Analysis of Genetic Diversity of Indian Buffalo Breeds by DNA Markers

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Abstract: Buffalo is an important species contributing significantly to Indian economy. There is urgent need to study genetic diversity between and within breeds using appropriate set of microsatellite markers which can be further supplemented by using Genome sequencing data, phonemics and high density arrays. Animal resources are valuable assets of a country. Present buffalo breeds have gene pools of wide representation and valuable combination of genes. Genetic variability also provides the opportunity for tracing the history of populations, species, and their ancestors through DNA based markers. For the overall breed improvement and to meet future challenges there is an urgent need to characterize buffalo breeds. It is our responsibility to conserve, preserve and maintain the animal genetic diversity.

Keyword: Genetic diversity, buffalo bread, DNA markers.

1. INTRODUCTION

It is generally believed that buffaloes were first domesticated in the Indus civilization 5000 years ago [1]. However, according to Chen and Li [2], this species was domesticated in China during the fifth millennium BC. The present domesticated buffaloes are the descendants of Bosarni buffaloes found in wild state even today in the North-Eastern parts of India.

Water buffalo (*Bubalus bubalis*) have been divided into swamp and river buffalo based on morphological, behavioral, geographical criteria and number of chromosomes. River buffaloes (*B. bubalis bubalis*) are generally large in size, mostly with curled horns, prefer to enter clear water, have 50 chromosomes. They are primarily used for milk production and, mainly found in India, Pakistan and some of the west Asian countries. Swamp buffaloes (*B. bubalis carabanesis*) are mostly stocky animals with marshy land habitats and have 48 chromosomes. They are primarily developed for draught power in paddy fields and transport. Swamp buffaloes are mostly found in South East Asian countries and few animals are also available in north eastern states of India. The difference in number of chromosomes is due to a telomere- centromere tandem fusion between two chromosomes in river buffalo [3].

India is gifted with rich genetic resources in terms of its buffalo breeds. River buffaloes are the main stay of the dairy industry in India. The buffalo is an integral part of agriculture in India, providing a source of milk, meat, skin, hides, fertilizer, fuel, and draft power. The buffalo is the main milk producing species in India and its contribution to the total milk production larger than that of cattle. India is the home tract for some of the best buffalo breeds. India has been the centre of dispersion of reputed breeds for improvement of the species elsewhere in the world.

Share of buffalo milk occupies the highest position in Indian dairy industry, contributing about 56 percent of total milk (110 MT) produced by its varied population which is less than half that of the cattle population [4]. It contributes substantially to economy of the country by producing 60.9 million metric tons of milk, over 1.6 million metric tons of meat. Apart from that about 7.5 million male and 0.3 million female buffaloes are also used as draught power.

2. IMPORTANCE

The main evolutionary forces of mutation, selection, and genetic drift have created a vast diversity which ended in the formation of many well-defined breeds used for a variety of purposes with differing levels of performance. The knowledge of the genetic variability and process that underlie its origins and maintenance is vital to provide critical insights into the structure and dynamics of populations. Genetic variability also provides the opportunity for tracing the history of populations, species, and their ancestors through DNA based markers. For the overall breed improvement and to meet future challenges there is an urgent need to characterize buffalo breeds. Detailed knowledge of genetic variation within and among different breeds is very important for understanding and improving traits of economic importance. Hence, the future improvement is dependent on genetic variation present within breeds

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Table 1: Trend of Buffalo Population (Millions) in Different Countries

Country	Year 1997	Year 2007	Change (%)
India	89.91 (56.48)	98.70 (55.7)	0.98
Pakistan	20.83 (13.08)	28.16 (15.88)	3.51
China	21.73 (13.65)	22.72 (12.81)	0.45
Asia	154.91 (97.31)	171.86 (96.96)	1.09
Rest of the World	4.28 (2.69)	5.38 (3.04)	2.58
world	159.19	177.28	1.13

Source: Fao.org/stat 2008 (Figures in parenthesis are %).

and between breeds variation.

3. BUFFALO STATISTICS

The estimate of world buffalo population is approximately 177.247 million dispersed in 42 countries, out of which 97 percent (171 million) are found only in Asia (Table **1**, FAO [5]). India has approximately 55.7 percent (98.7 million) of the total world buffalo population. From 1997 to 2007, the world buffalo population increased by approximately 18 million, which is annual increase of about 1.13 per cent. This increase is due to increase in population in Asian countries. In India, percent increase was about 1.0 as compared to 1.09 in Asia and 2.58 in rest of the world.

4. BREEDS OF RIVER BUFFALOES

It is essential to understand the genetic architecture and relationships among different breeds so as to design rational breeding strategies for optimum utilization and conservation of available genetic variability in India. The diversity within domestic livestock species is perceived generally in terms of differences that are referred to as breed. Turton [6] defined breed as “a homogenous, sub-specific group of domestic livestock with definable and identifiable external characters that enable it to be separated by visual appraisal from other similarly defined groups within the same species, or a homogenous group where geographical separation from phenotypically similar groups has to general acceptance of its separate identity”. Each breed is the product of mutation and genetic drift, as well as separate adaptation and evolution, with differing selection pressures imposed by climate, endemic parasites and diseases, available nutrition and criteria imposed by man.

These breeds have been developed mainly as a result of farmers’ wisdom over the past thousands of years [7]. The Toda breed is maintained by a Toda tribe in the Nilgiri hills is endangered as this breed has less than 1000 heads. Approximately 70 percent buffaloes in India are nondescript as these animals do not resemble any of the ten well characterized breeds. The well recognized breeds in India [8] are:

4.1. Breeds

1. ***Murrah:*** The breeding tract of this breed is Rohtak, Hisar and Jind of Haryana, and Nabha and Patiala districts of Punjab. The colour is usually jet black with white markings on tail, and face. It has short horns and tightly curved. It has long tail reaching up to the fetlocks. The average milk yield per lactation is 1500 to 2500 kg.

2. ***Bhadawari:*** It is found in the Bhadawari Tehsil in Agra district and Etawah district of Uttar Pradesh and Gwalior district of Madhya Pradesh. Its colour is usually light or copper. The tail is long with black and white or pure white markings reaching up to fetlock. The average milk production is 800 to 1,000 kg.

3. ***Jaffarabadi:*** Its breeding tract is Kutch, and Jamnagar districts of Gujarat. The colour is usually black. The forehead is very prominent. The horns are heavy, inclined to droop at each side of the neck and then turning up. The average milk yield is 1,000 to 1.200 kg.

4. ***Surti:*** The breeding tract of Surti is Kaira and Baroda districts of Gujarat. The colour is black or brown. The head is long with prominent eyes. The horns are sickle shaped, moderately long and flat. Its milk yield ranges from 900 to 1,300 kg.

5. ***Mehsana:*** Its breeding tract is Mehsana, Sabarkanda and Banaskanta districts of Gujarat. Its colour is usually black to grey, with white

markings often on face, legs or tail-tips. The milk yield is 1,200 to 1,500 kg.

6. ***Nagpuri:*** It is found in Nagpur, Akola and Amrawati districts of Maharashtra. The horns are long, flat and curved, bending backwards on each side of the back almost to shoulders. Long and thin face and has comparatively short. The milk yield is 700 to 1.200 kg per lactation.

7. ***Nili-Ravi:*** The breed is found in Sutlej valley in Ferozpur district of Punjab and in the Sahiwal district of Pakistan. Its colour is black with white markings on forehead, face, muzzle and legs. The peculiarity of the breed is the wall eyes. Small and tightly coiled horns. The milk yield is 1,500 to 1,850 kg per lactation.

8. ***Godavari:*** The home tract of Godavari breed is Godavari deltaic areas and, Krishna deltaic areas. The colour is predominantly black with a sparse coat of coarse brown hair. Its horns are short, flat, curved slightly downward, backward and then forward with a loose ring at the tip.

9. ***Toda:*** These animals of this breed are quite distinct from other breeds and are native to Nilgiri hills. The head is heavy with horns set wide apart, curving inward, outward and forward. It has thick hair coat all over the body.

10. ***Pandharpuri:*** It is an important breed in south east Maharashtra. Its horns are very long, curved backward, upward and usually twisted outwards. Its colour varies from light black to deep black.

4.2. Breed Distribution State Wise

Buffaloes are spread over almost all states with varying density in India. Northern and western states comprising Haryana, Punjab, Uttar Pradesh, Rajasthan, Gujarat and Maharashtra have the majority of the population (72%). Most of the milch breeds are also found in these regions. Among different states, Uttar Pradesh has highest buffalo population (22914,000) followed by Andhra Pradesh (10630,000) and Rajasthan (10416,000) (Table **2**). However, highest growth percent in buffalo population was recorded in Haryana (3.80) followed by Gujarat (3.55) and Rajasthan (3.45). The large population of non-descript buffaloes are found in Madhya Pradesh, Bihar and Jammu & Kashmir.

5. REASONS FOR LOSS OF ANIMAL GENETIC RESOURCES

There are a number of factors which can cause loss to animal genetic resources. However, the common reasons for loss are:

- Use of a few breeds by artificial insemination and embryo transfer, which lead to the erosion of genetic resources [9].
- Poor agricultural policies,
- Changing market requirements,

Table 2: Number of Buffaloes (Thousands) and Annual Growth Rate in Different States

S. No	States	1992	2003	Growth rate (%)	Main breeds
1	Haryana	4373	6035	3.80	Murrah, Murrah grade
2	Gujarat	5268	7140	3.55	Jaffarabadi, Surti, Kundi, Murrah grade
3	Rajasthan	7743	10416	3.45	Surti, Murrah grade
4	Uttrakhand	—	1228	2.04	Tarai, Murrah grade
5	Andhra Pradesh	9154	10630	1.61	Godavari, Jerangi, Ganjam, Murrah grades
6	Punjab	5238	5995	1.44	Nili-Ravi, Murrah, Nili, grade, Murrah grade
7	Uttar Pradesh	20066	22914	1.42	Bhadawari, Murrah, Tarai, Marathwada, Murrah grade
8	Maharashtra	5448	6145	1.28	Pandharpuri, Nagpuri, Marathwada, Murrah, Murrah grade
Total		57290	70503	2.31	
Other states and Union Territories		26109	27419	0.46	
Grand total		83499	97922	1.57	

Source: Department of Animal Husbandry & Dairy, Govt. of India.

- Natural disasters,
- Political unrest and war
- Deterioration of ecosystems,
- Movement of high producing buffaloes to metropolitan cities from the breeding tract to meet the milk requirement further leads to genetic erosion of valuable germplasm due to slaughter of these animals after completion of lactation.

Lack of genetic diversity in a population/breed affects its performance by:

- Fitness of the population is reduced,
- Achievement of breeding objectives is affected,
- Decline in number or even breed extinction,
- Infectious diseases spread more easily,
- Sustainability of rare domestic breeds can be endangered,
- Restrict the options available to meet unpredictable future requirements.

Loss of variation within breeds is continuously countered by the introduction of new variation through mutation [10], but the variation among breeds cannot be readily regenerated. So by using genomic techniques, genetic variation can be assessed and accordingly remedial measures be taken to preserve genetic diversity for the future. The genetic diversity of low-production breeds is likely to contribute to current or future traits of interest [11, 12], they are considered essential for maintaining future breeding options.

6. POPULATION GENETICS ANALYSIS

6.1. Within-Breed Analysis

Expected heterozygosity or allelic richness within breeds indicates the influence of drift on breed diversity, where decreased heterozygosity is associated with increased drift. Differences between expected and observed heterozygosity as well as departure from Hardy-Weinberg equilibrium indicate nonrandom mating or the existence of population substructures [13]. The presence of inbreeding can be tested by F statistics [14], in particular by testing if the *F*IS parameter is significantly greater than zero.

6.2. Among Breeds Analysis

Total diversity can be partitioned in a within-breed and among-breeds component of variation. These components and others can be quantified by analysis of molecular variance (AMOVA) analysis [15] and reflect history and breeding practices. Normally, 50 to 90 per cent of the total diversity corresponds to the within-breed component which dependent upon the breeds sampled. The calculation of genetic distances among breeds is based on gene frequency data followed by visualization of relationships in trees, and networks [16]. Nei's standard genetic distance *D*s has been used most commonly in studies of natural populations in evolutionary genetics and has the advantage that it is linear in time [17]. Distance measures based on Wright's *F*ST statistic, which may be estimated *via* the *D*R distance [18, 19] may be more appropriate for short-term evolution such as the divergence between livestock breeds.

7. CHARACTERIZATION OF ANIMAL GENETIC RESOURCES

Domestic animal diversity is main component of global biodiversity. Different species of domestic animals and poultry contribute to meeting the needs of human population by providing meat, fibre, milk, eggs, draught animal power, skins, and manure. More than 7000 breeds and strains [9] constitute the animal genetic resources (AnGR) that are of crucial significance for food and agriculture. The present pattern of diversity of AnGR is the result of a long and complicated history, particularly by local adaptation, artificial selection, mutations and genetic drift creating diversity with vast differences in appearance, physiology and economic traits leading to the emergence of breeds. One of the Strategic Priority Areas of the *Global Plan of Action* is the characterization; inventory and monitoring of trends of AnGR diversity in order to properly assess value of breeds to use in breeding programmes. Autosomal microsatellite markers allow estimation of expected heterozygosity and allele frequencies which may reveal effects of genetic isolation, inbreeding, population bottlenecks, introgression and subdivision in within breeds. However, trees, networks, clustering diagrams can be used to establish the relationships between breeds.

7.1. Molecular Characterization of Diversity and its Maintenance

Molecular data have become progressively more relevant for the characterization of domestic genetic

diversity [20]. In 1993, a FAO working group formulated the *Secondary Guidelines: Measurement of Domestic Animal Diversity* (MoDAD) for characterization of AnGR [21] with recommendations for the molecular analysis of domestic animal diversity on a global scale (http://www.fao.org/docrep/meeting/ 022/am652e.pdf).

FAO MoDAD report succeeded in creating awareness to monitor AnGR diversity and establishing a standard procedure for molecular genetic characterization. The work in many countries to characterize locally available breeds is undertaken. This proposal has helped to motivate many national and international projects supported by International Livestock Research Institute (ILRI) and the World Bank, which help in achieving the objectives of MoDAD. Novel combinations of alleles at different loci can be tested by designing appropriate breeding schemes. The maintenance of a diverse range of genetic backgrounds essential to provide wide sources of variation as some of these novel allele combinations may result in superior genotypes whose performance may be beyond that would not be possible by planned selection.

Comprehensive knowledge of the breed characteristics, its population size and structure, geographical distribution, the performance in economic traits, and within- and between-breed genetic diversity is required to have effective management of farm animal genetic resources.

8. BREED CHARACTERIZATION

Awareness of the value of genetic resources has stimulated the study of genetic diversity of native breeds. For improvement of economic traits and for breed characterization, detailed knowledge of genetic variation within and among breeds is very important. Various types of markers such as morphological, chromosomal, biochemical and DNA markers are used for this purpose. Morphological / phenotypic (e.g., coat color, horn types or other features) markers usually have low level of variation. Biochemical markers have been tried extensively but were not found encouraging, as they often express low level of polymorphism, are sex limited and age dependent. Further these markers reflect variability in the coding sequences, which constitute less than 2 percent of the total genome [22].

8.1. Phenotypic Markers

Phenotypic / morphological markers include colour, shape, size and performance of the individual/breed in different traits of economic importance. They are usually easy to observe, but it is difficult to have a large number of them. One of the most obvious ways to characterize breeds is by their coat colour, e.g. Murrah has jet black colour. The first step in characterization is to identify the homogenous groups (breeds) within the heterogeneous assemblage and establish how the breeds are themselves grouped**.** The different breeds are also characterized on the basis of their performance in different traits of economic important.

8.2. Protein Markers

Proteins are the product of gene expression. Different alleles of genes may result in proteins with different amino acid compositions, and size. Differences in charge and size can be easily detected using gel electrophoresis and can be used as markers. As specific gene products, could indicate genetic specify of genotypes, and therefore could be used as markers for characterization of breed and to resolve taxonomic relationships. Barker [23] compared the protein polymorphism observed on the basis of 25 different proteins and with the polymorphism detected by using 21 microsatellite markers in river and swamp buffalo (Table **3**).

F statistics estimates show no significant departure from Hardy Weinberg expectation for either buffalo

Table 3: F Statistics for River and Swamp Buffalo Estimated Using 25 Protein Coding Loci and 21 Microsatellite Loci

	FIS	FST	FIT
Protein coding loci			
River	-0.068 (0.048)	0.108 (0.036)	0.048 (0.059)
Swamp	-0.004 (0.062)	0.182 (0.041)	0.181 (0.083)
Microsatellites			
River	0.031 (0.028)	0.038 (0.008)	0.068 (0.029)
Swamp	0.047 (0.027)	0.168 (0.018)	0.207 (0.034)

types whether based on protein coding loci or on microsatellites. *FIT* is the deviation from Hardy-Weinberg proportions in the total population. *FIS* is the average deviation from Hardy-Weinberg proportions in subpopulations. *FST* is Wright's standardized variance.

8.3. DNA Markers

Rapid developments in molecular genetics have made it possible to detect genetic polymorphism at the DNA sequence level, i.e. using DNA markers. DNA marker is a DNA sequence whose pattern of inheritance can be followed generation after generation and it must be polymorphic.

The features of DNA markers which make them as unique are:

- They exhibit high level of polymorphism,
- Detection is independent of tissue, age, environment or sex,
- Distributed throughout the genome,
- Detection is efficient and reliable.

DNA markers most widely used to study genetic diversity in various species of livestock are randomly amplified polymorphic DNA (RAPD-PCR), microsatellites, and single nucleotide polymorphism **(**SNP). These markers represent non-coding region of the genome, and hence they are selectively neutral.

8.3.1. Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR)

RAPD-PCR is a means of detecting polymorphisms for genetic mapping and strain identification [24, 25]. The technique is generally faster, less expensive than any other method for detecting DNA sequence variation and does not require prior sequence information. The fact that RAPDs survey multiple loci in the genome makes the method attractive for analysis of genetic distance and phylogeny reconstruction [26]. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of molecular characterization of the genome of the species in question [27]. It is a powerful tool in DNA fingerprint analysis of various animal species, gene mapping studies, population analysis and identification of breeds. The analysis for RAPD markers is quick and simple. In RAPD, typically, 5–12 bands can be observed per primer, which are distributed throughout the genome.

RAPD technique was employed to analyze the genetic variation in cattle and buffalo breeds. The mean average percent difference (MAPD) revealed lower values (24.16 ± 3.55) between Murrah and Surti breeds of buffaloes than Jersey crossbred and Ongole breeds (28.10 ± 10.53) of cattle according to Aravindakshan and Nainar [28]. Genetic identity index pooled over the 11 random primers was 0.596±0.037 between Bhadawari and Murrah breeds. The highest MAPD estimate (53.9) between the two breeds was obtained with the primer BG27. Saifi *et al.* [29] also reported the primers OPA-04 and BG-15 resolved a band of 460 bp, which was present only in animals of Bhadawari breed whereas primers OPA-14, BG-27 and BG-28 produced Murrah specific fragments of 730 and 1230 bp.

Sodhi *et al.* [30] indicated high level of genetic similarity between Murrah and Nili-Ravi breeds by using RAPD analysis. Two breed specific RAPD alleles were observed in each of Murrah (OPA02 and OPG16) and Nili-Ravi (OPG09) DNA pools. Within breed, band sharing values were relatively greater than those of interbreed values.

Sonika *et al.* [31], Barwar *et al.* [32] and Anand *et al.* [33] used RAPD to estimate genetic diversity in Murrah, Nili-Ravi Bhadawari breeds. Sonika *et al.* [31] reported within breed genetic distance of 0.26 and 0.18 in Murrah and Nili-Ravi breeds, respectively, as compared to genetic distance of 0.53 between breeds. Four primers viz. OPB-06, OPI-01, OPI-04 and OPI-07 resolved Murrah breed specific amplicons. However, Barwar *et al.* [32] observed higher within genetic similarity of 0.81 and 0.80 in Murrah and Bhadawari breed, respectively, while between breed genetic similarity was 0.31. Six random primers OPU01, OPU02, OPU05, OPU07, OPU14, OPV14 in Murrah breed and five random primers OPU05, OPU07, OPU14, OPU19, OPV14 in Bhadawari breed were found to be breed specific. Anand *et al.*, (2009) reported primers OPU-05, OPU-14, OPU-19, OPV-14, OPV-20 in Murrah, OPU-01, OPU-05, OPU-07, OPU-14, OPV-14 in Bhadawari and OPU-02, OPU-05, OPU-19, OPV-01, OPV-14 in Nili-Ravi resolved breed specific bands. However, RAPD-PCR has certain limitations. RAPDs are dominant markers with limited reliability. Detection of polymorphisms is still limited.

8.3.2. Microsatellites Markers

The microsatellite markers are highly polymorphic, distributed throughout the genome, locus specific and co-dominant. They have an edge over other genetic markers for comparative studies of evolution, genetic variation, parentage assessment and gene flow. Microsatellite markers have been used for studying polymorphism and genetic diversity in many livestock species [34]. They are simple DNA sequences (*e.g.* AC), usually 2 or 6 bases long, repeated a variable number of times in tandem. They are easy to detect with PCR and a typical microsatellite marker has more variants than those from other marker systems.

The FAO and the ISAG/FAO Advisory Group on Animal Genetic Diversity have proposed panels of 30 microsatellite markers for nine major livestock species (www.globaldiv.eu/docs/Microsatellite%20markers.pdf). The microsatellite marks recommended by FAO for the genetic distancing studies in buffaloes are AGLA293, CSSM038, CYP21, BMC1009, CSSM041, BRN, CSSM008, CSSM043, ETH121, CSSM013, CSSM045, ERH131, CSSM015, CSSM046, HMH 1R, CSSM019, CSSM047, MGTG4B, CSSM022, CSSM075, TGLA48, CSSM029, CSSM060, TGLA57, CSSM032, CSSM061, TGLA126, CSSM033, CSSM038, TGLA227, CSSM036, CSSM062, TGLA263. Majority of the markers are common for both cattle and buffalo breeds. Ideally, all 30 markers should be used for characterization of populations. Working group recommended the following criteria to select appropriate microsatellites

- Free access to microsatellite markers.
- Microsatellite loci should be present on different chromosomes.
- Markers should follow Mendelian inheritance.
- Each locus should exhibit at least four alleles.
- Marker loci which can be used on several related species are preferable, e.g. cattle primers are used in buffalo.

Vijh *et al*. [35] generated data on 24 microsatellite loci from 3 buffalo populations viz. Bhadawari, Tarai and Kerala buffaloes was subjected to analysis for estimation of genetic distances. The dendrograms were prepared using both UPGMA and NJ algorithms. The Tarai and Bhadawari populations were close to one another and this was expected because of the contiguity of their breeding tract resulting in increased gene flow.

Kumar *et al.* [7] identified the Toda, Jaffarabadi, and Pandharpuri breeds as one lineage each, and the Bhadawari, Nagpuri, Surti, Mehsana and Murrah breeds as admixture on the basis of microsatellite markers. Analysis of molecular variance refuted the earlier classification of these breeds proposed on the basis of morphological and geographical parameters. The Toda buffaloes, reared by Toda tribe, represent an endangered breed from the Nilgiri hills in South India. Divergence time of the Toda buffaloes from the other main breeds, calculated from Nei's standard genetic distances, suggested separation of this breed approximately 1800–2700 years ago.

One breed specific marker was found in each of Mehsana (BM1818) and Bhadawari (ILSTS030) and four were found in Jaffarabadi (BM1818, ILSTS030, ILSTS054 and ILSTS011). Genetic distance (Ds) between the Mehsana and Bhadawari breed was the maximum (0.29), followed by Murrah and Mehsana (0.27), and Nili-Ravi and Bhadawari (0.26). The lowest Ds was found between the Jaffarabadi and Nagpuri breeds which was only 0.05. The highest divergence time of 1318 years was established between Mehsana and Bhadawari breeds whereas it was found to be lowest (272 years) between the Jaffarabadi and Nagpuri breeds according to Shukla *et al.* [36].

Vijh *et al.* [35] studied diversity and differentiation on 527 animals belonging to 10 recognized breeds and 2 additional populations of Indian buffalo by using 22 microsatellite loci. Relationships among buffalo breeds and populations were estimated based on genetic distances. The Bayesian analysis grouped 12 populations into 8 distinctive clusters. Geographically close breeds clustered together, except for the Jaffarabadi and Murrah, which were not in geographic contiguity. The phylogenetic relationship based on microsatellite loci supported the breed classification based on body size. The Toda breed, which is considered to be endangered, had genotypes similar to those of the surrounding buffalo populations.

Rupinder *et al.* [37] observed heterozygosity 0.49 and 0.53 in Bhadawari and Murrah breed, respectively, while mean heterozygosity in both breeds was 0.51. The average PIC value over all loci was 0.51 in Bhadawari and 0.52 in Murrah breeds. The mean F_{IS}, F_{IT} and F_{ST} for all loci was – 0.12, 0.16 and 0.04, respectively. The standard genetic distance (D_s) between Bhadawari and Murrah breeds was 0.13 and the genetic identify was 0.88. Four private alleles in Bhadawari breed and three private alleles in

Murrah breed were also observed. Bhuyan *et al.* [38] observed mean heterozygosity in Murrah found to be 0.5438. Both observed and expected heterozygosity was above 0.5 which shows that there is sufficient variability in the population and reflects presence of large number of polymorphic loci in Murrah breed.

Jakhesara *et al.* [39] estimated mean allele diversity (9.63), mean observed heterozygosity (0.66), mean expected heterozygosity (0.77) and mean polymorphism information content (0.738) values showed substantial within-breed genetic variability on the basis of 11 microsatellite markers in Mehsana breed.

Mishra *et al.* [40] constructed phylogenetic tree using chord distance estimates revealed the distinctness of Banni and Jaffarabadi buffaloes from other river buffalo breeds of the region. Multi dimensional scaling display of pair-wise FST values revealed the close proximity of Mehsana, Surti and Murrah buffaloes while Banni and Jaffarabadi buffaloes were placed separately. This genetic structure was further supported by Bayesian clustering analysis which revealed three inferred clusters with Banni and Jaffarabadi forming separate clusters each while the remaining three breeds viz. Mehsana, Surti and Murrah together formed a single cluster. The results thus revealed the genetic uniqueness of Banni buffalo among other buffalo breeds of the region.

8.3.3. Single-Nucleotide Polymorphisms (SNPs)

SNP occurs due to change in the nucleotide at a particular location within the genome of a species / breed/population. SNP usually have only two alleles. The potential number of SNP markers is very high, and possible to find them throughout the genome. They may represent either neutral or functional genetic diversity. http://www.fao.org/docrep/ meeting/022/ am652e.pdf

A variety of methods can be used for assaying of SNP like, KASPar® and TaqMan® assays by which many animals are to be genotyped. Alternatively, microarrays can be used to obtain genotypes at low cost.

Advantages of SNP over microsatellites

- Allele scoring is unambiguous.
- The cost of genotyping per SNP is much less than with microsatellites.
- The high number of SNPs can allow a description of individual and breed relationships with high accuracy.
- SNP can reveal functional, as well as neutral genetic variation.
- High density SNP screens can identify multiple SNPs in linkage disequilibrium.

There is need to development of high-density SNP panel for studying genetic diversity studies in buffaloes. Their analysis will yield a large amount of data, which will require additional computing infrastructure for bioinformatics, genetic analysis and data management. Further in developing high density panels, care is required to have unbiased estimation of relationships among breeds by avoiding SNPs that may be monomorphic in certain breeds.

8.3.4. Mitochondrial DNA Markers

The complete water buffalo (*Bubalus bubalis*) mtDNA is 16355 bp in length. The length of the genome appears to be highly specific, as no tandem repeats have been found [41, 42]**.** This is in contrast to those observed in horse, and sheep were the length of the mitochondrial genome is highly influenced by the occurrence of a different number of tandem repeat [43]. Mitochondrial markers have been instrumental in identification of wild ancestors, localization of domestication centres and reconstruction of colonization and trading routes [11, 20, 44]. Most studies with mtDNA target the hypervariable control region (D-loop). Domestic river and swamp buffalo arose independently based on differences in their cytochrome b sequences [45, 46] and they also suggested that these two types were domesticated independently.

8.3.5. Y-Chromosomal Markers

Y-chromosomal variation is a powerful tool to trace gene flow by male introgression [47]. It is the most powerful marker in human population genetics and is used more and more in domestic animal species.

9. DATABASES/SOFTWARE

9.1. Molecular Databases

Large scale sequencing projects are running on different species and the sequence data generated are usually deposited in any one of three major databases like GenBank, which would be required for assessment of genetic diversity. NCBI databases does allow for the

submission of individual, even redundant, sequences, including microsatellites and SNPs (both stored in dbSNP; http://www.ncbi.nlm.nih.gov/projects/SNP/) [48]. Status of INRA buffmap Database on September 2011 is microsatellites 66; assigned microsatellites 15; polymorphisms 39; 323 breed polymorphism records on 3 breeds (http://dga.jouy.inra.fr/cgi-bin/lgbc/summary.operl?BASE= buffalo). In addition, numerous databases on livestock genomics are available whose contents range from genome maps including annotations, SNPs, QTL data, whole genome shotgun libraries and microsatellites to extensive link lists. These databases remain valuable resources for the development of markers as well as for fundamental research on livestock animals.

9.2. Breed Description Databases

To create awareness through information transmission is considered an important component in conserving and utilizing of animal genetic resources. Accordingly, three groups of databases can be identified to address issues often with different perspectives:

First, breed societies maintain websites to describe their populations, to advertise their own genetic resources. Second, national websites have been created by each country with a complete coverage of breeds which are part of national heritage. Third, at the international level like The _Breeds of Livestock_ website run by the University of Oklahoma (http://www.ansi.okstate.edu/breeds/) describes a respectable number of breeds of livestock, including buffalo, cattle, goats, horses, sheep, pigs, camelids and poultry, with differing degrees of detail.

9.3. Whole Genome Sequencing of Water Buffalo

Genome sequencing in farm and other animals has advanced significantly in recent years. The sequence data are available in the public domain as on March 1, 2010, there are 2,509,850 cattle, 3,237,358 pigs, 2,195,532 chicken, 6,259,791 and sheep, 470,489 sequences available in the GenBank nucleotide databases (http://www.ncbi.nlm.nih.gov). Whole genome sequencing has been completed in buffalo, cattle, horse, and chicken. A total of 66,935 nucleotide sequences for the water buffalo have been deposited in the GenBank database and are mainly 64,212 whole genome shotgun sequences, while the rest includes 974 mitochondrial genomic sequences and 1,748 nuclear gene/genomic DNA sequences [49]. The latter may be further classified into nuclear gene-related sequences (981), satellite-related sequences (689) and others (78). The 689 satellite sequences involve satellite (17), microsatellite (311) and minisatellite (361) sequences, respectively.

The Buffalo genome was sequenced at 17X-19X depth and 91-95% coverage through collaboration between the National Bureau of Animal Genetic Resources (NBAGR) in Karnal; the Central Institute for Research on Buffaloes (CIRB) in Hisar; and the Animal Science Division of the Indian Council of Agricultural Research (ICAR). http://210.212.93.85/ buffalogenome.html. The assembly has 185,150 contigs with the median contig length of 2.3 Kb and the largest contig length of 663 Kb. This assembly of the Water Buffalo is the first deep sequencing project that provides the resources to better understand the genomic basis of adaptable traits and genetic variation that distinguishes buffalo from cattle [50].

9.4. Database Management

NBAGR, Karnal is the central agency dealing with livestock diversity, has of late initiated some schemes to continue or revive the use of purebred breeds. A broad legislation on biodiversity, currently being formulated which include measures to check the erosion of animal diversity. The NBAGR should prepare a breed directory and an animal watch list for regular monitoring of livestock genetic resources by Department of Animal Husbandry and Dairying, Government of India.

FAO has developed a Domestic Animal Diversity Information System (DAD-IS) (http://www.fao.org/dad-is/); providing a summary of national breed level information on various aspect like its origin, population, special characteristics, morphology, performance of breeds and their risk status. DAD-IS has been recognized as an early warning tool for animal genetic resources (AnGR). The recently adopted Global Plan of Action for Animal Genetic Resources, calls on FAO to continue to develop DAD-IS to strengthen these roles.

9.5. Bioinformatics Software for Phylogenetic Analysis

Bioinformatics software mostly freely available for phylogenetic analysis are:

a. **DISPAN** is used to **c**alculate diversity statistics, genetic distances and constructs phylogenetic trees.

(http://homes.bio.psu.edu/people/Faculty/Nei/Lab/dispan2. htm).

b. **MEGA** is used to **ca**lculate a wide variety of population genetics statistics and convenient tree reconstruction program. (http://www.megasoftware.net/).

c. **PAUP** It is a comprehensive package handling nexus files for tree reconstruction. (http://paup.csit.fsu.edu/).

d. **PHYLIP** is also comprehensive package requiring its own file format for tree reconstruction. (http://phylip.com).

e. **SPLITSTREE** is used to construct neighbour-joining tree. It offers many graphical output options. (http://www-ab.informatik.uni-tuebingen.de/software/ splitstree4/welcome.html).

f. **TREECON** is used to draw phylogenetic trees. (http://bioinformatics.psb. ugent.be/software/details/TREECON).

10. FUTURE PROSPECTS

In the near future, new technologies such as high throughput SNP typing or even whole-genome sequencing are likely to revolutionize our perception into the diversity and uniqueness of breeds, with the ultimate objective of gaining a fuller understanding of the molecular basis of functional diversity. Phenomics will enable the development and adoption of high-throughput and high-dimensional phenotyping, and is the natural complement to genome sequencing. Phenomic-level data are necessary to understand which genomic variants affect phenotypes, to understand pleiotropy and to furnish the raw data that are needed to decipher the causes of complex phenomena, including health, yields, disease and evolutionary fitness [51]. This will help in better management of animal genetic resources and the breeds will be in better position able to face the future eventualities. Genetic resources banking (germplasm banks) are essential for species conservation and maintaining diversity and to enable the recovery of rare breed after total wipe out. Conservation of the genetic diversity of a particular species and involves a wide representation of genes of the species without conservation of the phenotype with an aim to preserve a desirable combination of gene representing of a particular breed.

An overall integrated view of an entire set of biological molecules involved in complex biological processes is emerging. New "–omics" scientific disciplines and at a still higher level of complexity, systems biology [52]. The inquiry into the biological complexity is a new frontier which requires high-throughput molecular technology, high computer speed and memory, new approaches to data analysis, and integration of interdisciplinary expertise (ftp://ftp.fao.org/docrep/fao/010/ a1250e/a1250e17.pdf).

11. CONCLUSION

Buffalo is an important species contributing significantly to Indian economy. There is urgent need to study genetic diversity between and within breeds using appropriate set of microsatellite markers which can be further supplemented by using Genome sequencing data, phonemics and high density arrays. Animal genetics resources are valuable assets of a country. Present buffalo breeds have gene pools of wide representation and valuable combination of genes. It is our responsibility to conserve, preserve and maintain the animal genetic diversity.

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