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Mitochondrial genetic diversity analysis of cattle using high density SNP array

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Abstract
Background: Animal mitochondrial DNA is small, extra chromosomal genome and self-replicating. The mtDNA has proved to be valuable in the study of genetic diversity as it shows maternal inheritance and changes rapidly than single copy nuclear DNA in mammals.
Aim: This study aim to analyse mitochondrial diversity and Lineage of diverse cattle breeds of India.
Method: Total 132 individuals DNA samples of seven diverse Indian native cattle breeds (Gir, Sahiwal, Tharparkar, Vechur, Hariana, Kangayam and Ongole) distributed over various geographical regions as well as covering different utility purposes (Milch/Dual/Draught/) were included in the present study. 132 mitochondrial DNA were isolated and SNP genotyping was done by Illumina Bovine HD chip. Data were analyzed by using Genome Studio software (Illumina). The data were filtered by using different parameters like SNP call rate (≤ 95%), and minor allele frequency (≤ 0.05) using PLINK version 1.07.Analysis of After pruning the data using above mentioned quality control parameters, various software's were used for SNP data analysis as per the need. data were analyzed based on, different haplotypes and their frequencies, haplotype diversity (XD), Nucleotide Diversity (Pi) and pairwise differences (K value) between all the possible sets for each population. AMOVA and Global Fixation index were done.
Result: Analyses revealed 81 haplotype with the haplotypic diversity range from 0.9333±0.0477 to 0.9883±0.0210, Nucleotide diversity was ranging from 0 to 0.0862+/- 0.0446. AMOVA shows 2.61% variation among population and 97.39% variation within population. F_{ST} value is significantly different from 0 for all pair wise combinations representing significant amount of Genetic differentiation between populations. F_{ST} estimates between the population shows that Sahiwal and Vechur are more genetically closer (-0.00477) while animals from Gir and Kangayam are genetically more differentiated (0.06601). Multidimensional Scaling indicate milch breeds are in same lineage as compare to dual and draft.

Keywords: Mitochondrial DNA, SNP array, Indian cattle, mitochondrial diversity

Introduction
Indian cattle (*Bos indicus*) play a significant role in the economy of small and marginal farmers, as they are a potential source of milk, drought power and manure. Animal mitochondrial DNA is small, extra chromosomal genome and self-replicating. The animal mitochondrial DNA is circular molecule of 15-20 kb in length [15]. The copy number of bovine mt DNA molecules, ranges from 220 to 1720 per cell, and has applicability even in severely decomposed sample in which nuclear DNA has been already degraded [30]. The mitochondrial chromosome displays exclusively maternal inheritance (Wallace, 1993). mtDNA is only a small portion of the DNA in eukaryotic cell as most of DNA can be found in the nucleus. The mtDNA has proved to be valuable in the study of genetic diversity as it shows maternal inheritance and changes rapidly than single copy nuclear DNA in mammals [6]. Mitochondria are the principle energy source in all cells of eukaryotes. Size of mitogenome in *B. Taurus* and *B. indicus* is 16,338 and 16,339 bp, respectively, and differs at 237 positions. With few exceptions all mitochondrial genomes contain the same 37 genes, all of which are involved in the production of energy and its storage in ATP. 22 genes among these are encoded by transfer RNAs. Remaining genes encode proteins (13 genes) involve in electron transport and oxidative phosphorylation and ribosome RNA (2 genes) that translate the protein genes within the mitochondria. The only noncoding area of the mtDNA is the control region typically 1kb, involved in the regulation and initiation of mtDNA replication and transcription, In fact each mitochondrion has several copies of its own genome, and there are several hundred to several thousand mitochondria per cell. Mitochondrial DNA is highly polymorphic due to high rate of evolution (5-10 times of nuclear DNA) (14).

Mitogenome consist of 1) control region, 2) D- loop, 3) hypervariable region. Replication of mtDNA can occure in two different ways, both starting in the D-loop region. One way continues replication of the heavy strand through a substantial part of the circular molecule, and then replication of the light strand begins. Some study mode starts at a different origin within the D-loop region and uses coupled-strand replication with simultaneous synthesis of both strands, control region refers to the fact that this region contains the signals that control RNA and DNA synthesis. The polymorphism is mostly concentrated in hyper variable region of the D- loop. This region has a rate of nucleotide substitution 5-10 times higher than the nuclear DNA [6]. So mtDNA can estimate relationship between both closely related and distantly related breed population. It permits an evaluation of relatedness among individuals in the population. This is an ideal tool for studying genetic diversity and population structure, because it has unique features of maternal inheritance, has a relatively fast rate of evolution and lack recombination. To describe the population genetic diversity and structure within and between the populations, there is need to investigate the patterns of mitochondrial D-loop sequence variation. It is important to generate the information on genetic diversity and composition of population for adopting the correct methodology for genetic improvement of the breed. The different breeds in the adoptive herd at various part of the country. Thus, before the application of breeding strategies and planning for the genetic improvement and conservation of germplasm it is mandatory to assess the genetic diversity and population structure of the individuals comprising the herd.

Experimental animals/ Resource Populations

Total 132 individuals DNA samples of seven diverse Indian native cattle breeds, distributed over various geographical regions as well as covering different utility purposes (Milch/Dual/Draught/) were included in the present study.

Table 1: Experimental animals

Breed	No. of samples	Utility
Sahiwal	19	Milch
Tharparkar	17	Milch
Gir	16	Milch
Ongole	24	Dual
Hariana	18	Dual
Kangayam	18	Draft
Vechur	20	Miniature
Total	132	

Mitochondrial DNA SNP genotyping

Mitochondrial DNA was isolated from whole blood using commercially available DNA isolation kit, following the prescribed protocol as per the manufacturer's instructions. The isolated DNA then was outsourced for genotyping of the mitochondrial genome using Illumina Bovine HD chip.

Data analysis

Quality control

The SNP genotype data were analyzed by using Genome Studio software (Illumina). The data were filtered by using different parameters like SNP call rate (≤ 95%) and minor allele frequency (≤ 0.05) using PLINK version 1.07 [25].

Analysis of genetic diversity

After pruning the data using above mentioned quality control parameters, various software's were used for SNP data analysis as per the need. GenAIEx version 6.503 [23] was used to find out the different haplotypes and their frequencies in different populations of the cattle under study. Arlequin version 3.5.2 [10] was used to estimate haplotype diversity (XD), Nucleotide Diversity (Pi) and pairwise differences (K value) between all the possible sets for each population.

AMOVA Analysis

Along with the frequency of molecular markers even the mutational differences between different genes can be obtained for molecular data. Analysis of Molecular Variance (AMOVA) which estimates the population differentiation directly from molecular data is more useful than using just Mendelian frequencies. Analysis of Molecular Variance (AMOVA) was carried out using programme Arlequin version 3.5.2 [10]. AMOVA was utilized further to quantify the extent of population differentiation and the distribution of genetic variation in the sample population.

Global fixation index (F_{ST})

The fixation is a measure of population differentiation due to genetic structure. It is frequently estimated from genetic polymorphism data such as single nucleotide polymorphism (SNP). F_{st} at a given locus is based on the variance of allele frequencies between populations and on the probability of identical by descent. The values range from 0 to 1. A zero value implies a complete pan-mixia that is two populations are interbreeding freely. A value of one implies that all genetic variation is explained and the two populations do not share any genetic diversity. F_{st} in the present study was calculated by using Arlequin version 3.5.2 [10]. Based on population pairwise F_{st} is used to construct multi dimension scaling (MDS) with the help of Software SPSS 16.0

Results

Total, 132 samples from 7 Indian cattle breeds covering different utility and geographic area were included in the study, with the aim to screen the haplo group, haplotypes and thus the genetic diversity of different cattle breeds of India. The results obtained during the course of present study are presented with discussion under various heads.

Quality Control

Samples that had more than 10% missing genotypes were excluded. SNPs with call rate (CR) (≤ 95%), minor allele frequency (MAF) (≤ 0.05), and HWE (p≤0.001) were also excluded. After these all filtration a total of 343 SNP were left.

Allelic patterns across populations

Total 132 individuals of 7 breeds were analyzed by GenAIEx ver 6.503 [23], Result revealed number of different allele (Na) ranging from 11 to 18 and effective allele (Ne) ranging from 8 to 15.696. The Shannon information index was ranging from 2.253 to 2799. Unbiased Diversity is above 93% for all the Breeds (as Table no.4.1) which indicate high amount of diversity.

Table 2: Allelic Patterns across population

Breed	Na (No. of Different Alleles)	Ne (No. of Effective Alleles)	I (Shannon's Information Index)	h(Diversity)	uh (Unbiased Diversity)
Hariana	12	9.529	2.37	0.895	0.948
Ongole	18	14.4	2.788	0.931	0.971
Kangayam	12	8.526	2.322	0.883	0.935
Tharparkar	15	12.565	2.639	0.92	0.978
Sahiwal	17	15.696	2.799	0.936	0.988
Vechur	16	14.286	2.718	0.93	0.979
Gir	11	8	2.253	0.875	0.933

Table 3: Genetic Diversity indices

Breed	No. of sample	No. of haplotype	Nucleotide Diversity (pi)	Avg. Nucleotide Differences(k)	Haplotype diversity
Hariana	18	12	0.0004±0.0006	0.2222±0.2758	0.9477±0.333
Ongole	24	18	0.0862±0.0446	14.5724±6.7623	0.9710±0.0208
Kangayam	18	12	0.0004±0.0006	0.2222±0.2758	0.9346±0.0409
Tharparkar	17	15	0.0050±0.0030	3.2352±1.7545	0.9779±0.0313
Sahiwal	19	17	0.0038±0.0028	1.2280±0.8153	0.9883±0.0210
Vechur	20	16	0.0031±0.0027	0.7421±0.5730	0.9789±0.0214
Gir	16	11	0.0000±0.0000	0.0000±0.0000	0.9333±0.0477
Total	132	101	0.0141±0.0081	2.888±1.488	0.962±0.309

Analysis of genetic diversity

Haplotype and nucleotide diversity

Total 81 haplotype were find out of 132 individuals. Identical sequence consider as same haplotype. Haplotypic frequency breed wise shown in Table No. 4.3. Within population wise demography indices, the number of haplotype (H) were 12 (Hariana), 18 (Ongole), 12 (Kangayam), 15 (Tharparkar), 17 (Sahiwal), 16 (Vechur), 11 (Gir) whereas Haplotypic Diversity (HD), Nucleotide Diversity (Pi), Average nucleotide Differences (k) mention in Table no.3. Nucleotide diversity (Pi) was found to be in between 0.000±0.000 to 0.0862±0.0446 with overall Nucleotide diversity of 0.01413±0.0077 (Table 3). Overall average number of nucleotide differences (K) among the populations was highest in Ongole (14.5724±6.7623) and lowest in Gir 0.000±0.000. The overall avg. number of nucleotide differences was 2.888±1.488. (Table no. 3).Haplotype diversity highest observed in Sahiwal (0.9883±0.0210) and lowest is in Gir (0.9333±0.0477) with overall haplotypic diversity was 0.962±0.309. The indices indicated presence of substantial genetic diversity and differentiation within the all breeds. This constitutes the good diverse population to be considered as a base population for genetic improvement of this breed in India.

Table 4: Haploid Allele Frequencies by Population

Breed	Hariana	Ongole	Kangayam	Tharparkar	Sahiwal	Vechur	Gir
Sample Size	18	24	18	17	19	20	16
1						0.05	
2		0.042					
3		0.042					
4		0.042					
5		0.042					
6		0.042					
7		0.083					
8		0.042					
9	0.111	0.083					
10		0.042					
11	0.056	0.042	0.111				
12			0.222				
13	0.056	0.125	0.167				
14		0.042					
15		0.042					
16	0.056	0.042					
17		0.042					
18		0.125	0.056				
19		0.042	0.056				
20		0.042					
21					0.053		
22					0.053		
23					0.053		
24					0.053		
25					0.053		
26						0.05	
27						0.05	
28					0.053		
29			0.056				

30						0.05	
31						0.05	
32						0.05	
33					0.053		
34				0.059			
35							0.063
36				0.059			
37							0.063
38				0.059			0.063
39					0.053		
40							0.063
41							0.063
42							0.063
43							0.063
44	0.056						
45	0.111						
46			0.056				
47	0.056						
48						0.05	
49					0.053		
50					0.053		
51					0.053		
52				0.059			
53						0.05	
54	0.056						
55	0.056						
56				0.059			
57					0.105		
58				0.059	0.053	0.1	
59				0.059	0.053	0.1	0.25
60				0.059			
61						0.05	
62	0.056						
63	0.167						
64			0.056				
65						0.05	
66				0.059			
67				0.059			
68				0.176	0.105	0.05	0.125
69							0.125
70			0.056				
71						0.05	
72					0.053	0.1	0.063
73			0.056				
74			0.056				
75	0.167		0.056				
76						0.1	
77				0.059			
78				0.059			
79				0.059			
80				0.059			
81					0.053		

Analysis of molecular variance (AMOVA)

To understand the partitioning of the levels of genetic diversity of the 7 different populations of Indian cattle. The analysis of molecular variance (AMOVA) was conducted. The results of the AMOVA (Table No. 5) revealed that 97.39% of total genetic diversity existed among the individuals within the populations and only 2.61% of total genetic diversity accounted for differences among populations

Global Fixation Index (F_{st})

Fst values were significantly different from 0 for all pair wise combinations representing significant amount of Genetic differentiation between population. FST value ranged from -0.00477 to 0.06601. Pair-wise genetic differentiation between populations are represented in Table 4.5. F_{st} value was highest between population of Gir and Kangayam with 0.06601 and lowest between population of Sahiwal and Vechur with a value of -0.00477. Here minus value we was consider as zero because range of F_{st} value is 0 to 1 only. Where zero is indicating same genetic material individuals have, and 1 indicate completely genetically unrelated. These F_{st} estimates between the population shows that Gir and Kangayam more genetically differentiated while Sahiwal and Vechur are genetically closer.

Table 5: Analysis of molecular variance (AMOVA)

Source of Variation	df	Sum of Square	Variance components	Percentage of variation
Among Population	6	4.345	0.01291	2.61
Within population	125	60.185	0.48148	97.39
Total	131	64.53	0.49439	

Table 6: Global Fixation Index (F_{st})

Breed	Hariana	Ongole	Kangayam	Tharparkar	Sahiwal	Vechur	Gir
Hariana							
Ongole	0.02002						
Kangayam	0.035	0.0126					
Tharparkar	0.03724	0.0256	0.04381				
Sahiwal	0.03191	0.02046	0.03842	0.00452			
Vechur	0.02574	0.00853	0.02683	0.01284	0.00477		
Gir	0.0594	0.04725	0.06601	0.01902	0.00956	0.03138	

Table 7: Significance of F_{st} value

Breed	Hariana	Ongole	Kangayam	Tharparkar	Sahiwal	Vechur	Gir
Hariana		+	-	+	+	+	+
Ongole	+		-	+	+	-	+
Kangayam	-	-		+	+	+	+
Tharparkar	+	+	+		-	-	-
Sahiwal	+	+	+	-		-	-
Vechur	+	-	+	-	-		+
Gir	+	+	+	-	-	+	

Based on F_{st} value MDS (multi dimension Scaling) form which shows cluster formation milch breeds that indicate same lineage of origin of milch cattle, whereas Kangayam and Hariana breeds are far away from all breeds which indicate independent lineage. Ongole was showing intermediate lineage in between milch and draft Indian breeds.

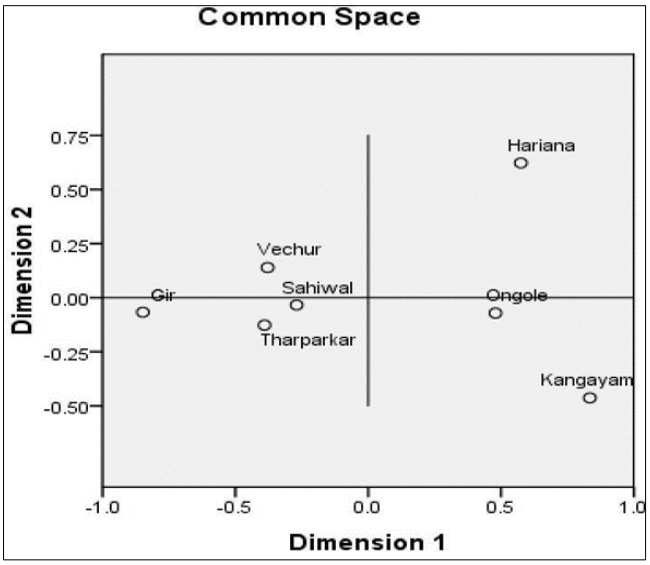

Fig 1: Multidimensional Scaling

Discussion

Table 8: Mitogenome of domestic animals

Sr. No.	Name Of Species	Complete Sequence (bp)
1	Human	16569[1]
2	Mouse	16295[4]
3	Cattle	16338[2]
4	Chicken	16775[9]
5	Cat	17009[19]
6	Sheep	16616[12]
7	Dog	16782[16]
8	Pig	16613[18]
9	Japanese Quail	16697[21]
10	Goat	16640[24]
11	Buffalo	16355[22]

Lie *et al.* (2006) [17], worked on mitochondrial D-loop sequences (910 bp), in 82 cattle from 4 breeds in Guizhou province revealed 31 mitochondrial haplotypes, 65 polymorphic sites, covering 7.14% of the entire length of the sequence. The nucleotide diversity and haplotype diversity estimated from mtDNA D-loop region in 4 cattle breeds in Guizhou varied from 2.16% to 2.61% and 0.695 to 0.909 respectively showing abundant mitochondrial genetic diversity exists in Guizhou cattle breeds. Wang *et al.* (2009) [29] studied Chinese Leiqiong cattle and analyzed complete mtDNA cyt b genes and three haplotypes of 18 individuals were identified from 2 polymorphic sites with length of 1140 bp. The average haplotype diversity and nucleotide diversity were 0.0741 and 0.0012 showing less genetic diversity in leiqiong cattle. A neighbor joining tree was constructed and revealed that leiqiong cattle only originated from *Bos indicus* and had no direct relationship with *Bos Taurus*, *Bos grunniens* and *Bos javanicus* cattle. Cai *et al.* (2007) [7] tried to clarify the genetic diversity of indigenous cattle breeds of china, they carried out phylogenetic analysis of representatives of those breeds by determining mitochondrial gene polymorphism. Cyt b gene sequences (1140 bp) were determined for a total of 36 individuals from 18 different breeds and these sequences were clustered into two distinct genetic lineages of taurine and zebu. Analysis of polymorphism showed declining south to north gradient of female zebu introgression and geographical hybrid zone of *Bos Taurus* and *Bos indicus* in china. Shangang *et al*. (2007) [26] studied complete mtDNA D-loop region from 123 cattle of 12 Chinese breeds and two individuals of Germany yellow cattle breed. The 13 cattle breeds were divided into two main groups-*Bos Taurus* and *Bos indicus*. Apei-jiaza cattle breed of Tibet, which was similar to that of yak was at a higher level than other cattle breeds providing enough evidence of introgression of genes from yak. Genetic diversity of 277 nucleotides in the mitochondrial DNA control region of crossbreed beef cattle as well as in Nellore samples (*Bos indicus*) were studied by Henkes *et al.,*(2005) [11]. More than fifty mutations were found in Brangus-Ibage comprising. Sharma *et al.,* (2015) [27] analyzed 170 mitochondrial D-loop sequence from 11 Indain cattle breeds. The result revealed 60 haplotype found with average haplotypic diversity of 0.9024 and average nucleotide diversity was 0.02688. Two major cluster of haplotype were found. Result shows that south Indian breed Ongole was distinct from north/central Indian breeds. Correia *et al.,* (2017) [8] analyzed total 40 samples to check mitochondrial DNA D- loop (521-bp) based diversity of four breed of main Portuguese Lidia bovine populations and clarify their genetic relationships with Spanish Lidia lineages. The mtDNA diversity recorded was similar to that observed in Lidia cattle. Haplotype T_3 was the most common (62.5%), followed by the African T_1 haplotype (25%); very low frequencies were recorded for haplotypes T_2 (2.5%) The results support the existence of two major ancestral lines for the Lidia breed: European and African, similar to other Mediterranean breeds. Bhuiyan *et al.,* (2007) [3] analyzed mtDNA displacement loop (D-loop) sequences of 48 samples along with 22 previously published sequences from Bos indicus and Bos taurus breeds. 25 haplotypes were identified in Red Chittagong cattle that were defined by 44 polymorphic sites and nucleotide diversity was 0.0055±0.0026. The phylogenetic studies showed Red chittahong cattle clustered with Bos indicus lineage with two distinct haplogroups representing high genetic variability of this breed. Hoda *et al.,* (2014) [13] analyzed 77 mtDNA D-loop sequences from six different Albanian goat breeds. The result revealed 67 different haplotypes, with haplotype diversity ranging from 0.864 to 1 and nucleotide diversity values ranging from 0.016 to 0.106 and analysis indicated that 98.7% of the variation was found within the goat breeds and only 1.3% among them. Ming *et al., (*2017) [20] conducted study on camel population of 113 individuals representing 11 domestic breeds by examining 809 bp MtDNA and found 15 different haplotypes and the phylogenetic analysis suggests domestic and wild Bactrian camels have two distinct lineages and the analysis of domestic Bactrian camels from different geographical locations had no significant genetic divergence in China, Russia and Mongolia 18 haplotypes and more than sixty nucleotide changes in Nellore. The data indicated sequence identities of 99.6 and 92.1% between the *Bos Taurus* reference sequence and Brangus-Ibage and Nellore respectively. The comparison of data with sequence data for 612 individuals recovered from Gen Bank showed a total of 205 haplotypes defined by 99 polymorphic sites. Most of the variability was due to differentiation within breeds

Conclusions

The results indicated higher haplotype diversity of mitogenome within breeds (~93% and above). The milch breeds shared the common ancestry and was different from the dual and draft breeds these results provide basic information about genetic diversity and structure of Indian cattle which should have implications for management and conservation of Indian cattle diversity.

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