

Survey and characterization of South Kanara buffaloes in India

P. Kathiravan¹, D.K. Sadana¹, B.P. Mishra¹, R.S. Kataria¹, P. Kaur¹, A. Kumar² & N.S. Jayaprakash²

¹National Bureau of Animal Genetic Resources, Karnal, India

²Department of Animal Husbandry, Shimoga, Karnataka, India

Summary

Kanarese buffaloes are medium built animals distributed in the South Kanara region on the west coast of South India. These buffaloes are dual purpose animals used mainly for agricultural operations in wet fields and for some milk production. They are famous for racing in water-bound fields. South Kanara buffaloes are moderate milk yielders producing about two to seven litres daily for a lactation period that ranged between 210 to 360 days. Genetic diversity analysis was performed using 10 microsatellite markers in a panel of 48 unrelated animals. The average number of alleles was estimated to be 6.30 with an average heterozygosity of 0.62 per locus. The population showed departure from the Hardy-Weinberg equilibrium at all of the 10 loci tested. The heterozygote deficiency was estimated as 9.2% suggesting the presence of considerable inbreeding in the population. The allele frequency distribution followed the normal L-shaped form suggesting that the breed had not encountered a genetic bottleneck in the recent past.

Résumé

Les buffles Kanarese sont des animaux de taille moyenne qui se trouvent dans le Sud Kanara, région de la côte Ouest du Sud des Indes. Ces buffles sont des animaux à doubles propos utilisés surtout dans les travaux agricoles des régions humides et pour quelque production de lait. Ils sont connus pour les courses dans les champs inondés. Les buffles du Sud de Kanara ont une production de lait limitée, environ deux à sept litres par jour pendant la période de lactation, qui va de 210 à 360 jours. L'analyse de la diversité génétique a été réalisé en utilisant 10 marqueurs microsatellites sur un échantillon de 48 animaux sans relations. Le nombre moyen d'allèles a été estimé à 6,30 avec une moyenne d'hétérozygosity de 0,62 par locus. La population montrait une déviation de l'équilibre de

Hardy-Weinberg dans tous les 10 loci testé. Le manque d'hétérozygosity a été estimé à 9,2%, ce qui indique la présence importante de consanguinité dans la population. La fréquence de distribution des allèles suit la forme normale de L, ce qui indique que la race n'a pas rencontré une limite génétique récemment.

Resumen

Los búfalos Kanarese son animales de tamaño medio que se encuentran en el sur de Kanara, en la región de la costa oeste del sur de la India. Estos búfalos son animales de doble propósito utilizados sobre todo en los trabajos agrícolas en zonas húmedas y para algo de producción de leche. Se conocen también por las carreras en campos inundados. Los búfalos del sur de Kanara tienen una producción de leche limitada, alrededor de dos a siete litros por día durante el periodo de lactación, que va de 210 a 360 días. El análisis de la diversidad genética se ha realizado utilizando 10 marcadores microsatelitares sobre una muestra de 48 animales sin relación entre sí. La media de alelos se ha estimado en 6,30 con una media de heterocigosis de 0,62 por locus. La población muestra una desviación del equilibrio de Hardy-Weinberg en todos los 10 loci analizados. La falta de heterocigosis se ha estimado en 9,2%, lo que indica la presencia de consanguinidad en la población. La frecuencia de distribución de los alelos sigue la forma normal de L, lo que indica que la raza no ha encontrado recientemente ningún límite genético.

Keywords: Status, Characteristics, Genetic diversity, Microsatellites, Bottleneck.

Introduction

India is a virtual repository of buffalo genetic resources and is the major buffalo rearing country

in the world. Around 57% of the world buffalo population is found in India and buffaloes contribute 54% of the total milk produced in the country. Furthermore, they play an important role in the rural economy through other means as well, including draught power, dung, urine and other minor products. The genetic diversity of Indian buffaloes is represented by ten recognized breeds and 16 lesser known populations. Many of these are known for their adaptability to harsh climatic conditions, tolerance to tropical diseases and survival under meager feeding and poor management practices.

South Kanara buffaloes are one such lesser-known populations, but are famous for their speed in '*Kambla*' racing and wet-field agricultural operations. Also known as '*Kanarese*' and '*Malabar*' buffaloes, these animals were originally found in the coastal regions of the former South Kanara district in Karnataka state, which included the Mangalore and Udupi regions. There was a sect of Hindus known as '*Jain Bants*' who owned and developed this hardy breed of buffaloes. Buffalo racing has traditionally been one of the great sources of amusements of these people and every rich Bant kept his own '*Kambla*' field for buffalo racing.

South Kanara buffaloes were first mentioned by Gunn (1909) and subsequently by Littlewood (1936) and Cockrill (1974), who described the famous '*Kambla racing*' involving these animals in this region. However, information on characteristics, performance levels and management practices are scant in the literature. In the present study an attempt has been made to characterize this breed through a systematic survey in the breeding tract and genetic diversity analysis using microsatellite markers.

Material and Methods

A survey was conducted in 13 villages selected randomly from different parts of the breeding tract, to collect information on various aspects of buffalo husbandry in the region. Data collection on various management practices followed in the breeding tract and different performance traits were generated by interviewing the farmers using a structured questionnaire. A total of 47 farmers were interviewed to record the habitat, housing system, feeding management and breeding practices followed in the tract. Performance traits like daily milk yield, lactation length, age at first calving and calving interval were recorded on 48 animals based

on the reports of the farmers. Physical characteristics regarding colour, appearance, horn pattern, head, face and barrel were recorded in adult buffaloes of both sexes (N=63) and udder characteristics were recorded in adult females (N=51) during the survey. Eight different body measurements were recorded on 107 animals of different age and sex, and were analyzed accordingly. The body measurements recorded included body length, height at withers, heart girth, paunch girth, face length, ear length, horn length and tail length.

A total of 48 blood samples were collected randomly from unrelated animals in different regions of the breeding tract following the MoDaD guidelines (Measurement of Domestic Animal Diversity, FAO, Rome). DNA was extracted from whole blood using standard protocol (Sambrook *et al.*, 1989). The DNA isolation procedure involved lysis of red blood cells, digestion of proteins using Proteinase-K and precipitation of proteins using phenol:chloroform:isoamyl alcohol. A set of 10 microsatellite markers originally identified in cattle and evaluated in buffaloes (Navani *et al.*, 2002) were utilized for the present study. PCR was performed with 100 ng of genomic DNA in a 25 μ l reaction volume with an initial denaturation of 95°C for 2 minutes, 30 cycles of 92°C for 45 s, 55°C for 45 s, 72°C for 45 s and finally extension at 72°C for 10 minutes. The amplified products were resolved on 6% denaturing Urea-Polyacrylamide gels (Sequi GT system, Bio-Rad, USA) and alleles were detected by silver staining (Bassam *et al.*, 1991).

The fragment sizes were calculated by comparing the electrophoretic mobility of the alleles with standard size DNA marker ladder which was run along the PCR product. The allele frequencies, observed and expected heterozygosities and departure from Hardy-Weinberg equilibrium were calculated using POPGENE software (Yeh, *et al.*, 1999). Polymorphism Information Content was calculated using the formula given by Botstein *et al.* (1980). The population was tested for the occurrence of any recent reduction in the effective population size using the BOTTLENECK program (Piry *et al.*, 1999).

Results and Discussion

Breeding tract

South Kanara buffaloes are medium built animals distributed originally in the former South Kanara

region around Mangalore and Udupi on the west coast of India. This region is bounded by coast line on the west while a range of mountains called the Western Ghats separates it from the east. The Western Ghat region is characterized by natural shoal forests which are rich in highly diversified fauna and flora. Towards the east, the Western Ghats gradually fade into semi-mountainous regions and the plain lands of the Shimoga district. However, the presence of buffaloes in their original habitat has decreased substantially while more such animals are found in the adjoining Shimoga district (Figure 1).

Ecological settings

Geographically, the breeding tract is situated between 12°57' to 14°39' N latitude and 74° to 75°52' E longitude. The altitude is about 600 metres above mean sea level. The total geographical area of the breeding tract is 14 849 square kilometres. The entire breeding tract is classified into three agro-climatic zones based on rainfall pattern, soil and climate namely the coastal zone, hilly zone and

southern transitional zone. The coastal zone includes the strip of land along the west coast in the Dakshin Kannad and Udupi districts. The hilly zone includes the region around the Western Ghats in the Dakshin Kannad and Shimoga districts. The southern transitional zone includes the eastern plains of the Shimoga district.

The general climate of the district is classified as humid with medium to heavy rainfall in the Kharif season (June – September) followed by a mild winter (October – January). The summer months are moderately hot with scanty rainfall. The average annual rainfall is around 180 cm. Rice is the major crop of the region with maize, cotton, sugarcane and groundnut also being cultivated.

Status of buffaloes in the breeding tract

According to the 17th Livestock census (2003), the cattle and buffalo population of Karnataka state is 9.54 and 3.99 millions, respectively. The State has 5.2% and 4.1% of the total cattle and buffalo population of the country respectively. The trend of the buffalo population from 1972 to 1997 in the

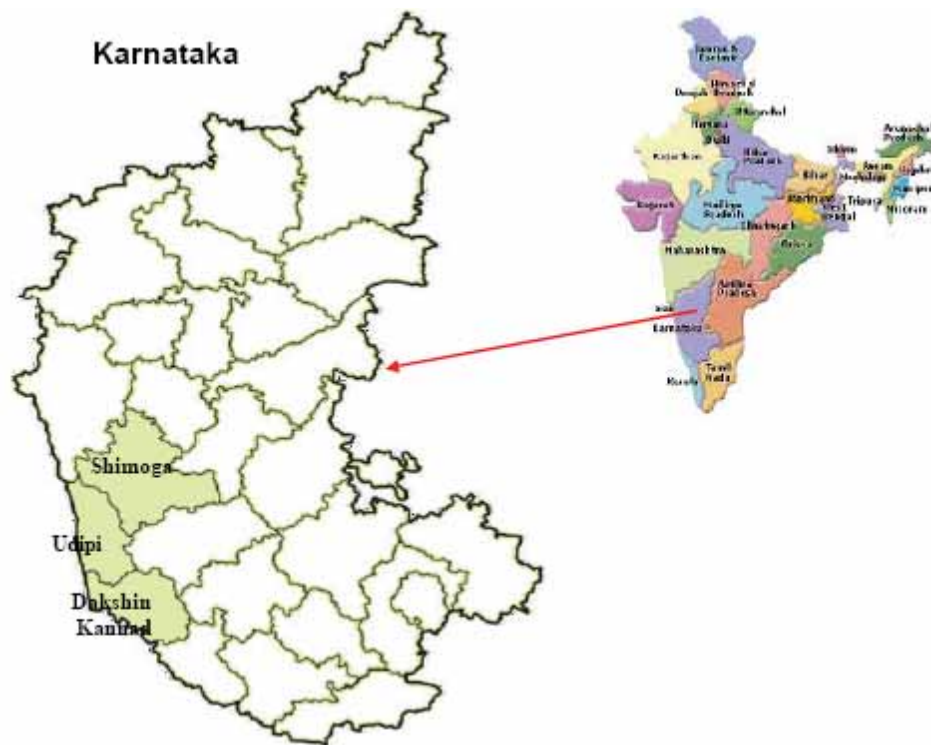


Figure 1. Breeding tract of South Kanara buffaloes.

State showed an increase of 33.6% while it declined by 8.7% from 1997 to 2003. There was also a decline in the total cattle population of 11.9% in the corresponding period (1997 to 2003) with higher reductions in indigenous animals (16.8%). The crossbred cattle population increased by 23.9% during this period. This shows a trend of shifting preference towards crossbred cattle as preferred milk animals in the region.

In the districts of Dakshin Kannad, Udupi and Shimoga which comprise the breeding tract of South Kanara buffaloes, the total buffalo population is 269 200 (Table 1). This consists of animals of the South Kanara type, graded Murrah and Surti and non-descript animals. Murrah/Surti graded buffaloes constitute 3.5% of the total buffalo population in the breeding tract. Although the buffaloes of the South Kanara type are found to be more common in all three districts, the population of non-descript animals is relatively higher in urban areas than in the countryside. Among the three districts, the population of buffaloes in Dakshin Kannad and Udupi are considerably lower, where crossbred cattle have replaced them as dairy animals. However, in Shimoga district, the population of buffaloes is four times higher than that of crossbred cattle suggesting a preference for these animals. The possible reason for this scenario is a difference in the agricultural pattern between

these areas. Dakshin Kannad and Udupi districts are in the coastal zone and are comparatively more fertile than the interior Shimoga region, a large area of which is covered by forests. Most of the remaining area of this district is rain-fed with irrigation facilities restricted to only two circles (*Taluks*). These South Kanara buffaloes are thus able to thrive better than crossbreds in the comparatively low input system of the Shimoga region.

Buffalo husbandry practices

Housing

Animals are housed close to the human dwellings. In most cases, closed housing is provided (81.8%). In most instances (62.5%), the animals and humans are housed in different parts of the same building, with separate structures in the remaining cases. Most of the constructions are permanent (63.6%) with thatched roofs covered with paddy straw or tiled roofs. Floors are generally uneven without proper drainage facilities (Figure 2). In peri-urban areas, the animals are overcrowded with less than the minimum required floor space of 3.5 square meters (ICAR, 2002) being provided. In rural areas, the practice of allowing the animals to wallow in the nearby water sources is prevalent (66.7%). Mostly the animals wallow around noon after grazing in the fields under a hot sun (Figure 3).

Table 1. Population status of cattle and buffaloes in the breeding tract.

Species	Dhakshin Kannad	Shimoga	Udupi
<i>Buffalo male</i>			
< 1 year	833	14 911	898
1-3 years	1 431	11 289	887
Breedable males	375	1 606	208
Work animals	14 949	11 350	37 731
Breeding and work	584	576	328
Others	123	670	129
<i>Buffalo female</i>			
< 1 year	1 373	26 814	1 865
1-3 years	1 532	25 616	1 378
In milk	2 875	55 993	3 824
Dry females	1 564	37 508	1 473
Heifers	345	6 408	374
Others	78	1 263	92
Total	26 062	194 004	49 187
<i>Cattle</i>			
Crossbred	109 193	59 012	91 053
Indigenous	222 349	465 939	235 004
Total	331 542	524 951	326 057

Feeding

Paddy straw, dry mixed grasses and green grasses are the main sources of roughage. Wheat bran, cotton seed cake, groundnut cake and rice bran are given as concentrates. About half (45.5%) of the farmers provide concentrates to the milking animals; 0.5 to 2 kg of concentrate is usually given to the lactating animals at the time of milking. Some farmers even feed the animals with kitchen wastes and hotel wastes; this practice is more prevalent in the urban areas.



Figure 2. Typical animal house in the breeding tract.



Figure 3. South Kanara buffaloes wallowing in a pond.

Breeding

Breeding of buffaloes is highly disorganized in the breeding tract. Natural service is commonly practiced with only 9.9% of the farmers in the urban and peri-urban areas using artificial insemination. In the rural areas A.I. is completely absent and even the availability of breeding bulls is inadequate with usually no more than two to three animals per village. Although A.I. services are available in some urban areas, semen of South Kanara buffaloes is not available and the farmers have to opt for either Murrah or Surti semen. As a result, the proportion of graded South Kanara buffaloes and non-descript animals are more common in the urban areas.

Physical characteristics

South Kanara buffaloes are well-built medium-sized animals. The coat colour varies from brown to silver grey and black. Their skin is black. Below the knees, the hair colour is generally brownish white. The head is fairly long with a broad forehead. Ears are moderately long and erect. The neck is long with a moderately thick dewlap. Horns are flat, corrugated and curved, projecting backward, sideward and downward at the neck. Shoulders are long and slope smoothly with the body. The barrel is well built and medium in size with a straight and wide back. Legs are strong with hard hooves. The udder is moderately developed with teats of medium size and squarely placed behind the hind legs. The tail is fairly long, thin and flexible ending in a black switch. Figures 4 and 5 show typical male and female South Kanara buffaloes, respectively.

Body measurements

The mean and standard error of eight different body measurements in different age groups are presented in table 2. The body length, height at withers, heart girth and paunch girth ranged from 84 to 188 cm, 93 to 189 cm, 117 to 181 cm and 131 to 196 cm, respectively. Face length and ear length ranged from 37 to 55 cm and 15 to 28 cm respectively. The length of horns varied from 23 to 87 cm in the adult animals.

Table 2. Body measurements of South Kanara buffaloes in different age groups.

Parameters	Young Calves	Calves	Young Stock	Adults	
	(< 2 weeks) (17)	(6 – 12 months) (13)	(> 1 to < 3years) (14)	Male (12)	Female (51)
Body length (cm)	49.4±2.3	60.3±1.6	68.8±2.5	124.5±2.5	112.8±3.3
Height at withers (cm)	61.4±1.4	73.3±1.2	85.5±1.6	119.0±1.0	113.1±2.9
Heart girth (cm)	59.7±1.6	80.2±1.8	97.8±5.0	169.5±0.5	154.2±2.9
Paunch girth (cm)	57.1±1.7	88.0±2.6	104.5±4.6	170.0±2.0	165.2±3.2
Face length (cm)	21.3±0.4	27.9±0.6	31.8±1.3	44.5±1.5	42.5±0.7
Horn length (cm)	-	-	11.5±3.3	51.0±5.0	42.1±2.2
Ear length (cm)	12.7±0.5	16.4±0.3	6.8±0.3	20.5±1.5	20.9±0.6
Tail length (cm)	31.6±1.3	42.3±1.0	51.8±3.8	92.0±2.0	72.5±1.8

Figures in parentheses indicate number of observations.

Table 3. Measures of genetic variation at 10 microsatellite loci in South Kanara buffaloes.

Locus	Allele size range	n _o	n _e	Heterozygosity		PIC	F _{is}
				Observed	Expected		
ILSTS 017	113-125	7	6.15	0.896	0.846	0.817	-0.069
ILSTS 073	143-149	3	2.32	0.000	0.575	0.477	1.000
ILSTS 052	139-185	10	6.98	0.750	0.866	0.841	0.125
HEL 013	168-188	6	3.25	0.563	0.700	0.651	0.188
ILSTS 061	137-163	8	5.73	0.521	0.834	0.803	0.369
ILSTS 058	123-153	10	6.81	1.000	0.862	0.837	-0.172
ILSTS 026	140-150	4	2.67	0.800	0.633	0.582	-0.279
ILSTS 008	129-159	4	1.60	0.455	0.378	0.319	-0.217
ILSTS 095	201-219	6	2.63	0.745	0.626	0.561	-0.203
ILSTS 036	126-170	5	2.33	0.468	0.577	0.542	0.179
Mean	-	6.30	4.05	0.620	0.690	0.643	0.092

n_o = Observed no. of alleles; n_e = effective no. of alleles; PIC=Polymorphism Information Content.

Production performance

South Kanara buffaloes are moderate milk producers and normally give two to seven litres of milk daily. Some animals in villages reach a peak yield of more than 10 litres per day, however. The average daily milk yield was 3.9 ± 0.3 litres ($n=48$) as reported by the farmers. The length of lactation varied from 210 to more than 360 days with an average of 313.6 ± 10.2 days. The lactation milk yield

varied from 420 to 2520 litres with a mean of $1\ 206.8 \pm 110.1$ litres ($n=44$). South Kanara buffaloes have relatively long productive life spans as demonstrated by animals with more than five calvings commonly found in the villages. Age at first calving and calving interval varied from 30 to 60 months and 12 to 36 months respectively. The average age at first calving was estimated to be 41.4 ± 1.9 months ($n=38$) and the mean calving interval was 543.4 ± 51.3 days ($n=36$).



Figure 4. South Kanara bull.



Figure 5. South Kanara she-buffalo.



Figure 6. South Kanara males in wet fields operations.



Figure 7. South Kanara working buffalo.

Utility

South Kanara buffaloes are dual purpose animals used for milk production as well as agricultural operations in wet fields. They are better suited than are local cattle to ploughing and puddling the wet fields meant for paddy cultivation. They are active, fast moving, hardy and can work continuously for four to six hours in the wet fields. Although both males and females are used for the purpose, males are preferred (Figures 6 and 7).

Genetic characterization using microsatellite markers

All 10 microsatellite loci amplified successfully in the samples from South Kanara buffaloes and produced definite banding patterns from which individual genotypes could be ascertained (Figure 8). Different measures of genetic variation estimated in South Kanara buffaloes are presented in table 3. Across the 10 microsatellites studied, a total of 63 alleles were identified. The mean observed number of alleles (6.3) was marginally

lower than that of other Indian buffaloes as reported by Kumar *et al.* (2006).

Eight of the 10 loci with the exception of ILSTS 008 and ILSTS 073 had Polymorphism Information Content (PIC) values of more than 0.5, suggesting that they are informative for population genetic analysis. (Botstein *et al.* 1980). Observed heterozygosity varied between zero (ILSTS 073) to one (ILSTS 058) and the mean observed heterozygosity of 10 loci was less than average expected heterozygosity (0.69). The mean observed heterozygosity of South Kanara buffalo population is higher than that of Marathwada (Kathiravan *et al.*, 2008), Bhadawari and Tarai (Arora *et al.*, 2004) buffaloes while it is slightly lower or comparable to that of other Indian buffalo breeds (Kumar *et al.*, 2006). The average observed heterozygosity estimation in this study thus shows that South Kanara buffaloes are harbouring a good amount of genetic variation.

The test for Hardy-Weinberg equilibrium (HWE) showed that all the 10 loci deviated significantly

(Table 4). Departure from HWE is mostly due to heterozygote deficiency which may result from one or more of the following reasons:

1. presence of null alleles;
2. small sample size; and
3. Wahlund effect i.e. presence of fewer heterozygotes in a population than predicted on account of population subdivision.

In farm animal species, the prevalence of sire lines selected for economic traits leads to increased consanguinity. Such a breeding system produces reduced heterozygosity within a sub-population in a breed. However in our case, although selection and use of extensive A.I. are absent, the availability of very few breeding bulls in the tract might have contributed to increased consanguinity. This is further supported by the estimated mean value of F_{IS} in the population which was positive and equal to 0.09. Thus, the shortage of breeding bulls in the population and confinement of these buffaloes to a small geographical area could be the possible reasons for the deficiency of heterozygotes.

Table 4. Test for Hardy-Weinberg equilibrium at 10 microsatellite loci in South Kanara buffaloes

Locus	No. of observations	DF	Chi Square	P-value
ILSTS 017	48	21	38.14	0.012
ILSTS 073	48	3	103.51	0.000
ILSTS 052	48	45	64.39	0.030
HEL 013	48	15	36.36	0.002
ILSTS 061	48	28	68.79	0.000
ILSTS 058	47	45	63.61	0.035
ILSTS 026	45	6	18.60	0.004
ILSTS 008	44	6	19.34	0.000
ILSTS 095	47	15	48.02	0.000
ILSTS 036	47	10	20.24	0.027



Figure 8. Resolution of genotypes at microsatellite locus ILSTS 058 in a silver stained Urea-PAGE gel.

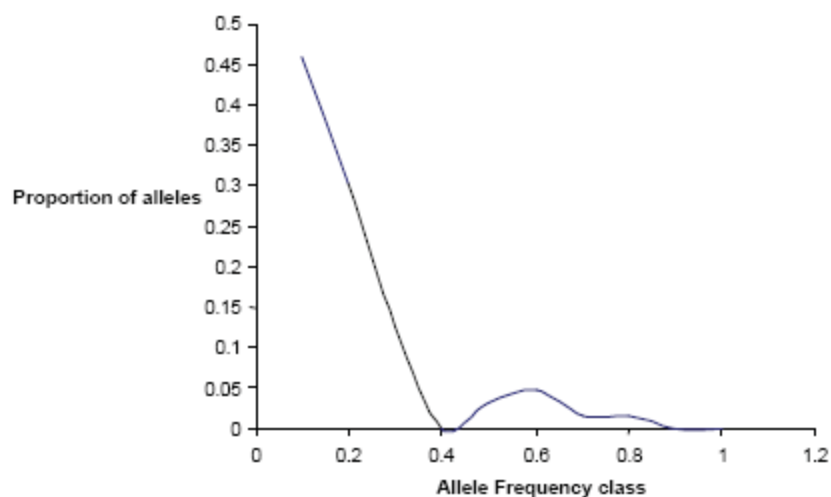


Figure 9. Normal L-shaped curve of distribution of allelic proportions in different allele frequency classes indicating absence of recent bottleneck in South Kanara buffaloes.

The test for BOTTLENECK did not show any significant reduction of effective population size in the recent past. The allele frequency spectrum visualized by the qualitative graphical method of Cornuet and Luikart (1996) is shown in the figure 9. The distribution followed the normal L-shaped form suggesting that the breed had not encountered a genetic bottleneck in the recent past.

Conclusion

South Kanara buffaloes are hardy, dual purpose animals reared for both milk and draught purposes. These buffaloes are able to thrive well in low input systems forming an integral part in the livelihood of farmers in the region. Sufficient genetic diversity was found to exist in the South Kanara buffalo population as revealed by microsatellite data, however steps need to be taken for the genetic improvement as well as conservation of this precious germplasm of the country.

Acknowledgement

The authors are grateful to Dr. R. Raveendra, Deputy Director, Department of Animal Husbandry and Veterinary Services, Shimoga and to Dr. M.G. Govindaiah, Dean, Veterinary College, Shimoga, Karnataka for their help and cooperation during the study.

List of References

- Arora, R., Lakhchaura, R.B. Prasad, M.S. Tantia & R.K. Vijn 2004. Genetic diversity analysis of two buffalo populations of northern India using microsatellite markers. *J. Anim. Breed. Genet.* 121, 111-118.
- Bassam, B. J., G. Coetano-Anolles & P.M. Gressho. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 196, 80-83.
- Botstein, D., R.L. White, M. Skolnick & R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32, 314-331.
- Cockrill, W.R. 1974. The husbandry and health of domestic buffalo. Food and Agriculture Organization, Rome, Italy.
- Cornuet, J.M. & G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144, 2001-2014.
- Gunn, W.D. 1909. Cattle of Southern India. Vol. III, Bulletin No. 60. Department of Agriculture, Madras.
- ICAR. 2002. Handbook of Animal Husbandry. 3rd Edition, Indian Council of Agricultural Research, New Delhi, pp. 364-383.

.....

Kathiravan, P., B.P. Mishra, R.S. Kataria & D.K. Sadana. 2008. Evaluation of genetic architecture and mutation drift equilibrium of Marathwada buffalo population in Central India. *Livest. Sci.* (In Press) doi. 10.1016/j.livsci.2008.06.023.

Kumar, S., J. Gupta, N. Kumar, K. Dikshit, N. Navani, P. Jain & M. Nagarajan. 2006. Genetic variation and relationships among eight Indian riverine buffalo breeds. *Molecular Ecology*, 15: 593-600.

Littlewood, R.W. 1936. *Livestock of Southern India.* Government of Madras, India.

Navani, N, P.K. Jain, S. Gupta, B.S. Sisodia & S. Kumar. 2002. A set of cattle microsatellite DNA markers for genome analysis of riverine buffalo (*Bubalus bubalis*). *Animal Genetics*, 33, 149–154.

Piry, S., G. Luikart & J.M. Cornuet. 1999. Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* 90, 502–503.

Sambrook, J., E.F. Fritsch & T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Yeh Francis, C., R.-C. Yang Boyle, B.J. Timothy, Z.-H. Ye & X. Mao Judy. 1999. POPGENE version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada <www.ualberta.ca/~fyeh>.