5. DISCUSSION

India possesses the best buffalo germ plasm in the world. There are seven established buffalo breeds and numerous nondescript animals spread across the country. There has been large scale migration and slaughter of the animals of the established breeds, while some of the best animals have also been exported. Indiscriminate and unscientific breeding programmes have also brought about the dilution of these animals. On the other hand, amongst the nondescript buffaloes, there are several buffalo groups which are named after the region in which they are located. Not many efforts have been made to delineate the characteristics of these animals and to find out if there are any special features in these animals.

Conservation of indigenous germ plasm is the need of the hour. One of the prerequisites for effective conservation is to study genetic differentiation of the populations within the buffalo species. Information on polymorphic loci can be employed to detect population specific alleles, to measure the amount of genetic diversity in each species and to evaluate the change in variation in breeds over time periods. Morphological, blood group, biochemical and cytogenetic studies have been carried out in the established breeds, while these studies have been lacking in the other buffalo groups within the species.

In the present study, genetic characterization of Gowli buffaloes inhabited in the northern districts of Karnataka state was carried out on the basis of phenotypic traits and cytogenetic and RAPD-PCR techniques.
5.1 Gowli buffaloes

Gowli buffaloes are being maintained by the people of the Gowli tribe generations together in the northern parts of Karnataka. However, these animals are not yet bred and reared in organized farms as is being followed for the recognized buffalo breeds of India. Since there are no recognized farms for these animals, there are no studies on morphology, biochemical, cytogenetic or molecular genetics in these animals, although much work has been carried out on these aspects in the other recognized buffalo breeds of India.

5.1.1 Habitat and Management

The major breeding tract of Gowli buffaloes lies in the Belgaum and Dharwar districts of Karnataka, which fall under the Northern Transition zone. The climatic soil water balance indicates safe cropping period of 150 to 165 days, giving scope for double cropping, thus ensuring availability of sufficient green fodder to the animals.

The Gowli buffaloes take their name after the tribal community called Gavali or Gowli who normally habitat in the dense woods and peri-urban areas in the northern districts of Karnataka, viz., Belgaum, Dharwar and Uttar Kannada. Gowli people live in small hamlets comprising of 20 to 30 families. Buffaloes owned by these people practically depend on grazing the whole day in the forest area and/or common property resources (CPR) with nominal concentrate feeding at the time of milking.
Normally either aged people or young children take the herds of buffaloes for grazing during day time. Buffaloes enjoy mud splashing or wallowing in the stagnated or running water or streams/nullas. The women folk of the community are ardent lovers of animals and totally dedicated to rearing of buffaloes for milk production. Their main occupation is dairying and derives their income from the selling of milk and home made milk products. The women generally carry head loads of milk and milk products like curds, ghee and khoa for selling at the door steps of the customers either at villages or the townships, which makes their living. The people are innocent and simple unaffected by the modern civilization and arts of modern dairy husbandry practices.
5.2 PHENOTYPIC CHARACTERS IN BREED CHARACTERIZATION

Characterization of recognized buffalo breeds in India has been carried out by the state agricultural universities (SAUs) and national institutes. However, not much work has been done to characterize the other local buffaloes, including Gowli buffaloes, which are spread all over the country.

Survey, Characterization and Evaluation of different livestock species including buffaloes has been initiated by National Bureau of Animal Genetic Resources (ICAR), Karnal, Haryana. However, this is restricted to the recognized breeds available in India. One such completed work was the survey, evaluation and characterization of Nagpuri buffaloes in its breeding tract in Vidharbha region by Sirothia et al. (2004). Another network project completed was the Survey and characterization of Jaffarabadi Buffalo, while the other buffalo breeds studied were the Toda and Pandharapuri.

5.2.1 Morphological traits

a. Colour

The colour of the skin and hair coat was black in majority of the animals, while it was light brown in few younger animals tending to light black and later whitish colour with advancement in age. Two animals of the age group upto one year had white forehead, while four animals had wooly hair coat. This observation conforms to the report of Govindaiah and Rai (1984) that Dharwari buffaloes are generally black in colour. In Nagpuri buffaloes, the coat colour was observed to be black in 82.05 per cent of the animals, whereas 9.53 per cent to 8.42 per cent of the animals
were of light brown to deep brown in colour, respectively (Sirothia et al., 2004). The muzzle colour was totally black and moist in all the animals observed in the field. The tail switch colour was overall found to be white in 84 animals out of the 129 animals studied. Similarly, Sirothia et al. (2004) also reported that a majority (85.41%) of the Nagpuri animals had blackish muzzle, while 85.92 per cent of the animals possessed white tail switch.

b. Head

The animals had straight forehead with a face length of 50.63 ± 0.675 cm in females and 50.33 ± 1.424 cm in males. The face width was 21.53 ± 0.616 cm in females and 18.33 ± 0.726 cm in males. These values were in close proximity with the values reported in Nagpuri buffaloes, the values for head length being 47.96 ± 0.31 cm in females and 46.98 ± 0.68 cm in males, and those of head width being 19.81 ± 18.29 cm in females and 18.29 ± 0.69 cm in males (Sirothia et al., 2004). Patil and Ulmek (2002) reported almost similar values for head length and head width as 43.63 ± 0.27 cm and 20.28 ± 0.16 cm, respectively, in Pandharpuri buffaloes.

In Dharwari buffaloes, Govindaiah and Rai (1984) observed that the head was carried a little high, being proportionately built to body size but appearing a little long. The forehead was narrow, with the face thin with straight profile, moderately long and tending to taper towards the muzzle. The nostrils were wide open, while the neck was smooth, slender and long. The ear length values obtained in the present study were slightly lower than the values recorded in Nagpuri buffaloes (Sirothia et al., 2004).
c. Horns

The horn length recorded in the present study was higher (79.16 ± 2.439 cm in females and 76.00 ± 3.686 cm in breeding bulls) than the values of 61.55 ± 0.48 cm and 54.83 ± 2.96 cm in Nagpuri buffaloes (Sirothia et al., 2004). The poll distance in the present study was 21.41 ± 0.576 cm in females and 20.33 ± 0.333 cm in males. In the Dharwari buffaloes, Govindaiah and Rai (1984) observed that the horns were long flat and sweeping in nature, generally projected laterally and usually carried backwards, downwards and parallel to neck and upwards almost crossing the shoulder blade. In some cases, the horns were carried backward downwardly almost touching the ground. Usually, the horns ended up with sharp points.

d. Tail

The tail length was 81.77 ± 1.028 cm in females and 85.33 ± 2.167 cm in males, which was considerably above the values recorded for tail length in Nagpuri buffaloes (Sirothia et al., 2004).

5.2.2 Body measurements

The body measurements recorded included ear length, horn length, poll distance, face length, face width, body length, height at withers, height at hip bone, height at pin bone, height at elbow, height at hock, rump slope, tail length, heart girth, body girth, distance between hip bones, distance between pin bones.

5.2.2.1 Females and Males above 4 years of age
a. **Height at withers, body length, heart girth and body girth**

The average body measurements of Gowli buffaloes were recorded for the dimensional traits viz., height at withers, body length, heart girth and body girth (table 4.1).

The value obtained for height at withers in the present study was in conformity with the values reported for Nagpuri (Sirothia *et al.*, 2004) and Pandharpuri (Patil and Ulmek, 2002). However, Bhat *et al.* (1981) had reported higher values of 130 cm for females and 140 cm for males in Nagpuri/Ellichpuri buffaloes, whereas Govindaiah and Rai (1984) reported value in the range of 150 – 175 cm in Dharwari males.

The mean value for body length in the present study conformed to the report of Sirothia *et al.* (2004) in Nagpuri females, while slightly lower values were reported in males of Nagpuri (Sirothia *et al.*, 2004) and Pandharpuri (Patil and Ulmek, 2002) buffaloes. However the values for both females and breeding bulls were much lower than the values reported for Nagpuri/Ellichpuri (150 cm for females and 180 cm for males) by Bhat *et al.* (1981) and in Dharwari buffaloes (Govindaiah and Rai, 1984).

The overall average chest girth value in the present study was in close proximity with that of Patil and Ulmek (2002) in Pandharpuri buffaloes, but was much higher than the value of 175.02 ± 0.53 cm reported in milking Nagpuri buffaloes (Sirothia *et al.*, 2004).
The value for body girth recorded in the present study falls within the reports of 202.67 ± 0.61 cm in Pandharpuri buffaloes (Patil and Ulmek, 2002) and 219 cm in Nagpuri/Ellichpuri females (Bhat et al., 1981).

5.2.2.2 Young Males and Females between 1-4 years age group

The average height at withers for animals between 1 and 4 years of age was 105.3 ± 4.137 cm, while the values were 104.3 ± 5.569 cm for body length, 143.5 ± 6.075 cm for heart girth and 155.0 ± 8.351 cm, for body girth.

The body length obtained in the present study was similar to the report of Sirothia et al. (2004). However, the values for chest girth and height at withers obtained in the present study were higher than the values in Nagpuri buffaloes.

5.2.2.3 Young Males and Females below 1 year age group

The average height at withers for animals upto 1 year was 90.60 ± 3.409 cm, while the corresponding values in the four groups were 79.32 ± 3.925 cm for body length, 109.6 ± 5.960 cm for heart girth and 114.8 ± 6.189 cm for body girth.

Lower values than in the present study for average chest girth, body length and height in Nagpuri females and males below one year of age were recorded by Sirothia et al. (2004). The overall average chest girth of Nagpuri buffaloes male was 87.02 ± 5.93 cm and that in female was 90.87 ± 2.38 cm. The overall body length of male was 60.87 ± 1.83 cm and that of female was 62.80 ± 1.36 cm.
The height of male and female recorded was 73.14 ± 1.51 cm and 77.14 ± 1.74 cm, respectively.

### 5.2.3 Peak yield

The peak milk yield recorded in Gowli buffaloes in the present study averaged 7.75 ± 0.257 litres/day. This value is in conformation with the value of 7.14 kg in Nagpuri buffaloes reported by Sirothia et al. (2004) who also reported average lactation yield of 1038.9 kg. In Dharwari buffaloes, Govindaiah and Rai (1984) estimated the mean lactation yield as 765.2 ± 8.9 litres, ranging from 426.8 ± 27.4 to 1196.6 ± 57.1 litres. These values indicate similarities between the different groups of buffaloes. In case of farm bred Surti buffaloes maintained at AICRP on buffaloes at Dharwar, Jahageerdar et al. (1997) had observed values of 5.5 ± 0.1 kg and 1232 ± 38 kg for peak yield and total lactation yield, respectively. The high peak yield and higher range of lactation yield indicated that there was ample scope for genetic improvement in both peak yield and lactation yield through proper selection and management.

From the overall breed profile of Gowli buffaloes with respect to various to body measurements at different ages, it appeared that there was close resemblance with Nagpuri and Pandharpuri buffaloes. Noting that the breeding tracts of these animals also fall close by covering the interior of Maharashtra and the border areas of Maharashtra and Karnataka, it may indicate a common ancestry and gene pool for these different buffaloes. Also, Govindaiah and Rai (1984) had also indicated that Dharwari buffaloes almost resembled Pandharpuri buffaloes and that Dharwari buffaloes were variously called as Holesal, Mundargi and Gowli depending on the
locality or ownership. The values reported by Bhat et al. (1981) and Govindaiah and Rai (1984) were recorded much earlier and are higher than those recorded in Gowli, Nagpuri and Pandharpuri in the last few years. This may indicate a slight decrease in the body characteristics of these buffaloes over the years, which observation has also been made in Deoni cattle by Appannavar (2001). Therefore, there is a need for detailed studies on type, contribution and potential of non-defined buffalo sub-populations/breeds/strains called differentially in Karnataka and Maharashtra, through adoption of latest techniques in statistical methodologies.

5.3 CHROMOSOME PROFILE IN BREED CHARACTERIZATION

5.3.1 Blood collection and transportation

Blood samples collected using heparinized vacutainers gave acceptable cultures. The blood samples were collected from places far off from the laboratory and transported by road in ice box at approximate temperature of 8 to 15°C. The cultures were set from these samples within 20 to 24 hours after collection of blood samples. The long interval of time elapsed between collections of samples and setting up of cultures, besides transportation from for a long distance by road, did not exert adverse effect on the lymphocyte cultures.

5.3.2 Lymphocyte culture technique

5.3.2.1 Culture medium
The buffalo lymphocytes were cultured in McCoy’s 5A (w/L Glutamine) enriched with L-methionine and yielded consistently good growth of lymphocytes. In this study, about 0.5 ml of whole blood was added to 10 ml of culture medium. Phytohaemagglutinin-M (PHA-M) was used separately as mitogen, with which sufficient number of metaphase spreads were obtained for further examination.

5.3.3 Chromosome number and morphology

In the present study the observed diploid chromosome number was 2n = 50, in Gowli buffaloes. This was in accordance with the findings of Chandra (1968), Gupta and Raychaudhuri (1978), Rathnasabhapathy and Ganesh (1980) and Joshi and Govindaiah (1997) in river buffaloes of India. The diploid number of 2n = 48 in river buffaloes reported by earlier investigators (Malkino, 1944; Dutt and Bhattacharya, 1952; DeGirolamo, 1956) might be attributable to the poor methodologies available at that time.

The chromosome number in buffaloes varied from 46 in Bubalus depressicornis fergusam to 54 in Syncerus caffer nanus (Hsu and Benirschke, 1969). The diploid chromosome number of Bubalus bubalis varied from 48 in swamp type to 50 in riverine type (Fisher and Ulbrich, 1968; Chandra, 1968; Scheurmann et al., 1974). The variation in the chromosome number in the group of bovidae was mainly due to centric fusion or centric fission with a fundamental number of 58 to 62 (Wurster and Benirschke, 1968). The difference in karyotype of Murrah and swamp type could be due to a balanced tandem fusion between both the
members of the chromosome numbers 4 and 9 of the Murrah karyotype (Bongso and Hilmi, 1982).

The karyotype analysis in Gowli buffaloes revealed 5 pairs of submetacentric, 19 pairs of acrocentric autosomes and one pair of acrocentric sex chromosomes. The X chromosome was the largest acrocentric, and the Y chromosome was smallest acrocentric. This finding was in conformity with the earlier karyological reports on river buffaloes made by Gupta and Raychaudhuri (1978), Chakrabarti and Benjamin (1980), Yadav (1981) and Joshi and Govindaiah (1997). Although no difficulties encountered during karyotype preparation in identifying all the five pairs of submetacentric chromosomes and the X chromosome, identification and location of the Y chromosome exhibited problems in most of the karyotypes. The problem faced in identification of Y chromosome was attributable to its inconspicuous size and shape. Such problems were also pointed out by the earlier workers (Wurster and Benirschke, 1968; Yadav, 1981; Joshi, 1995). Toll and Halnan (1976a) and Gupta and Raychaudhuri (1978) stated that 'Y' was the smallest acrocentric, while Yadav and Balakrishnan (1982) mentioned that the length of Y chromosome ranged between 19th and 20th pair, creating problems in identifying and cataloguing the chromosome.

5.3.4 Banding pattern of chromosomes

5.3.4.1 G-banding technique

The trypsin G-banding technique adopted under this study yielded a characteristic G-banding pattern of chromosomes in
Gowli buffaloes, analogous to the chromosome preparations obtained using this technique in other breeds of riverine buffaloes.

The chromosomes were identified and arranged in accordance to their size and banding pattern. The major bands observed on chromosomes were found to be consistent in all the spreads of all the animals studied. The centromeric region of the chromosome was found to be G-band negative. The banding patterns observed in the present investigation were in close agreement with the G-band features reported by earlier workers in the river type buffaloes (Gupta and Raychaudhuri, 1978; Bongso and Hilmi, 1982; Thiagarajan, 1987; and Joshi and Govindaiah, 1997). The appearance of minor bands was found to be associated with the duration of trypsin digestion and length of chromosomes.

5.3.5 Chromosomal morphometrical parameters

5.3.5.1 Relative length

The concept of relative length of chromosomes makes the length of the chromosomes in different karyotypes prepared by different workers a comparable parameter.

The per cent relative length of first pair of submetacentric autosomes recorded was 7.90 ± 0.17 in males and 7.37 ± 0.09 in females, while that of the last pair was 1.70 ± 0.09 in males and 1.63 ± 0.07 in females. The corresponding values obtained by earlier workers were varying and were found to be 5.51 and 1.96 in Murrah (Gupta and Raychaudhuri, 1978), 5.70 and 0.86 in Paralakhemundi buffaloes of Orissa (Bidhar et al. 1986), 8.75 and 1.86 in Murrah, 6.92 and 2.1 in Surti and 6.5 and 2.27 in Mehsana
buffaloes (Kumar and Yadav, 1991), 6.80 ± 0.17 in South Kanara to 7.38 ± 0.12 in desi buffaloes and that of the last pair ranged from 1.67 ± 0.03 in South Kanara to 1.83 ± 0.06 in Murrah (Joshi, 1995).

Sarkhel (1988) attributed the differences in the relative length of chromosome to differential degree of exposure of cultures to colchicine resulting in a variation in the degree of condensation of chromosomal arms, whereas Long (1990) opined that the errors associated with the measurement of the chromosomes could be high, due to practical difficulties in detecting the points where the two arms really end and the exact location of the centromere. Similar problems were also encountered in the present study.

The variations noticed in the present study in respect of relative length conforms with the observation of Ford (1973) who pointed out that even the members of same pair of the homologous chromosomes in the same cell line exhibited some degree of variation in the length of the chromosomes.

The X chromosome contributed more than six per cent to the total length of haploid genome in both male and male buffaloes in the present study. Similar results were observed by earlier workers in buffaloes (Gupta and Raychaudhuri, 1978; Kumar and Yadav, 1991; Ramesha and Hegde, 1992; Joshi, 1995). In comparison to the present observation, the contribution of X chromosome to the total haploid genome was about 5.0 per cent in sheep and goat (Hansen, 1973), zebu cattle (Yadav, 1981) and taurus cattle (Gustavsson, 1969), highlighting the fact that the higher value of relative length of X chromosomes obtained in buffaloes could be a species difference.
Due to absence of distinguishing features in terms of length and morphology, proper identification of Y chromosome was difficult. In the present study the Y chromosome was placed as the smallest acrocentric. Gupta and Raychaudhuri (1978) and Chakrabarti and Benjamin (1980) had considered the Y chromosome as one of smaller acrocentrics, and that it was not always identifiable. On the contrary, based on G- and C-band studies, Yadav and Balakrishnan (1982) attributed that Y chromosome was equal in length to that of chromosome pairs 19 and/or 20.

Considerable variability was obtained in the relative length of the smaller acrocentric autosomes and Y chromosome, which could be attributed to the fact that the small sized chromosomes were difficult to pair and measure with accuracy in most of the karyotypes.

Highly significant (P ≤ 0.01) differences due to sex were obtained for 1st and 2nd biarmed chromosomes, while significant differences (P ≤ 0.05) were observed for the 3rd, 4th, 7th, 8th, 9th, 10th and 12th pair of autosomes.

Joshi (1995) had observed significant differences (P ≤ 0.05) due to the effect sex for 12th pair. Besides, he had also recorded significant (P ≤ 0.05) individual effect for 4th, 7th, 8th to 10th, 12th, 14th, 15th and 17th to 20th autosomes and X chromosomes. However, Yadav (1981) and Kumar and Yadav (1991) reported that the breed and sex effects were not contributing to relative length of any of the chromosomes while, the individual effects were significant on few pairs of chromosomes.
5.3.5.2 Centromeric index

The centromeric index was found to be highest for fifth pair ($38.49 \pm 1.08$ in males and $36.82 \pm 1.25$ in females) and it was lowest ($26.19 \pm 0.88$ in males and $29.37 \pm 0.96$ in females) for first pair of autosomes. These values indicated that the third and fifth pairs were more metacentric than submetacentric and the first pair had the smallest short arm among all the biarmed chromosomes. These findings were in conformity with the earlier reports of Yadav (1981) and Joshi et al. (1999a). However, Gupta and Raychaudhuri (1978) considered only the fifth chromosome to be nearly metacentric in Murrah buffaloes.

Testing of means of centromeric index of all the five biarmed chromosomes revealed highly significant difference of sex for the fourth pair, while significant differences were obtained for the first and second pair of biarmed chromosomes. This was in confirmation with the observations of Joshi (1995) who had observed significant differences between animals for the first and fourth autosome pairs.

It was felt that studies involving a larger number of animals within a breed would help to develop an index which may be of practical utility in eliciting and measuring the differences between animals within a breed and also the genetic diversity among breeds. Further investigations are therefore needed on more number of animals involving different breeds to draw inferences.

G-bandning technique did not reveal any specific chromosome marker in the present study. Similar observations were also made
by Joshi and Govindaiah (1997) who could not establish any differences in banding pattern and morphology of chromosomes among Murrah, Surti, South Kanara and deshi buffaloes. Hence, it could be postulated that the observed similarities in chromosomes of Gowli and other genetic groups of buffaloes of Karnataka indicate their common origin. More detailed study using advanced cytogenetic and molecular genetic techniques could delineate variations, if any, in chromosomes between breeds of buffaloes.

5.4 MOLECULAR GENETIC MARKERS IN BREED CHARACTERIZATION

Prior to the detection of molecular genetic markers, breed characterization was dependent on morphological, biochemical and cytogenetic profiles. However, these profiles had inherent problems of less number of markers. However, molecular genetic markers can detect polymorphisms at the DNA level and can generate larger number of polymorphisms, and were thus preferred for genetic characterization of livestock breeds.

5.4.1 Random amplification of polymorphic DNA

Random amplified polymorphic DNA markers were identified as fingerprint products generated based on random amplification of DNA by PCR. RAPD technique involves use of short, decamer oligonucleotide primers annealing at low temperature. The number and size of the RAPD products depend on the complimentarity of sequences of the particular primer and template DNA. Different primers were found to produce different RAPD polymorphisms.
RAPD-PCR is a simple technique and easy to analyze. It does not require radioactively labeled nucleotides. RAPD markers follow Mendelian laws of segregation. RAPD-PCR technique requires very small amount (in nanogrammes) of genomic DNA, which is subjected to PCR using random sequence oligonucleotide primers. In RAPD-PCR, only a single random oligonucleotide primer is employed, whereas in standard two primer mediated PCR amplification, two primers are required. RAPD does not require any prior knowledge of nucleotides since primers are chosen arbitrarily and many organisms can be mapped with the same set of primers. It is based on the principle that, when the primer is short (e.g. 8 to 10 mer), there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation.

The potential use of RAPD technique was evaluated as a source of development of alternative genetic marker system for studying variation in the genomes of buffalo (Annapoorani, 1996; Aravindakshan and Nainar, 1998), cattle (Gwakisa et al., 1994; Nagaraja et al., 2003; Appannavar et al., 2002), sheep (Clouscard et al., 1995; Cushwa et al., 1996; Dodgson et al., 1996) and horse (Bailey and Lear, 1994). The above procedure was also adopted in the present study for evaluating genomes of Gowli and South Kanara buffaloes.
DNA Isolation

In the present study, DNA from each 10 ml of blood of 50 Gowli and 50 South Kanara buffaloes was isolated by using high salt method.

5.4.2 RAPD Primers

In the present study, commercially available ten random primers were tested for amplification of pooled genomic DNA of Gowli and South Kanara buffaloes. Out of these, eight primers amplified genomic DNA and produced low to high polymorphic fingerprints. Individual primers were separately used for studying variation in fingerprints between pooled samples of Gowli and South Kanara buffaloes.

Rao et al. (1996), using fourteen arbitrary primers, observed clear and distinct RAPD patterns with a higher level of polymorphism between buffalo, cattle, sheep and goat, while fewer polymorphisms were found within the species. Almost similar polymorphic patterns were obtained in the current study also.

5.4.2.1 Primer ILO 868

In the present study, primer ILO 868 produced moderate fingerprints among DNA pools of Gowli and South Kanara buffaloes. Three breed specific fragments of size 1064 bp, 1393 bp and 1755 bp were amplified in South Kanara buffaloes which were not seen in Gowli buffaloes. Using the same primer, Shashidhara (2002) obtained a RAPD fragment of 110 bp specific to Murrah breed, which was not observed in South Kanara buffaloes. This
RAPD fragment was also not observed either in Gowli or South Kanara buffaloes in the present study.

5.4.2.2 Primer ILO 876

Primer 876 was found to be quite polymorphic. It produced six bands in Gowli buffaloes and eight bands in South Kanara buffaloes. Six fragments ranging from 369 to 1265 bp were produced in Gowli buffaloes. On the other hand, two additional fragments of sizes 502 and 862 bp were detected only in South Kanara buffaloes.

These observations conform to the report of Aravindakshan and Nainar (1998) who obtained average number of 7.7 bands in Murrah and 7.8 bands in Surti breeds.

5.4.2.3 Primer OPG 05

Of the eight fragments produced with primer OPG 15, two fragments (350 and 1859bp) were found only in South Kanara buffaloes. In Gowli buffaloes, the products were found to range from 523 bp to 1448 bp, while the range was from 350 to 1859 bp in South Kanara buffaloes.

Three of the bands amplified in the present study were in close proximity to the report of Saifi et al. (2003) who obtained three amplicons with OPG 05 in both Murrah and Bhadawari breeds, the band sizes being 550, 715 and 870 bp.
5.4.2.4 Primer OPG 11

Primer OPG 11 was found to be less polymorphic, producing five fragments ranging from 398 to 1435 bp. Of these five, fragment of 918 bp was observed only in Gowli buffaloes.

Primer OPG 11 was found to resolve seven amplicons in Murrah and Bhadawari buffaloes, of which one amplicon of size 2445 bp was found to be Bhadawari specific (Saifi et al., 2003). The fragment sizes for this primer were found to be higher (730 to 2445 bp) than the range obtained in the present study.

5.4.2.5 Primer OPG 13

The primer OPG 13 was found to be moderately polymorphic. It produced maximum number of bands in both the breeds (ten fragments in Gowli and 13 numbers in South Kanara) studied. The products ranged from 355 to 1742 bp in the Gowli and South Kanara buffaloes. Fragments of size 1136, 543 and 484 bp were detected only in South Kanara buffaloes.

Using the same primer, Saifi et al. (2003) resolved seven amplicons in Murrah and Bhadawari buffaloes, ranging from 420 to 1935 bp.

5.4.2.6 Primer OPAV 15

Primer OPAV 15 was next to OPG 13 in the number of bands produced, with eight and nine bands, respectively, in Gowli and South Kanara buffaloes. Band sizes ranged from 328 to 1136 bp,
while fragment of 850 bp was detected only in South Kanara buffaloes.

These values were in close proximity with the report of Aravindakshan and Nainar (1998) who observed an average of 6.3 bands in Murrah animals and 6.8 bands with Surti buffaloes with OPAV 15.

**5.4.2.7 Primer OPAX 19**

Primer OPAX 19 has not been reported elsewhere in buffaloes. In the present study, primer OPAX 19 was found to be the least polymorphic, with only one fragment of size 430 bp being detected in Gowli buffaloes. As this did not produce any polymorphic fragments, it is least useful in breed characterization. However, in the South Kanara buffaloes, in addition, four other fragments of size 817, 672, 622 and 303 bp were detected.

**5.4.2.8 Primer BG 28**

Primer BG 28 was observed to be highly polymorphic. A total of 15 fragments were detected with primer BG 28, ranging from 270 to 1407 bp. Of these, seven were common for both groups of buffaloes, while fragment 713 bp was observed only in South Kanara buffaloes.

Saifi *et al.* (2003) also obtained more number of bands with BG 28, being common for both Murrah and Bhadawari buffaloes.

On the whole, several workers have used different primers to obtain RAPD fingerprints using a few breeds of buffaloes for
comparision. Several breed specific fragments have been reported. Shashidhara (2002) showed that Primer ILO 1127 produced two fragments of 428 and 461 bp specific to female South Kanara buffaloes, while Primers ILO 868, BG 86 and OPAC 4 produced Murrah breed specific RAPD fragments of 110 bp, 638 bp and 325 bp, respectively.

Saifi et al. (2003) suggested that primers OPB 07, OPG 05 and 13 and BG 28 could be useful in exploring genetic polymorphisms in buffalo breeds as they amplified buffalo genome satisfactorily. They reported a Bhadawari specific amplicon of 2445 bp using primer OPG 11. Primer BG 27 was found to resolve two distinct breed specific bands of 905 and 2385 bp in Murrah breed pool and three bands (650, 1050 and 1465 bp) unique to Bhadawari breed pool.

In another study, Saifi et al. (2004) observed that primers OPA04 and BG15 resolved a band of 460 bp, which was present only in animals of Bhadawari breed.

**5.4.3 Band sharing**

Based on the number of bands resolved with individual primers, it was observed that the band sharing between the breeds was higher with primer BG 28 than with ILO 876 and ILO 868, the values being 0.63, 0.75 and 0.91, respectively. Further it was observed that band sharing for these primers was higher in Gowli than in South Kanara breed, indicating that there was more homogeneity in Gowli than in South Kanara buffaloes.
Based upon RAPD studies in South Kanara and Murrah buffaloes by Shashidhara (2002), the order of preference of different primers for genetic characterization of the two breeds was BG 86, ILO 868, OPAC 4 and ILO 1127. In the present study, primer ILO 876 was observed to be the primer of choice for genetic characterization of buffaloes. For primer ILO 876, Aravindakshan and Nair (1998) observed intrabreed variation to be higher than interbreed variation in Murrah and Surti buffaloes, which finding is corroborated by the present results. Using 11 random primers, Saifi et al. (2004) obtained a genetic identity index of 0.596 based on band frequency, and concluded that the genetic identity between Bhadawari and Murrah breeds was of a moderate level.

Several workers have expressed their apprehension about the applicability of RAPD technique referring to the reproducibility of the reaction products (Hedrick, 1992; Riedy et al., 1992; Scott et al., 1992 and Meunier and Grimont, 1993). Most of the major bands had high reproducibility in the present study excepting for some faint minor bands and not much variation was observed between these products. Variations were however observed in the intensity of amplified bands. But, the presence or absence of the minor bands did not affect the analysis as very minor bands were not scored.

Variation in the intensity of each band has earlier been attributed to the fact that when the pooled DNA samples were used for RAPD analysis, particular fragments did not appear consistently in all the animals forming the pool. Hence, in a pooled analysis, one of the reasons for variation in the intensity of RAPD bands could be attributed to the absence of such an allele in all the animals constituting the pool. The variations in the intensity of
bands between individuals may also be probably due to variation in the number of copies of different fragments of same size that were amplified by a particular primer. When the number of products is high, the intensity of the amplified DNA fragments resolved on agarose gel was also found to be more.

In spite of such considerations, it has been shown that RAPD gave highly reproducible results (Welsh and McClelland, 1990; Carlson et al., 1991; Williams et al., 1990; Rothuizen and Wolferen, 1994). The present findings support the above observations that polymorphic highly reproducible amplification products can be generated by this technique. Optimal conditions are, however, very important to obtain reliable results.

It is suggested that the different primers available should be tested on the other breeds under similar laboratory conditions to find out whether they are specific to one or more breeds.

Further, using molecular genetic studies, it would be appropriate to evaluate and compare Gowli with other buffalo breeds such as Nagpuri/Ellichpuri and Pandharpuri buffaloes on a common platform. This would ascertain whether they are similar, as the breeding tracts of these breeds appear to overlap and all these animals bear close resemblances in their morphological and functional characteristics, evidencing a common origin.
CONCLUSIONS

1. Phenotypic characterization revealed a close resemblance of Gowli buffaloes with Nagpuri and Pandharpuri buffaloes, indicating a common origin.

2. A normal chromosomal complement of $2n = 50$ was observed in Gowli buffaloes, which is a characteristic of river buffaloes. The karyotype comprised of 5 pairs of submetacentric and 19 pairs of acrocentric autosomes and a pair of acrocentric sex chromosomes. The X chromosome was the largest acrocentric, while the Y chromosome was the smallest acrocentric.

3. RAPD is a simple and highly reproducible technique which can be efficiently used for genetic characterization of buffalo breeds.

4. Individual and breed variations can be detected efficiently by RAPD-PCR technique through testing different primers using large sized populations across various geographical locations.

5. Primers ILO 868, ILO 876 and BG 28 were found to be useful for differentiating buffalo breeds, with the primer ILO 876 being the primer of choice.