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Genetic characterization of farm animal genetic resources of India: A review

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ABSTRACT

India's vast native animal genetic resources have evolved over generations to adapt to the different agro-climatic conditions and to meet the socio-economic needs of people. In recent past, a number of native breeds are facing fast genetic degradation and dilution because of intensive production system and unplanned introduction and use of exotic germplasm. This scenario, if continued, might result in depletion of the invaluable native germplasm having better potentiality for production, draught capacity, resistance to diseases and heat tolerance ability. Awareness of the value of genetic resources for sustaining the animal agriculture system has lead to several tangible efforts to understand the existing genetic diversity of indigenous livestock breeds of our country. The application of DNA based markers particularly, the microsatellites, has been well documented in different livestock species worldwide for characterization of livestock genetic resources. This information is valuable for filling gaps in documentation of breeds. The National Bureau of Animal Genetic Resources has taken a step forward in a holistic manner for complete breed characterization using phenotypic and genotypic information. Various research programs have been undertaken for molecular genetic characterization of native breeds of livestock and poultry breeds using microsatellite markers. The compiled information on molecular genetic characterization of indigenous farm animal breeds reviewed here would be helpful in better understanding of the farm animal biodiversity and formulating action plans on inventorization, conservation and management of animal genetic resources of our country.

Key words: Animal genetic resources, Farm animals, Characterization, Diversity, Microsatellite, Molecular characterization, Native breeds

Livestock is the major asset for the resource poor landless, marginal and small land holding farmers and contribute significantly to their income. Apart from being the source of milk and meat, they are an important source of draught power and traction, and are able to convert otherwise indigestible crop residues into nutritious human food. Also, livestock manure plays an important role in nutrient recycling that helps to sustain crop production. Livestock agriculture accounts for 25–30 % of the agricultural GDP of developing countries and is thus an important component in their economies. Maintaining animal genetic resource diversity is essential to enable farmers, pastoralists and animal breeders to meet current and future production challenges including climate change.

There are a number of technologies that have been developed for or adapted to livestock in both developed and developing countries. The major technologies that are used effectively in livestock conservation across the world include molecular genetic techniques. This article reviews the use of different molecular markers/technologies being used for characterization and conservation of farm animal genetic resources.

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Brief history

In principle, genetic diversity can be measured on the basis of polymorphism occurring at different levels, viz. morphological, biochemical, cellular and DNA level. Previously, blood groups and biochemical polymorphisms in blood cell and serum proteins were extensively used to study evolutionary relationships in cattle and other species (Khanna and Braend 1968, Khanna 1979, Schlotterer 2004). But it was difficult to measure genetic divergence and gene flow among closely related populations with these classical markers because of their low discriminating power (Van Zeveran *et al.* 1995*,* Goldstein and Schlotterer 1999). The main reason for low informativeness of such classical markers was attributed to extremely low mutation rate due to which the occurrence of any new mutation and subsequent fixation in a population was minimal. Consequently, these markers had a small number of alleles and low heterozygosity. With recent advances in genome studies and genotyping

techniques, much attention has been focused to the ultimate level of variability i.e. at the DNA level. "Molecular markers" or "Genomic markers" capable of detecting the genetic variation both within coding as well as non-coding sequences of DNA have been developed. It is now possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for the evaluation of genetic basis for the observed phenotypic variability. The exploitation of DNA polymorphism as molecular markers has opened many vistas in genetic characterization, conservation, improvement and molecular evolution studies in livestock species.

Adequate maternally, paternally, and bi-parentally inherited genetic markers are available for particular species, and can be used to estimate evolutionary history, population subdivision, dispersal, gene flow, effective population size, extended pedigrees, levels of relatedness, breeding structure, etc. (Blott *et al.* 1998, Goldstein *et al.* 1999). Broadly, based on their functionality, genetic markers have been grouped into two types (O'Brien 1992): Type I markers are gene based and possess a relatively low degree of polymorphism, but have extensive evolutionary conservation, whereas Type II markers usually have no identifiable biological function but they are highly polymorphic and not well conserved between species. All these markers reflect differences in DNA sequences usually with a trade-off between precision and convenience.

Several techniques are available to type molecular markers for detecting diversity and breed differentiation. These techniques include the restriction fragment length polymorphisms (RFLP), variable number of tandem repeats (VNTR), single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), random amplified polymorphic DNA (RAPD) and also the method which makes use of polymorphism of the short tandem repeats called the microsatellites. The marker system that is contributing most in diversity studies are microsatellites/SSRs due to their highly polymorphic nature, abundance in the genome and ease of amplification by PCR (Dodgson *et al.* 1997).

Challenges and need for conservation of AnGR

Livestock genetic resources have originated from the respective wild ancestor species and evolved in diverse environments. These have been shaped through the processes of mutation, genetic drift, and natural and artificial selection and carry unique genes that define productive and adaptive capabilities. The genetic diversity found in domestic breeds allows animal breeders to develop new characteristics in response to changes in environment, diseases or market conditions. However, during the recent past, there has been a net loss of diversity because of an increased rate of disappearance of some of the traditional breeds of livestock (FAO 2007a). It is also contemplated that species involved in production and marketing systems with fast structural change show high proportions of breeds currently at-risk and already extinct. This includes 38% of chicken breeds, 35% of pig, 33% of horse, and 31% of cattle breeds (FAO 2009).

Worldwide there are roughly 3000 breeds of the seven major mammalian species viz., cattle, pig, sheep, goat, horse, donkey and buffalo. It is estimated that in the 20th century, about 600 breeds have already been lost (Hall and Ruane 1993). This situation is even more alarming as per Food and Agricuture Organization (FAO) estimates, where it is expected that every week at least one traditional breed of livestock becomes extinct.

On the poultry side, the picture is even worse as over half the breeds of the five major species chicken, domestic duck, muscovy duck, goose and turkey are thought to be endangered or critical (FAO 2007a). The scenario for India and other developing countries would be of concern, as many of the breeds would be lost without ever having been adequately characterized or studied (Kohler Rollefson 1997, FAO 2007). This loss of genetic diversity has been accelerated by the rapid intensification of livestock production, failure to characterize local breeds and their importance, and inappropriate breed replacement or crossbreeding facilitated by the availability of high performing breeds (FAO 2007b). The extinction of a breed or population means the loss of its unique genes/germplasm responsible for special adaptive attributes that are the results of complex interactions between the genotype and the environment. Even though, these kinds of naturally evolved breeds are not as productive as their temperate breed counterparts, their loss may affect future of livestock diversity and sustainable animal productivity.

Further, local breeds are also important to meet food security under low or zero input production systems and harbor genetic diversity to mitigate anticipated changes in food quantity and quality demand in future with respect to climate change. Hence, use of locally adapted livestock and development of appropriate intervention are effective routes to help secure livestock assets of the animal keepers. To achieve this goal, characterization, conservation and utilization of the indigenous farm animal genetic resources are of paramount importance.

Need for characterization of AnGR

Genetic variability in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new emerging demands. To preserve the maximum amount of genetic diversity and further set conservation priorities, it is important to characterize different breeds so as to know how unique or different a breed is from other native populations (Eding *et al.* 2002). The underlying assumption is that the taxonomically distinct breeds are most likely to have special adaptation and gene combination not found in other breeds (Hall and Bradley 1995). Therefore, genetic characterization

is the first step to answer questions on taxonomy, evolution, domestication processes, management of genetic resources and setting conservation plans (Groves and Ryder 2000, Oakenfull *et al.* 2000, Jansen *et al.* 2002) for their effective utilization.

Diversity of indigenous livestock

The farm animal genetic resources in India are represented by a broad spectrum of domesticated livestock and poultry breeds including large number of well documented and registered breeds of cattle-34, buffalo-12, sheep-39, goat-21, poultry-15, camel-8, equine- 6 and many more populations of these species that have not been characterized so far. Beside many populations other species including mithun, yak, ponies and donkeys have so far not been characterized into breeds.

Many of these breeds have potential of surviving and also to sustain production, reproduction and draught power under various harsh climatic conditions and some breeds have unique attributes. The Indian native cattle, viz. Tharparkar, Hariana, Sahiwal, Rathi, Gir, and Nagori etc. can survive in extremes of agroclimatic conditions (Joshi *et al.* 2001, Singh *et al.* 2006, Vijh *et al.* 1994). Kankrej an important cattle breed, is known for its adaptability to high temperature, powerful draught capacity (Sodhi *et al.* 2006) and resistance to tick-borne disease. Similarly, India has excellent and diverse buffalo germplasm, known for high yield, fat rich milk- Murrah (Sadana *et al.* 2006); very high milk fat per cent-Bhadawari (Kataria *et al.* 2005); adaptaed to brackish water and salinity conditions- Chilika; drought tolerant, adapted to hot-dry climate and nocturnal grazing- Banni buffaloes of Kutch Gujarat; excellent draught power- Swamp buffaloes of northeast India. The indigenous sheep breeds also have remarkable diversity. Garole sheep is known world over for its high prolificacy (Davis *et al.* 2002) and adaptation to mangrove ecology. On the other hand, Malpura, Chokla, Marwari sheep (Jain *et al.* 2005, Gupta *et al.*2007) and many other breeds from arid zones are well adapted to desert conditions. Many breeds of goat are also adapted to different ecological niche with high temperature, humidity or both for example; Andaman goat is adapted to saline conditions, Changthangi is adapted to higher altitude temperate condition (Bhattacharya *et al*. 2004), whereas Jakhrana is well acclimatized to semiarid condition (Verma *et al.* 2005). Further, Zanskari, Spiti breeds of horses, ponies and double hump camel are well known for working ability under hypoxic conditions at higher altitude (Chauhan *et al.* 2004, Behl *et al.* 2005). Several native populations of poultry, viz. Ankleshwar, Assel (Pandey *et al.* 2005), Punjab brown, Kadaknath etc. are known, respectively, for their unique qualities in their respective native habitats.

Molecular markers in diversity analysis

Traditionally, phenotypic characterization based on morphological features, physical body measurements, production, reproductive and adaptive traits was used in the description of breeds. A number of reports are available (Kaura 1941, ICAR 1945, AHD 1961, Acharya 1982, Acharya and Bhatt 1984, Nivsarkar *et al.* 2000, Joshi *et al.* 2005) wherein assignments of indigenous breeds was based on phenotypic/subjective data and information gained from the local sources. However it is not always feasible to describe a breed on the basis of phenotypic characteristics alone because of the difficulty of combining the various measures used and interbreed phenotypic comparison is difficult or may not be reliable.

Realizing the limitations of the morphological characterization, efforts were focused on the characterization of genetic variation. Early reports on the detection of genome variation were focused on the analysis of protein and blood type variation (Naik *et al.* 1971, Singh and Khanna 1972, Singh and Bhat 1981, Das *et al.* 2002, Menrad *et al.* 2002, Mwacharo *et al.* 2005, Atta *et al.* 2009). These biochemical markers were used extensively, but were not very effective for characterization, as they often express low level of polymorphism (Goldstein and Schlotterer 1999) and also are sex limited and age dependent. Further, the analysis requires high quality samples through methods and reflects variability in the coding sequence only, which constitute less than 3% of total genome and are influenced by selection, development or by environmental factors (Van Zeveran *et al.* 1995).

The DNA based markers employed for genetic characterization were either multilocus, viz. restriction fragment length polymorphism RFLP, amplified fragment length polymorphism AFLP, random amplified polymorphic DNA RAPD, or single locus e.g., single nucleotide polymorphism SNPs and microsatellites (Sunnucks 2000). A number of studies were carried based on RAPD markers (Appa Rao *et al.* 1996, Joshi *et al.* 1998, Sharma *et al.* 2001, Ahlawat *et al.* 2004, Bhattacharya *et al.* 2004, Sodhi *et al.* 2006a, Yadav and Yadav 2007) to characterize the livestock genetic diversity. For diversity analysis, population genetics and uniqueness based on neutral loci has been considered as most effective and are being used worldwide (Barker 1994, Takezaki and Nei 1996, Ruane 1999, Sunnucks 2000, Eding and Laval 2002, Kalinowski 2002, Bruford *et al.* 2003).

Microsatellite marker

In India, the need for characterization and conservation of livestock genetic resources has been very well realized. The establishment of National Bureau of Animal Genetic Resources in 1984 can be considered as the beginning of the efforts for characterization and conservation of livestock genetic resources. Initial efforts by the NBAGR and other research groups in India were focused on the morphological (Joshi *et al.* 1995, Nivsarkar *et al.* 2000, Sahana 2000, Pundir *et al.* 2003 2004, Verma *et al.* 2005, Vij *et al.* 2006a) and biochemical marker based (Khanna *et al.* 1970, Singh and

Bhat 1980) characterization of the livestock. Later in 1997, decision was taken at the brain storming session held at National Bureau of Plant genetic resources, New Delhi, that microsatellite markers would be employed for molecular characterization of native livestock and poultry breeds. The decision was as per the suggestions of Food and Agricultural Organization (FAO) of the United Nations, which formulated an integrated programme for the global management of genetic resources of various livestock species. Project DADIS, MoDAD was formulated which suggested the use of species specific microsatellite loci. A list of 30 microsatellite loci per species (cattle, chicken, sheep and swine) to be used in diversity analysis was provided by FAO (http://dad. fao.org/).

Initial attempts for diversity analysis were based on the manual genotyping using urea-PAGE polyacrylamide gel electrophoresis denaturing sequencing gel. Here in, the allele sizes were determined by running a 10bp ladder along with the samples and recording the genotype of individual animal by direct counting method. Amongst the cattle breeds, the first breed focused was Sahiwal (Mukesh *et al.* 2004b) which has a decreasing population trend. The analysis revealed substantial diversity in this important milch breed of India, in spite of its small population size. Following the guidelines set by NBAGR, other research groups in the country also started the characterization of their regional breeds (Metta *et al.* 2004). A number of other breeds were characterized using this manual approach (Table 1, a-f). All these reports reinforced that microsatellite markers are useful in characterizing genetic diversity and observed the existence of substantial genetic diversity in studied cattle breeds. However the time consuming and cumbersome technique of manual genotyping was not very successful because of the difficulty in (i) accurate determination of the allele size, (ii) comparison of data across the different breeds, and (iii) its reproducibly. All these factors hinder the comparative studies across the various livestock and poultry breeds. With the advances in sequencing technologies and amenability of microsatellites to automation, the switch from manual genotyping to fragment analysis using sequencing technique was feasible. With the availability of high throughput systems, microsatellite markers have become the most frequently used markers in genetic characterization studies.

Microsatellite based diversity analysis of AnGR in India

Large number of projects studying genetic variation of domestic animals species has been performed with the aim of providing information for breed prioritization for conservation, mainly based on DNA markers information (Arranz *et al.* 1998, 2001, MacHugh *et al.* 1998, Schmid *et al.* 1999, Bjørnstad and Roed 2001, Kantanen *et al.* 2000, ArangurenMendez *et al.* 2002, Saitbekova *et al.* 2001). In a review on molecular genetic characterization studies (Baumung *et al.* 2004), worldwide, about 87 projects from 93 different countries have been reported, mostly on ruminants using microsatellite loci. The microsatellites, therefore, are the molecular markers that can help in assessing the genetic variation within and between breeds and provide a rational basis for ranking the breeds in terms of genetic uniqueness for conservation purpose. Also, microsatellite can help in solving the problems ranging from individual specificity, such as the questions of relatedness and parentage, the genetic structure of populations, the comparison among breeds/populations/species to the linkage analysis and gene mapping.

Initial attempts were made primarily to assess the genetic diversity of Indian livestock and poultry breeds. Almost all the descript breeds of buffalo (Table 1d), cattle (Table 1c), sheep (Table 1b), goat (Table 1a), horse (Table 1f) and poultry (Table 1e) have been characterized using microsatellite markers. Concerted efforts have also been put in to address the different issues related to molecular genetic characterization. The studies (Sodhi *et al.* 2005 and 2010, Mukesh *et al.* 2004a and 2008, Sharma *et al.* 2007 and 2008, Arora and Bhatia 2009) on Indian native breeds have indicated the presence of adequate genetic diversity in the analyzed breeds. The allelic diversity and heterozygosity in the Indian livestock breeds has been observed to be remarkably higher than that reported for the European counterpart. This might be attributed to lack of any artificial selection pressure and also point towards the existence of large effective population size of the Indian livestock breeds.

Considering the importance of buffalo in Indian agriculture, efforts have been made to evaluate the genetic diversity and relationship in Indian buffalo (Navani *et al.* 2002, Arora *et al.* 2003 and 2004b, Vijh *et al.* 2005b,c Tantia *et al.* 2006, Kumar *et al.* 2006, Mishra *et al.* 2008). Kumar *et al.* (2006) conducted a study on 8 recognized buffalo breeds of India and disproved the earlier classification of these breeds proposed on the basis of morphological and geographical parameters (Cockrill 1981). Based on morphology, buffalo breeds were categorized in 2 groups: (i) the Bhadawari, Nagpuri, Surati, Pandharpuri, and the Toda, and (ii) the Nili Ravi, Murrah, Mehsana, and the Jaffarabadi. However, clustering analyses showed distinct cluster of Jaffarabadi from Mehsana and Surti breeds in spite of the fact that they are in close geographical proximity. Also most of the Pandharpuri and Toda animals represent 2 different clusters in themselves which is not in accordance with the classification of Cockrill (1981). Vijh *et al.* (2008) also performed the analysis of molecular variance on the same buffalo breeds and did not observe significant differences between the 2 groups suggested by Cockrill's (1981). However, genetic classification by Vijh *et al.* (2008) was more or less in agreement with Cockrill's (1981) classification of buffaloes based on size. The microsatellite markers have also been helpful to identify genetic uniqueness of the breeds. Microsatellite based genetic diversity analysis

Table 1a. Status of genetic characterization of indigenous goat breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Attapaddy	Aggarwal *et al.* 2006 A (25), Dixit *et al.* 2010 (25)
Barbari	Ganai andYadav 2001 A(16), Ganai and Yadav 2005 A(12), Sharma *et al.* 2008 M (24), Rout *et al.* 2008A (23), Cauveri *et al.* 2009M (5), Ramamoorthi *et al.* 2009 M(21)
Beetal	Sharma *et al.* 2008 M(21)
Black Bengal	Behl *et al.* 2003 M(22), Tantia *et al.* 2004 M(21), Rout *et al.* 2008 A(23), Vijh *et al.* 2010 M(22)
Changthangi	Mishra *et al.* 2010 A(12)
Cheghu	Behl *et al.* 2003 M(22), Behl *et al.* 2007c M(25), Tantia *et al.* 2004 M(21), Vijh *et al.* 2010 M(22)
Ganjam	Dixit *et al.* 2010 A(25), Dixit *et al.* 2010 A(25)
Gohilwadi	Fatima *et al.* 2008 M(18), Kumar *et al.*2009 M(25), Verma *et al.* 2007c M(22)
Jamunapari	Ganai andYadav 2001 M(16), Ganai and Yadav 2005 M(12), Gour *et al.* 2006 M(23), Rout *et al.* 2008 A(23)
Jhakarana	Kumar *et al.* 2005b M(25) , Rout *et al.* 2008 A(23),Verma *et al.* 2007b M(25)
Kanniadu	Thilagam *et al.* 2006A(20), Cauveri *et al.* 2009M(5), Dixit *et al.* 2010b A(25), Dixit *et al.* 2011 A(25),
Kutchi	Dixit *et al.* 2008 A(22), Dixit *et al.* 2009 A(25)
Malabari	Verma *et al*. 2009 A(25), Bindu *et al.* 2010, Dixit *et al.* 2010 A(25)
Marwari	Rout *et al.* 2008 A(23), Dixit *et al.* 2007 A(22), Kumar *et al.* 2005a M(25).
Mehsana	Aggarwal *et al.* 2007 M(25), Dixit *et al.* 2009 A(25)
Osmanabadi	Dixit *et al.* 2010 A(25), Dixit *et al.* 2010 A(25)
Sangamnari	Dixit *et al.* 2010 A(25), Verma *et al.* 2010 A(25), Dixit *et al.* 2010b A(25)
Sirohi	Ganai and Yadav 2001 M(16), Ganai and Yadav 2005 M(12), Rout *et al.* 2008 A(23), Verma *et al.* 2007a A(25), Dixit *et al.* 2009A(25)
Surti	Patel 2004, Fatima *et al.* 2008M(18)
Zalawari	Fatima *et al.* 2008 M(18)

Table 1b. Status of genetic characterization of indigenous sheep breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Bellary	Kumar *et al.* 2007 M(20)
Bonpala	Pandey *et al.* 2009a M(24)
Changthangi	Sharma *et al.* 2010 A(25)
Chhotanagpuri	Arora and Bhatia 2010a A(25)
Chokla	Sodhi *et al.* 2006b M(25), Mukesh *et al.* 2006 M(11)
Coimbatore	Kumaraswamy *et al.* 2009 A(18)
Deccani	Arora and Bhatia 2010a A(25)
Ganjam	Arora and Bhatia 2010a A(25), Sharma *et al.* 2009 M(21)
Garole	Sodhi *et al.* M(25), Mukesh *et al.* 2006 M(11), Arora and Bhatia 2010a A(25), Mukesh *et al.* 2009 M(24)
Hassan	Sharma *et al.* 2006a M(10)
Jalauni	Arora *et al.* 2008A (25), Arora and Bhatia 2010b A(25)
Kheri	Bhatia and Arora 2008 A(25), Arora and Bhatia 2010b A(25)
Madras Red	Selvam *et al.* 2009 A(10), Prema *et al.* 2008 A(17)
Magra	Arora and Bhatia 2006 A(25), Arora and Bhatia 2008 M(13)
Malpura	Arora and Bhatia 2010b A(25)
Mandyal/Bandur	Arora and Bhatia 2010 M(25)
Marwari	Arora and Bhatia 2008 M(13), Arora and Bhatia 2010 M(25), Basanti J *et al.* 2010 M(20)
Munjal	Arora and Bhatia 2010b A(25)
Muzzafarnagri	Arora and Bhatia 2004 M(25), Arora and Bhatia 2008 M(13), Arora and Bhatia 2009 M(13), Arora and Bhatia 2010b A(25)
Nali	Sodhi *et al.* 2006b M(25), Mukesh *et al.* 2006 M(11)
Nellore	Arora and Bhatia 2010a A(25)
Nilgiri	Haris *et al.* 2007 M(25)
Patanwadi	Arora and Bhatia 2010b A(25), Basanti J *et al.* 2010 M(20)
Pugal	Mishra *et al.* 2009 M(22).
Rampur Bushair	Pandey *et al.* 2008 M(20)
Shahbadi	Pandey *et al.* 2009b M(18)
Sonadi	Arora and Bhatia 2008 M(13), Arora and Bhatia 2010b A(25)
Vembur	Pramod *et al.* 2009 M(25)

Table 1c. Status of genetic characterization of indigenous cattle breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Amritmahal	Prabhu, 2004
Bachur	Sharma *et al.* 2007 M(23)
Deoni	Metta *et al.* 2004 A(10), Mukesh *et al.* 2004a M(20),Kale *et al.* 2010M(7), Sodhi *et al.* 2005M(25)
Gangatiri	Sharma *et al.* 2006b M(22), Sharma *et al.* 2009 M(20),
Gaolao	Chaudhari *et al.* 2009M(25)
Gir	Kale *et al.* 2010 M(7), Mukesh *et al.* 2009 A(25)
Hallikar	Kumar *et al.* 2006 M(19)
Hariana	Mukesh *et al.* 2004a M(20)
Kangayam	Karthickeyan *et al.* 2009M(25)
Kankrej	Sodhi *et al.* 2007a A(25), Kale *et al.* 2010 M(7), Mukesh *et al.* 2009 A(25)
Kenkatha	Pandey *et al.* 2006b M(21), Sharma *et al.* 2009 M(20), Chaudhari *et al.* 2009 M(25)
Kherigarh	Pandey *et al.* 2006a M(21), Sharma *et al.*2009 M(20)
Krishnavalley	Karthickeyan *et al.* 2006 M(5)
Malvi	Thakur *et al*. 2010 A(24)
Mewati	Mukesh *et al.* 2009 A(25)
Nagori	Mukesh *et al.* 2009 A(25)
Nimari	Sharma *et al*. 2010 A(24)
Ongole	Karthickeyan *et al.* 2006 M(25), Karthickeyan *et al.* 2007 M(25), Metta *et al.*2004 A(10)
Ponwar	Sharma *et al.*2009 M(20), Sharma *et al.* 2006c M(24)
Punganur	Kesvulu *et al.* 2009 M(11), Tantia *et al.* 2008 M(24)
Rathi	Sodhi *et al.* 2008 A(23), Mukesh *et al.* 2009 A(25)
Red Kandhari	Sodhi *et al.* 2005 M(25)
Sahiwal	Mukesh *et al.* 2004a M(20), Mukesh *et al.* 2004b M(25)
Siri	Sharma *et al.*2008 A(23)
Tharparkar	Sodhi *et al.* 2006c M(25), Sodhi *et al.* 2008 A(23), Mukesh *et al.* 2009 A(25)
Umblachery	Karthickeyan *et al.* 2007 M(25)

Table 1d. Status of genetic characterization of indigenous buffalo breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Assamese	Mishra *et al.* 2010b M(23)
Banni	Mishra *et al.* 2009 M(24), Mishra *et al*. 2010a
Bhadawari	Arora *et al.* 2004b M(22), Vijh *et al.* 2005 M(22), Sukla *et al.* 2006 M(20), Tantia *et al*. 2006b M (22), Kumar *et al.* 2006 M(27), Vijh *et al.* 2008 A(22)
Chilika	Mishra *et al.* 2009d M(25)
Jaffrabadi	Kumar *et al.* 2006 M(27), Sukla *et al.* 2006 M(20), Vijh *et al.* 2008 A(22), Kataria *et al.* 2009a M(24), Mishra *et al.* 2009d
Marathwada	Vijh *et al.* 2008 A(22), Kathiravan *et al.* 2009b M(24)
Mehsana	Kumar *et al.* 2006 M(27), Sukla *et al.* 2006 M(20), Vijh *et al.* 2008 A(22), Kataria *et al.* 2009a M(24)
Murrah	Kumar *et al.* 2006 M(27), Vijh *et al.* 2008 A(22), Kataria *et al.* 2009a M(24), Mishra *et al.* 2008 (25), Mishra *et al.* 2009 M(24), Sukla *et al.* 2006 M(20).
Nagpuri	Kumar *et al.*2006 M(27), Sukla *et al.* 2006 M(20), Kataria *et al.* 2009b M(24), Mishra *et al.* 2009 M(24), Vijh *et al.* 2008 A(22)
Nili Ravi	Arora *et al.* 2004b M(22), Sukla *et al.* 2006 M(20), Vijh *et al.* 2008A(22), Kataria *et al.* 2009 M(24),
Pandarpuri	Kumar *et al.*2006 M(27), Vijh *et al.* 2008 A(22), Kataria *et al.* 2009a M(24)
South Kanera	Kathiravan *et al.* 2008 M(24)
Surti	Kumar *et al.* 2006 M(27), Vijh *et al.* 2008 A(22), Kataria *et al.* 2009a M(24), Mishra *et al.* 2009 M(24)
Toda	Kumar *et al.* 2006 M(27), Vijh *et al.* 2008 A(22), Mishra *et al.* 2008 M(25)

Table 1e. Status of genetic characterization of indigenous poultry breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Ankaleshwar	Pandey *et al.* 2005 A(26)
Aseel	Pandey *et al.* 2002M(25), Pandey *et al.* 2003 M(15),Vijh *et al.* 2004M(26), Vijh *et al.* 2004 M(26), Vij *et al.* 2006b A(26)
Busra	Vij *et al.* 2000 M(26)
Chittagong Malay	Ahlawat *et al.* 2008 M(25)
Danki	Vij *et al.* 2006 A(26)
Daothigir	Vij *et al.* 2007 A(25)
Ghagus	Vij *et al.* 2006 A(26), Ahlawat *et al.* 2008 M(25)
Kadaknath	Parmar *et al.* 2007 M(25), Ahlawat *et al.* 2008 M(25)
Kalasthi	Vij *et al.* 2006b A(26), Ahlawat *et al.* 2008 M(25)
Kashmir faverolla	Vijh *et al.* 2004 M(26)
Miri	Pandey *et al.* 2002 M(25), Pandey *et al.* 2003 M(15), Vijh *et al.* 2004 M(26)
Nacked neck	Pirany *et al.* 2009 M(9)
Nicobari	Pandey *et al.* 2002 M(25), Vijh *et al.* 2004 M(26), Pandey *et al.* 2003 M(15)
Punjab brown	Vij *et al.* 2006 a A(26)
Tellicheri	Ahlawat *et al.* 2008 M(25)

Table 1f. Status of genetic characterization of indigenous horse breeds

Breeds	References/genotyping method and number of microsatellite marker analyzed
Bhutia	Bhel *et al.* 2007a M(25)
Kathiawari	Koringa *et al.* 2008 M(24), Chauhan *et al.* 2010 M(20)
Manipuri	Bhel *et al.* 2007ab M(25), Gupta *et al.* 2007
Marwari	Gupta *et al.* 2005 M(26), Bhel *et al.* 2007a M(25), Chauhan *et al.* 2010 M(20)
Spiti	Bhel *et al.* 2007a M(25), Chauhan *et al.* 2004 A(25), Bhel *et al.* 2007b A(25), Chauhan *et al.* 2010 M(20)
Zanskari	Bhel *et al.* 2006A(25), Bhel *et al.* 2007a M(25), Bhel *et al.* 2007bA(25)

M: Manually analyzed using silver staining technique; A: Analysis using automated sequencing technique, fragment analysis. Number of microsatellite markers are indicated in the parenthesis.

have revealed Banni buffaloes of Kutch, Gujarat, as genetically unique population among other buffaloes of western India and thus indicated as a separate buffalo breed (Mishra *et al.* 2009a and 2010a).

Further, microsatellites have the potential to assess the admixture in the breeds and assignment of individuals to their specific population. This holds special importance as due to lack of breed associations and herd books for most of the Indian livestock breeds, there has been uncontrolled mixing of the breeds and consequently a large population of such interbred nondescript breeds has been evolved. Hence it becomes important to assess the genetic structure of economically important breeds of livestock. In one of such studies, genetic structure of important indigenous milch cattle breeds: Rathi, Tharparkar, Gir and other breeds Kankrej, Mewati and Nagori in close proximity was analyzed by Sodhi *et al.* (2010). Correspondence analysis plotted Rathi, Tharparkar, Gir individuals into 3 separate areas of multivariate space, whereas, Kankrej, Mewati and Nagori cattle showed low breed specific clustering. This reflected the existence of discrete genetic structure for Tharparkar, Rathi, Gir, the prominent dairy cattle breeds of the region, while admixture was observed for Kankrej, Mewati and Nagori individuals. Further 2 of the notable zebu cattle breeds developed in the western part of India are Deoni and Red Kandhari. Both these breeds are characterized by an extensive regional dispersal and coexisted in the same geographical semi-arid hilly areas for centuries. Microsatellite based analyses by Sodhi *et al.* (2008) suggested significant population differentiation between these 2 breeds with apparently small level of gene flow. These results further validate the usefulness of microsatellite markers in evaluation of genetic diversity and differentiation between closely related breeds.

The assignment of individuals to their population of origin has also been carried out in Indian chicken populations (Ahlawat *et al.* 2008). The assignment accuracy of 99% could be achieved in the analyzed 15 chicken populations utilizing only 21 microsatellite loci. With LOD score of 2 the assignment accuracy was 95% with 20 top ranked loci. The analysis revealed genetic distinctness of 6 chicken populations, viz. Ankleshwar, Danki, Kadaknath, Miri, Nicobari and Punjab Brown while remaining 9 populations were either admixed or have recent divergence. Efforts have also been made to assess the existing genetic diversity and establish the relationship among the indigenous Indian sheep

breeds. Evaluation of sheep breeds from different agroclimatic regions (Sodhi *et al.* 2003 and 2006a, Kumar *et al.* 2007, Arora and Bhatia 2006, Mukesh *et al.* 2006, Bhatia and Arora 2009, Arora *et al.* 2010a b) revealed a high level of genetic diversity in all the breeds analyzed. Genetic profiling of coarse wooled sheep breeds from Northwestern semi-arid zone of India (Arora *et al.* 2010a) revealed Kheri and Malpura to be the closest breeds while Muzzafarnagri and Sonadi were the distinct breeds. Seventeen sheep breeds from 3 different agroecological zones Northwestern arid and semi-arid, Southern Peninsular and Eastern zones have also been characterized by research group at NBAGR (Newsletter, NBAGR 2010) wherein many of the breeds were observed to be genetically diverse. The genetic distance and principal component analysis revealed separation of sheep breeds based on geographical proximity. Also, the Bayesian clustering approach suggested poor breed differentiation in the Northwestern arid and semi-arid region as compared to the breeds from Eastern and Southern Peninsular regions.

In Indian goat breeds, study by Dixit *et al.* (2010), suggested 3 major genetic groups of goat breeds according to their physio-geographical origin. Majority of the genetic variation (83.5%) was due to the differences among individuals within the breed. Different Indian native horse breeds have been analyzed by Behl *et al.* (2007a, b). The studies indicated higher within breed variability than between breed difference. All the Indian native horse breeds showed genetic distinctness from thoroughbred horse through independent clustering (Behl *et al.* 2007a).

Analysis of the 3 domesticated pig types native to India revealed high genetic variability in all the pig types with genetic distinctness of Ankamali pigs when compared with other native Indian pig types or exotic Large White pigs (Behl *et al.* 2006). The suitability of microsatellite marker analysis in Indian chicken breeds has also been evaluated at NBAGR. Analysis of poultry breeds from 17 locations across the country showed high level of heterozygosity, effective population size and presence of mutation equilibrium. Further, there was significant correlation between genetic and geographical distances pointing to isolation by distance. The addition of Red Jungle fowl and White Leghorn in analysis revealed a single cluster of all the indigenous populations (Vijh *et al.* 2004, Tantia *et al.* 2006a,). Molecular genetic characterization of duck populations has also been accomplished by Gaur *et al.* (2010).

Conservation genetics for selection of breeds

Large number of indigenous breeds are in danger of extinction and hence needs conservation, but the available resources are limited. Making a decision about which breeds should be targeted for conservation is challenging. A conservation strategy of animal genetic resources has to be directed towards maintenance of maximum genetic diversity in the global gene pool (Maijala and Kolstad 1992, Barker 1999) while maintaining within breed diversity to reduce inbreeding and preserve genetically differentiated groups. Diversity is generally measured as 1-f [f = average kinship or co-ancestry in a sub population] or 1- F [F = average inbreeding in a sub population] (Toro 2006). In order to manage genetic diversity it is best to minimize the average kinship in a population.

Maximization of genetic diversity for the next generation is achieved by minimizing the average relatedness of the parents. Optimal contributions have been applied in conservation of a sheep breed (Windig *et al.* 2007), goat breed (Mucha and Windig 2009), and pig breed (Fabuel *et al.* 2004). The principle of minimizing relatedness not only applies to the choice of parents for producing the next generation in breeding programs, but also to the choice of candidate breeds for conservation. Eding and Meuwissen (2001) worked out the principles to estimate average relatedness between different breeds based on microsatellite markers and to determine the optimal contributions of different breeds to a gene pool. Eding *et al.* (2002) also quantified the contribution of each breed to the maximum amount of genetic diversity and to identify important breeds for the conservation of genetic diversity in Northern European cattle breeds. In contrast to kinship approach, Weitzman's methods for selecting breeds for conservation give higher priority for inbred population. However, both methods rank the breeds as per the diversity content. Piyasatian and Kinghorn (2003) developed a method to balance genetic diversity, genetic merit and population variability in the establishment of conservation programmes. The method gives appropriate balance to the 3 issues: diversity, merit and variability. To sum up, conservation programmes should be based on wider information; in particular it should be based on a relatively large set of genetic marker loci by targeting good coverage of the genome. Further, other indicators of genetic variability and genetic uniqueness should also be included.

In our country, there is a need to complete the work of detailed genetic characterization and select the breeds for conservation by pooling the marker data with available information on degree of endangerment, traits of economical and ecological values, specific adaptive features, presence of unique genes/phenotypes and cultural and historical values. The decision for conservation of breeds can be taken on the basis of "independent selection principle" i.e. a breed can be conserved if it reaches a maximum value for at least one of the above mentioned criteria.

Conservation genomics

Recently, taking the advantage of the availability of sequence draft of different livestock species (chicken, 2004; cattle, 2005; rabbit, 2006; pig, 2007; sheep, 2009; camel, 2010), SNP arrays have been developed for chicken, cattle, pig and sheep and it is under development for other species. Multiplex SNP genotyping permits simultaneous high

throughput investigation of hundreds of thousands of loci with high measurement precision and provides a wealth of information useful for many aspects of livestock breed conservation. The genomic abundance and amenability to cost effective high throughput genotyping has made the SNPs as the most preferred class of genetic marker in genome wide association studies, genomic selection and the dissection of quantitative traits.

High density panel of SNP markers are being used to assess the true extent of autosomal diversity among cattle breeds. The whole genome SNP panel in different breeds of cattle identified several levels of population substructure with the greatest level of genetic differentiation detected between *Bos taurus* and *Bos indicus* breeds (McKay *et al.* 2008). Genetic differentiation among beef versus dairy and European versus Asian breeds could also be detected after exclusion of *Bos indicus* breeds. The efficiency of clustering breeds using SNP loci indicated that a large number of SNP markers are required for genetic diversity studies to replace FAO recommended 30 microsatellite markers. SNP array also offers the solution to know the extent of genetic diversity existing in the breeding stocks so as to take care of future needs. Global analysis of SNPs in chicken revealed that 50% or more of the genetic diversity in ancestral breeds is absent in commercial pure lines (Muir *et al.* 2008). The inability to incorporate ancestral alleles indicates that the extent of genetic diversity and the number of incorporated breeds in the breeding stock should be large.

Genome wide set of SNP has also been used to know the levels of genetic variability both within and between diverse samples of ovine populations. African and Asian populations revealed lower variability and genetic distance as compared to breeds of European origin. A weak phylogeographic structure, overlapping genetic similarity and low differentiation among sheep breeds have been reflected, which is consistent with their short evolutionary history.

The availability of dense SNP sets is driving investigations into the pattern of linkage disequilibrium LD, the dissection of quantitative trait loci QTL, the consequence of selection and genome wide selection as a method to accelerate genetic gain in livestock. Knowledge concerning the extent of genetic diversity and population substructure is critical to each of these applications. Whole genome LD scanning can provide a powerful strategy to map the genes that underlie complex traits especially in population isolates where it is hypothesized that the affected individuals may descend from a few common founders (Groenen 2008). Estimation of LD in cattle from Europe and Africa indicated that LD decrease with increasing physical distance across breeds revealing footprints of ancestral LD at short distances (Gautier *et al.* 2007).

The use of gene markers has attracted researchers in recent years as variation in these allele frequencies may provide information related to functional differences between breeds. Phylogenetic studies using gene markers or SNPs associated with traits of interest are relevant for breed utilization and conservation for future production needs.

Research accomplishments at NBAGR

In India the contribution of National Bureau of Animal Genetic Resources towards the characterization of Indigenous livestock and poultry genetic resources is substantial. A number of studies have been conducted by the bureau for molecular inventorization and determining the overall relationship amongst different Indian cattle breeds Table 1 (a-f). The bureau has nearly completed the task of genetic characterization of all the registered breeds of cattle 26/34, buffalo 12/12, sheep 28/39, goat 20/21, equine 6/6 and poultry 15/15. Based on the characterization and evaluation data, breed monographs/bulletins have been published. Similar efforts are being put forth for the lesser known breeds that have not been properly documented till date. Considering the need of the hour, NBAGR has taken a lead for breed registration of native animal genetic resources. Breed descriptors have been developed for a number of breeds of cattle, buffalo, sheep, goat and horses. Most of the documented livestock and poultry breeds (135) have been registered and accession numbers given. Additionally, *ex situ* conservation strategies have been implemented by preserving semen from important livestock breeds and the technique of somatic cell nuclear transfer has been standardized. Further, molecular genotyping of variations at important gene loci known to influence production and adaptive traits have also revealed genomic differentiation between Indian zebu and taurine cattle. The allele frequency spectra at specific candidate gene loci associated with milk production, disease resistance and thermoregulation trait generated across Indian zebu cattle breeds from different agro climatic zones pointed towards the existence of *Bos indicus* specific allelic profile that is different from *Bos taurus* breeds (Sodhi *et al.* 2007b, Mukesh *et al.* 2008, Mishra *et al.* 2009b). The status of A1/A2 alleles, the most abundant variants of ß-casein gene in dairy cattle was delineated systematically for the first time in Indian native cattle and buffalo breeds. In recent years, keen interest shown by several researchers on bovine ß-casein gene stems from the hypothesis that high consumption of A1 ß-casein milk increases the risk for a range of human diseases. The frequency distribution across analyzed breeds ranged between 0.95 and 1.0 indicating preponderance and fixation of A2 beta casein variant in Indian native cattle and buffalo breeds.

Similar studies were conducted in other species indicating the uniqueness of our native germplasm (Kumar *et al.* 2007, Tantia *et al.* 2008, Behl *et al.* 2009, Kathiravan *et al.* 2009a, Arora *et al.* 2010c). Such findings indicated that Indian livestock genetic resources have unique attributes which would open avenues for research to understand the mechanisms and the underlying genetic components

conferring positive attributes to the Indian livestock species and breeds.

Future challenges

From evolution theory of Darwin to first clone of animal-Dolly, domesticated livestock have guided our understanding of evolution and biology. Since the beginning of agriculture, livestock have been providing meat, milk, hides, hair, draught power and companionship and are now poised to give us even more due to new insights from genomics and molecular phylogenetics. Molecular markers used in genetic diversity studies give us information about the kinships between breeds and the domestication process. The development of tools for the analysis of DNA that has taken place in the past few decades has increased our capacity enormously to characterize variation within and between breeds, which form the bases for conservation and genetic improvement of the livestock and poultry breeds. Conserving the livestock breeds will be enabled by the existing and emerging molecular biotechniques. Genetic improvement of domesticated animals has been in practice for years by exploiting the existing variations. The approach was somewhat of the black box approach, as for most of the biological bases for these phenotypic traits were unknown. It is really a great challenge to place a definite time frame for the genetic characterization of all breeds of livestock available in India and to frame a roadmap as well as mobilization of adequate resources in such a vast and diverse country like India. The prospective availability of a large number of molecular markers and high throughput technology would help select animal for a given trait with more precision. The potential to enhance rates of genetic improvement by using molecular information clearly exists. While the researchers in India are mostly busy with AnGR characterization by using FAO recommended markers this only represent a part of reported variation. There is hardly any approach for additional marker development for a better genomic coverage. However the researchers in other parts of globe are working hard on methodology for breeding paneling through whole genome approach (SNP/Tilling Array based) and India should be equipped in terms of both infrastructure and trained manpower. Also, the allele mining of unique beneficial traits found in Indian livestock and technology for introgression of the superior alleles into elite germplasm for their optimum utilization are needed to be undertaken. The full realization of this potential will require further development and expansion in infrastructure and capacity building.

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