 | 1.329±0.002 | 1.559±0.002 | 1.499±0.002 |
| 22nd pair | 1.302±0.002 | 1.298±0.002 | 1.429±0.002 | 1.391±0.002 |
| 23rd pair | 1.203±0.002 | 1.187±0.002 | 1.303±0.002 | 1.211±0.002 |
| X | 5.982±0.004 | 5.519±0.004 | 6.038±0.004 | 5.629±0.004 |
| X or Y | 1.011±0.002 | 5.498±0.004 | 1.121±0.002 | 5.501±0.004 |

minutes and fixed thrice in 1:3 glacial acetic acid and methanol as a fixative. The slides were prepared by dropping the cell suspension from a height of 60cm by Pasteur pipette in such a manner that there was no overlapping of drops on the slides. The slides were air dried and stained with Giemsa stain solution (pH 6.8). Good quality metaphase spreads were photographed by using Carl-Zeiss photomicroscope (Fuke et al. 2004).

For identification and characterization of individual chromosome G-banding procedures were employed in this study. The modified trypsin digestion method was used for obtaining G-banding (Sumner et al. 1971 and Seabright, 1972). In this method previously air-dried glass slides were immersed in 2X SSC solutions (17.59gm of sodium chloride + 8.8 gm of distilled water) for 1 hour at 60$^0$C. Then the slides were flooded with a 0.25% trypsin in buffer solution for 45 seconds. Each slide was then rinsed with buffer, air-dried and stained in Giemsa satin [1ml of stock Giemsa solution (BDH) + 9ml of phosphate buffer at pH 6.8] for 4 minutes.

*Morphological study of chromosome*

*a)* Relative percentage length of chromosome

The relative percentage length of the chromosomes was calculated by using the formula:

$$\text{Relative length (\%)} = \frac{\text{Average length of two homologous chromosome}}{\text{Total length of haploid set of chromosomes}} \times 100$$

The above lengths were analyzed statistically as per the procedures of (Snedecor and Cochran, 1967). The transformed values were used for the calculation of standard error. It permits comparative analysis of an

**Table 2.** Mean relative percentage length of Geimsa stained and G-banded chromosome in crossbred (Riverine x Swamp) buffaloes

| Chromosome | Giemsa stained chromosome | | G-banded chromosome | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 1st pair | 7.681±0.005 | 7.576±0.005 | 8.698±0.004 | 8.586±0.004 |
| 2nd pair | 7.318±0.005 | 7.293±0.004 | 7.918±0.004 | 7.415±0.004 |
| 3rd pair | 7.102±0.005 | 7.019±0.004 | 7.239±0.004 | 7.215±0.004 |
| 4th a | 11.714±0.005 | 11.719±0.004 | 12.510±0.004 | 12.008±0.004 |
| 4th b | 6.781±0.004 | 6.615±0.004 | 7.008±0.004 | 6.798±0.004 |
| 5th pair | 6.579±0.004 | 6.681±0.003 | 6.919±0.004 | 6.697±0.003 |
| 6th pair | 6.201±0.003 | 6.112±0.003 | 6.398±0.004 | 6.227±0.003 |
| 7th pair | 5.119±0.003 | 5.017±0.003 | 6.009±0.004 | 5.219±0.002 |
| 8th pair | 4.987±0.004 | 4.798±0.003 | 5.117±0.003 | 4.819±0.003 |
| One of 9th pair | 4.717±0.004 | 4.519±0.003 | 4.729±0.003 | 4.695±0.003 |
| 10th pair | 4.609±0.004 | 4.485±0.002 | 4.698±0.003 | 4.591±0.003 |
| 11th pair | 4.417±0.004 | 4.219±0.002 | 4.529±0.003 | 4.329±0.003 |
| 12th pair | 4.211±0.003 | 4.007±0.003 | 4.311±0.003 | 4.119±0.003 |
| 13th pair | 4.008±0.005 | 3.917±0.003 | 4.118±0.003 | 4.005±0.002 |
| 14th pair | 3.678±0.003 | 3.549±0.003 | 3.801±0.003 | 3.659±0.002 |
| 15th pair | 3.171±0.003 | 3.016±0.003 | 3.311±0.004 | 3.116±0.003 |
| 16th pair | 2.901±0.003 | 2.817±0.003 | 3.011±0.003 | 2.917±0.003 |
| 17th pair | 2.797±0.003 | 2.591±0.003 | 2.895±0.003 | 2.691±0.003 |
| 18th pair | 2.697±0.003 | 2.415±0.003 | 2.798±0.003 | 2.559±0.003 |
| 19th pair | 2.598±0.002 | 2.393±0.003 | 2.698±0.003 | 2.427±0.003 |
| 20th pair | 2.297±0.003 | 2.199±0.004 | 2.314±0.004 | 2.309±0.003 |
| 21st pair | 2.008±0.002 | 1.889±0.002 | 2.109±0.002 | 1.995±0.002 |
| 22nd pair | 1.817±0.003 | 1.415±0.002 | 1.987±0.002 | 1.629±0.002 |
| 23rd pair | 1.697±0.002 | 1.297±0.002 | 1.798±0.003 | 1.419±0.002 |
| 24th pair | 1.601±0.003 | 1.107±0.002 | 1.705±0.002 | 1.295±0.003 |
| X | 6.981±0.004 | 6.589±0.005 | 7.001±0.004 | 6.775±0.004 |
| X or Y | 1.118±0.003 | 6.003±0.004 | 1.218±0.002 | 6.221±0.004 |

**Table 3.** Centromeric index and arm ratio of sub-metacentric chromosomes of Paralakhemundi and Crossbred buffaloes

| Chromo-some No. | Centromeric Index | | | | Arm Ratio | | | |
|---|---|---|---|---|---|---|---|---|
| | Paralakhemundi | | Crossbred | | Paralakhemundi | | Crossbred | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| 1st | 42.87 | 38.30 | 45.17 | 45.74 | 1.332 | 1.099 | 1.213 | 1.186 |
| 2nd | 43.94 | 46.91 | 42.88 | 43.37 | 1.275 | 1.332 | 1.332 | 1.385 |
| 3rd | 43.44 | 41.52 | 38.27 | 43.38 | 1.299 | 1.244 | 1.498 | 1.282 |
| 4th | 43.04 | 46.89 | 42.94 | 44.74 | 1.323 | 1.206 | 1.328 | 1.236 |
| 5th | 44.89 | 42.77 | 34.93 | 44.79 | 1.227 | 1.398 | 1.451 | 1.232 |
| 6th | 45.74 | 46.30 | 38.31 | 46.23 | 1.186 | 1.281 | 1.441 | 1.162 |
| 7th | 41.74 | 42.25 | 48.28 | 36.84 | 1.395 | 1.345 | 1.081 | 1.451 |
| 8th | 42.82 | 42.67 | 41.36 | 37.74 | 1.335 | 1.098 | 1.417 | 1.461 |
| 9th | 45.93 | 37.61 | 44.41 | 43.40 | 1.117 | 1.118 | 1.251 | 1.389 |
| 10th | 47.82 | 45.82 | 44.44 | 45.45 | 1.090 | 1.301 | 1.258 | 1.288 |

individual cell and individual subject.

*b)* Centromeric Index

The centromeric index (%) of the individual metacentric chromosome was calculated by using the formula:

$$\text{Centromeric Index (C.I)} = \frac{\text{Length of Short Arm (p)}}{\text{Chromosome lenght}} \times 100$$

c) Arm ratio

The morphology of a chromosome depends upon its total length and the position of the centromere. The position of the centromere is also indicated by the arm ratio, which is expressed by-

$$\text{Arm Ratio} = \frac{\text{Length of long Arm (q)}}{\text{Length of Short Arm (p)}} \times 100$$

## RESULTS AND DISCUSSION

In buffaloes most of autosomes and sex chromosomes are acrocentric in structure; therefore, identification problems are encountered while comparing with international standard (Gustavsson, 1999). As a result, conventional staining techniques fail to identify the individual autosome pairs with the exception of the largest and the smallest in the genome. But, with the recent advancements of technology several investigators have attempted to carry out unequivocal identification of the bovine chromosome through banding techniques. The G-banding was known to identify the homologous chromosomes in normal karyotype and also helpful for the identification of small parts of chromosomes involving translocation, deletion and in structural rearrangements (Rowley, 1973; Lin et al. 1977; Long, 1985). It had been postulated that DNA in G-positive band was A-T rich and in G-negative bands was G-C rich. Characteristic dark and light bands representing the heterochromatin and euchromatin area respectively had been observed in the present study. The euchromatin area (light bands) contains functional genes and DNA, which replicate early in S-phase. G-banding pattern indicated the centromeric heterochromatin unlike other heterochromatin did not take G-staining, however X-chromosome centromere

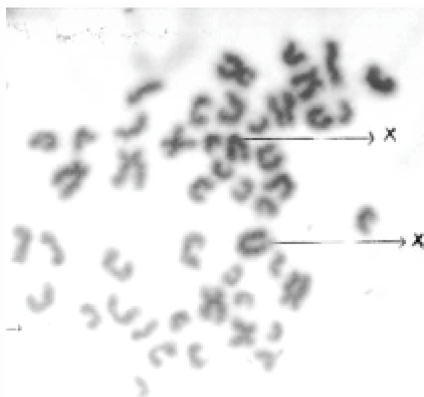


**Figure 1.** Mitotic metaphase spread of Giemsa stained chromosome of Paralakhemundi female buffalo

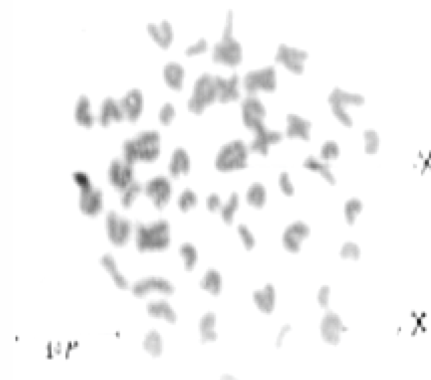


**Figure 2.** Mitotic metaphase spread of Giemsa stained chromosome of crossbred female buffalo

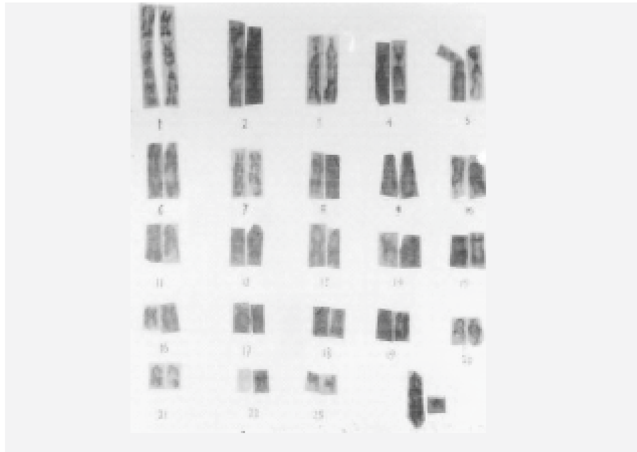

**Figure 3.** G-banded karyotype of Paralakhemundi buffalo in male

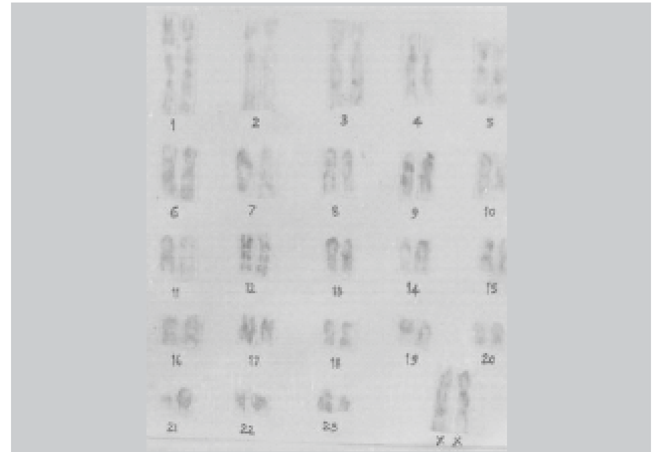

**Figure 4.** G-banded karyotype of Paralakhemundi buffalo in female

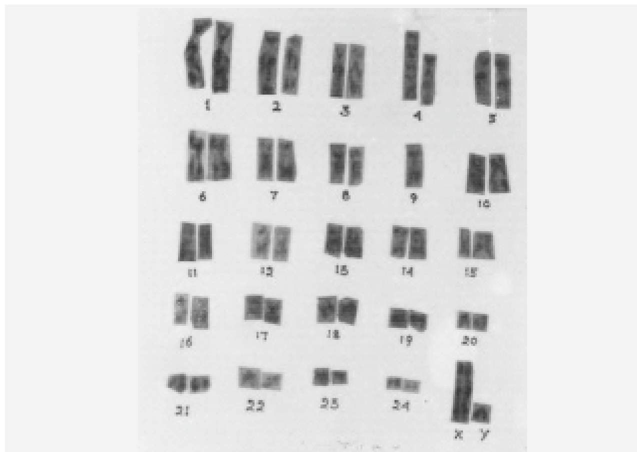

**Figure 5.** G-banded karyotype of crossbred (Paralakhemundi x Murrah) buffalo in male (Tandem fusion)

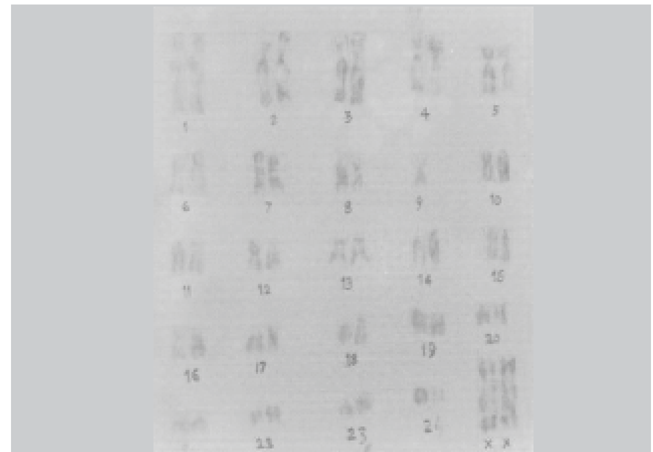

**Figure 6.** G-banded karyotype of crossbred (Paralakhemundi x Murrah) buffalo in female (Tandem fusion)

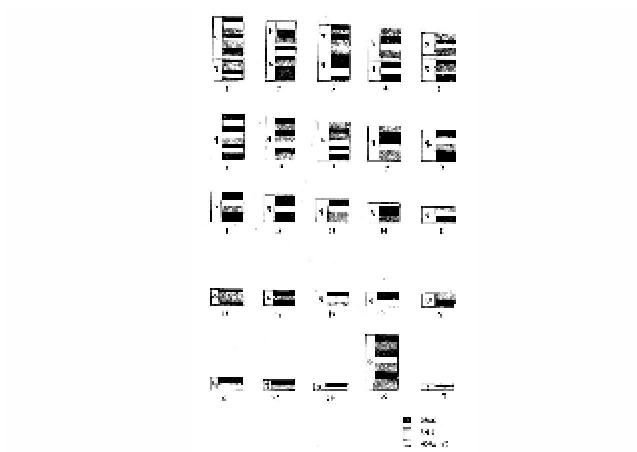

**Figure 7.** Diagrammatic presentation of the G-banded karyotype of Paralakhemundi buffalo in male

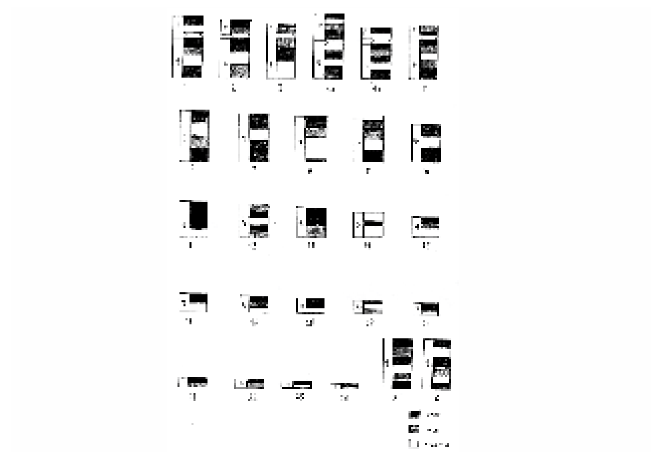

**Figure 8.** Diagrammatic presentation of the G-banded karyotype of Paralakhemundi buffalo in female

**Figure 9.** Diagrammatic presentation of the G-banded karyotype of crossbred (Paralakhemundi x Murrah) buffalo in male

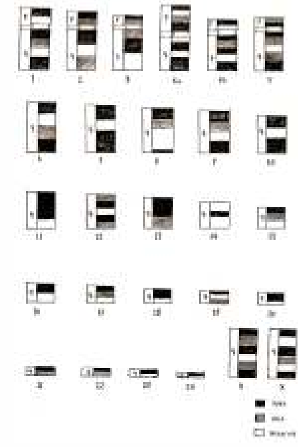

**Figure 10.** Diagrammatic presentation of the G-banded karyotype of crossbred (Paralakhemundi x Murrah) buffalo in female

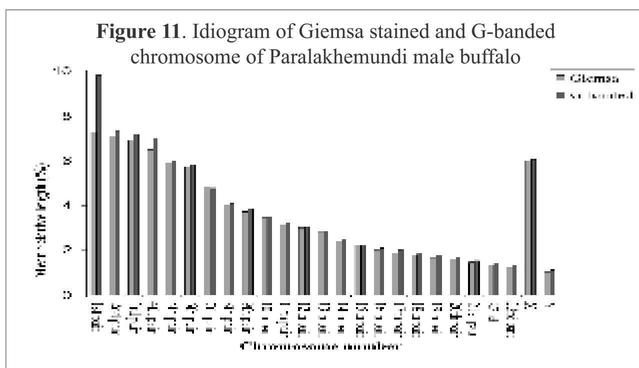


**Figure 11**. Idiogram of Giemsa stained and G-banded chromosome of Paralakhemundi male buffalo

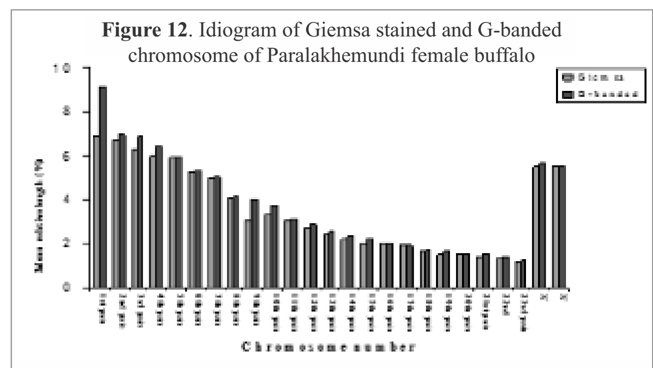


**Figure 12**. Idiogram of Giemsa stained and G-banded chromosome of Paralakhemundi female buffalo

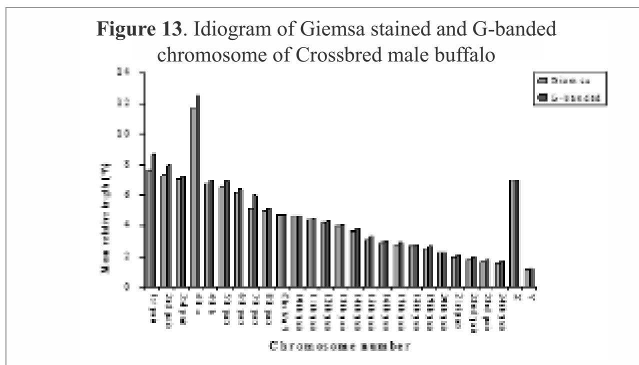


**Figure 13**. Idiogram of Giemsa stained and G-banded chromosome of Crossbred male buffalo

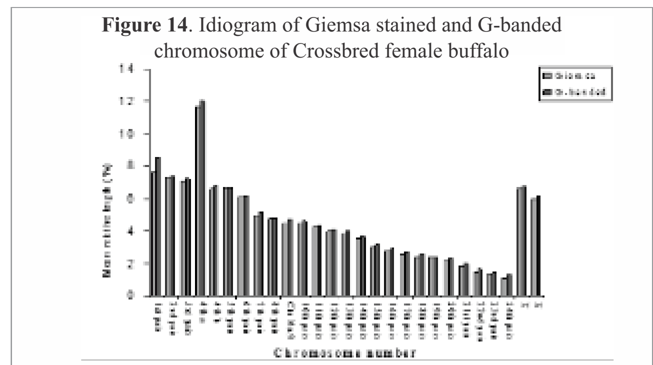


**Figure 14**. Idiogram of Giemsa stained and G-banded chromosome of Crossbred female buffalo

took G-staining, which were in agreement with the earlier results (Evans et al. 1973; Bharti and Verma, 2004).

The present study described the Giemsa stained chromosome (Figs 1 and 2), the karyotypes (Figs.3-6), diagrammatic presentation (Figs.7-10) and ideograms (Figs.11-14) of G-banded chromosomes of Paralakhemundi and crossbred buffaloes. It revealed that each sub-metacentric chromosome consists of two arms. Each arm (p=small arm and q=large arm) possessed different distinct G-banded regions.

In G-banding it was found that the variable bands like dark, pale and negative (no band) bands on the chromosome showed different degree of contraction due to different effects of trypsin treatment as presented in the diagrams (Figs. 7-10). The pale band represents the lighter band, where as, the dark band is deeply stained and there is no stain in negative band. The difference between positive and negative G-band may be due to distribution of chromosomal protein and DNA. Lin et al. (1977), Iannuzzi and Di-Meo (1995) and Nagpure et al. (2006) in cattle karyotypes and Long (1985) in sheep karyotypes reported different G-banding regions on the haploid chromosomes. The Paralakhemundi male buffalo has got seven bands in the 'p' arm and six bands in 'q' arm (Fig.7), whereas, the females possessed 6 bands on both p and q arm (Fig.8).

In the crossbred, the picture is completely different. Both male and female possessed two regions in the p arms, whereas, in q arm there were five regions in male (Fig.9) and four regions in female in the q arm (Fig.10). These types of differential banding patterns with different regions were also observed in all haploid chromosomes of Paralakhemundi and crossbred buffaloes in both sexes (Fig.7-10).

The diploid chromosome number obtained in G-banding procedure in Paralakhemundi and crossbreed buffaloes were 48 (24 pairs) and 49 (24 pairs+1), respectively, in both the sexes and both of them possessed 10 sub-metacentric chromosomes (Figs. 3-6). These findings were in agreement with the results obtained by Di-Beradino and Iannuzzi et al. (1990) and Bongso and Hilmi (1982). The chromosomes of crossbred buffalo possessed a longer sub-metacentric autosome at 4$^{th}$ pair in both the sexes in comparison to Paralakhemundi (Figs.5 and 6). It was a case of homologous translocation. It is evident that the numerical polymorphism is due to a balanced tandem fusion between both members of chromosomes 4 and 9 of the Murrah karyotype. A break in the vicinity of the centromere of acrocentric chromosome 9 resulted in fusion of this chromosome to the short arm of the sub-metacentric chromosome 4, which probably broke in its telomeric regions (Figs. 5 and 6). Using the G-banding technique, Rommelt-Vaster et al. (1978) reported that the tandem fusion occurred between chromosomes 2 and 9 in the Asian water buffalo. It was recently shown from R-banding patterns that the large metacentric chromosome of a Swamp buffalo in the Rome zoo resulted from a telomere-cetromere tandem fusion between 4p and 9 of the Murrah karyotypes (Di-Bererdino and Iannuzzi, 1981). Das et al*.* (2000) also reported the translocation as a telomere-centromere type tandem fusion between chromosomes 4 and 9 of the river buffalo (2n=50). Further, the results confirm the hypothesis of Wurster and Benirschke (1968), who suggested that a tandem fusion may have been responsible for the differentiations of the Swamp from the Murrah buffalo.

Comparison of mean relative percentage length of chromosome between Paralakhemundi and crossbred buffalo in the conventional Giemsa stained, G-banded metaphase chromosome (Tables 1 and 2) showed that the Giemsa stained chromosomes were smaller than the G-banded chromosomes (not considering the sex chromosome). The mean relative percentage length varied from 1.187±0.002 to 7.231±0.004 in Giemsa stained and 1.211±0.002 to 9.817±0.004 in G-banded Paralakhemundi chromosome (Table 1), which was in agreement with the reports of Bidhar (1986), Pradhan (1986) and Samal (1991) in swamp buffaloes. However, in crossbred buffaloes it varied from 1.107±0.002 to 11.714±0.005 in Giemsa stained and 1.295±0.003 to 12.510±0.004 in G-banded chromosome (Table 2). The higher length in the upper side in crossbred (i.e. 11.714 and 12.51) was due to translocation. In males the mean relative percentage length is comparatively more than the females irrespective of the banding patterns (Tables1 and 2). The Y-chromosome was the smallest acrocentric chromosome irrespective of breed and sex (Tables 1 and 2; Figs. 11 and 13), which was in agreement with the findings of Samal (1991), Gaikwad and Narayankhedkar (1995) and Shinde et al. (1997), and the X-chromosome was the largest acrocentric chromosome (Tables 1 and 2; Figs. 14 and 15), which agreed well with the reports of Prakash (1993), Naqvi and Baig (1994), Gaikwad and Narayankhedkar (1995) and Shinde et al. (1997). The centomeric index (%) of first ten pairs of sub-metacentric chromosomes varied from 37.61 to 47.82 in Paralakhemundi and 34.93 to 48.28 in crossbred buffalo (Table 3), which was similar with the report of Chauhan (2002). The arm ratio of first ten pairs of sub-metacentric chromosomes varied from 1.090 to 1.398 in Paralakhemundi and 1.081 to 1.498 in crossbred buffalo (Table 3). Thus, information on G-Banding profile could be important in physical gene mapping studies, which can be consider as one of the most important steps for molecular genetic improvement of the domestic animal.

REFERENCES

Barpujari D and Bhende SV. 1991. MVSc Thesis submitted to the Punjabrao Desmukh Krishi Vidyapeeth, Akola, Maharashtra.

Bharti and Verma SK. 2004. Chromosome homology and abnormalities studied by GTG banding in cattle. National Symposium on *Livestock Production Sustainable food Security and Livelihoods in Mountain area*s, GBPUAT, Pantnagar.

Bidhar GC, Pattnaik GR, Rao PK and Patra BN. 1986. Chromosome number and morphology of Paralakhemundi buffaloes in Orissa. *Buffalo Bulletin.* 5: 54-56.

Bongso TA and Hilmi M. 1982. Chromosome banding homologies of tandem fusion in river swamp and crossbred buffaloes (*Bubalus bubalis*). *Canadian Journal of Genetics and Cytology.* 24**:** 667-673.

Chauhan AK. 2002. Karyotyping of Tarai (Riverian) buffaloes. A M.V.Sc Tesis submitted G.B.P.U.A.T., Pantnagar, Uttarakhand.

Das D and Gogoi PK. 2000. Karyotyping of swamp buffaloes of Assam. *Indian Veterinary Journal.* 77: 499-500.

Di Beradino D and Iannuzzi L. 1981. Chromosome banding homologies in swamp and Murrah buffalo. *Journal of Heredity.* 72: 183-188.

Evans HJ, Buckland RA and Sumner AT. 1973. Chromosome homology and heterochromatin in goat, sheep and ox studied by banding techniques. *Chromosoma.* 42: 383.

Ford CE, Pollock DL and Gustavasson I. 1976. In: Proceedings of first international conference for standardization of banded karyotype of domestic animal. *Hereditas.* 92: 145-162.

Fuke NH, Sirothia AR, Sirothia KA and Joshi DK. 2004. Chromosome number and morphology of Purnathadi buffaloes of Maharshtra- Centromeric Index. *Livestock international.* 2: 5-7.

Gaikwad SM and Narayankhedkar SG. 1995. Chromosomal evaluation of Murrah buffaloes. *Journal of Bombay Veterinary College*. 4: 57-58.

Gustavsson I. 1980. Chromosome aberrations and their influence on the reproductive performance of domestic animals –a review. *Z. Tierz. Zuchtungsbiol.* 97: 176-195.

Iannuzzi L, Meo, RD, Perucatti A and Ferrara L. 1990. The high resolution of G- and R- banding patterns in chromosome of river buffalo (*Bubalua bubalis*). *Heriditas Landskrona.* 112: 209-215.

Iannuzzi, L. and Di-Meo, G. P. (1995). Chromosomal evolution in bovids: a comparision of cattle, sheep and goat G- and R- banded chromosomes and cytogenetic divergence among cattle, goat and river buffalo sex chromosomes. *Chromosome Research*. 3: 291-299.

Iannuzzi L, Gallagher DS, Di-Meo, Yang GPY, Womack JE, Davies SK and Taylor JF. 1999. Comparative FISH mapping of six expressed gene loci to river buffalo and sheep chromosome. *Cytogenetics and cell Genetics.* 84: 161-164.

Lin CC, Netwton DR. and Church RB. 1977. Identification and nomenclature for G-banded bovine chromosomes. *Candian Journal of Genetics and Cytology.* 19: 271-282.

Long SE. 1985. Standard nomenclature for the G-banded karyotype of the domestic sheep *Ovis aries*. *Heredita.* 103: 165-170.

Nagpure NS, Kharche SD and Koul GL. 2006. Comparative C- and G-banding studies in Hariana, Holstein Friesan and their crossbreds. *Indian J. Anim. Sci.,* 76 (10): 810-815.

Naqvi AN and Baig MA. 1994. Karyotype of Nill-Ravi buffalo. *Buffalo Bulletin.* 13:61-63.

Pradhan JK. 1986. Chromosome studied on some local varieties of buffalo in Orissa. M.V.Sc Thesis submitted to Orissa University of Agriculture and Technology, Bhubaneswar.

Prakash B, Balain DS, Lathwal SS and Mallick RK. 1994. Trisomy-X in a sterile river buffalo. *Vet. Rec*., 134: 241-242.

Rathnasabapathy V and Ganesh TN. 1980. White blood cell culture technique for the display of chromosomes in buffaloes. *Cherion.* 9: Pp.45.

Rommelt-Vaster C, Scheurmann E and Jainudeen MR. 1978. Chromosomes of Indian water buffalo (*Bubalus bubalis*). *Kajian Veterianary.* 10 (1): 8-14.

Rowely. 1973. *Genetics*, Wiley Eastern Limited. New Delhi, Bangalore, Bombay, Calcutta, Chenai and Hyderabad, pp 249-250.

Samal DD. 1991. Cytogenetic studies on the crossbred (indigenous X Murrah) buffaloes of Orissa. M.V.Sc Thesis submitted to Orissa University of Agriculture and Technology, Bhubaneswar.

Seabright M and Sumner AT. 1972. A rapid banding technique for human chromosome. *The Lancet.* 2: 971-972.

Shinde AS, Narayanhedkar SG, Nehta SB, Umirkar UD and Swane MP. 1997. A study on Murrah buffalo chromosomes. *Cherion.* 26: 182.

Snedecor GW and Cochran WG. 1967. Statistical methods. Oxford and IBH Publ. Co., New Delhi, India.

Sumner AT, Evans HJ and Buckland RA. 1971. New technique for distinguishing between human chromosomes. *Nature (London), New Biol*., 232: 31-32.