GENETIC RESOURCES AND DIVERSITY AMONG SHEEP BREEDS OF ASIA AND EUROPE

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Abstract: Sheep has been domesticated southwestern Asia for about 8000-9000 years ago and represented one of earliest livestock species. Ancestor relationship can be investigated through mtDNA data. There are many genetic markers to determine the relationship between and among the sheep breeds. Restriction fragment length polymorphisms are basic technique and have less variability and many restrictions as compared to Random amplification of polymorphic DNA. Due to specific amplification of Amplified fragment length polymorphisms it is more suitable than Restriction fragment length polymorphisms and Random amplification of polymorphic DNA. Microsatellites are widely used technique for the determination of genetic diversity. This technique provides information about the classification and characterization of sheep breeds. However this technique cannot provides information on breed functional traits. On the basis of mtDNA analysis haplotypes groups differ in sequences by any extent of Ovis species. Haplotypes A and B are two of most important haplotype groups. Haplotypes A carries Asian mouflon (Ovis orientalis) while Haplotypes B carries European mouflon (Ovis musimon). Actually Haplotypes A and B are both found in Asia while Haplotypes B only dominates in Europe so sheep descent from one or more Asian mouflon. Haplotypes C also found in Turkey, Portugal, Caucasus and China while Haplotypes D present in Karachai sheep from Caucasian. Haplotypes E which is very rare and found only in Turkey.

Keywords: Genetic markers, mtDNA, Microsatellites, Haplogroups, Relationship

1. Introduction
Sheep is the most important and economically breed which played a magnificent role in human society and after humans spread all over the world (Colladge et al., 2005; Chessa et al., 2009; Ulutay et al., 2018). Sheep has been domesticated around 8000-9000 years ago in the Fertile Crescent zone of Asia (Ryder, 1984; Ryder and Stephenson, 1968). Based on morphology sheep originated from urial (Ovis vignei) of Central Asian mountainous ranges (Ryder and Stephenson, 1968; Piper and Ruvinsky, 1997). Natural and Artificial selection resulted development of around 1400 breeds from their ancestors (Scherf, 2000). Human activities played an important role in breed development process in respect of flow of genes between population and breeds (Warmuth et al., 2012).

Molecular genetics provides the information among relationships between breeds and populations and inherited genes present within breeds. Genetic variation at continent level between the native sheep breeds can provide the understanding of their origin and dispersal with respect to how human played role in the distribution of breeds. Paleontology with the help of molecular genetics provides great information about origin and dispersal of sheep (Poplin, 1979; Hiendlieber et al., 1998; Pedrosa et al., 2005; Chessa et al., 2009; Meadows et al., 2007, 2011; Kijas et al., 2009, 2012; Demirci et al., 2013).

Autosomal microsatellites used for the understanding of population history (Forbes et al., 1995; Walling et al., 2004) as well as the relationship between the Europe and Asia sheep breeds (Arranz et al., 1998, 2001; Díez-Tascon et al., 2000; Tapio et al., 2003; Chu et al., 2003). As sheep is considered one of the earliest livestock species (Zeder et al., 2006; Sen et al., 2011) it has important role in domestication process. The domestic sheep interbred with Argali, Mouflon and Urail which lead to more complexities in finding the origin of sheep (Guo et al., 2005). From north part of Zargros to southeastern Anatolian region sheep were domesticated mostly nearly 10000-11000 B.C. or might be more earlier (Peter et al., 2005).

2. Role of Unconscious Selection in Domestication of Sheep
There are two types of selection; conscious selection which is also called selective breeding as selected by breeders according to trait of interest. Unconscious selection in which animals are isolated from their wild environment to man-made environment as a
result new traits automatically selected because of loss of activity of various adaptations which are necessary to survival in wild environments results in new characteristics of animals. As compared to plants less attention has been made to unconscious selection in domestication of animals (Zohary et al., 1998) when shift from wild to artificial environment some morphometrical, behavioral and physiological developments occur.

Unconscious selection play important role in domestication of sheep by two ways first is favor of specific morphometric or behavioral type by domesticator and provide founder effect and second one is the culling of young males. Culling of young males is closely associated to changes in morphometry (Zohary et al., 1998).

### 3. Genetic Polymorphisms

Analysis of genetic diversity can be measured with the help of different genetic markers. Each marker is based on DNA sequence polymorphisms and used to detect other than itself the presence of any specific genotype or phenotype which is difficult to measure. With the help of genetic markers evolutionary history of any species can be determined by phylogenetic analysis as well as genetic studies of population and gene mapping.

There are different classes of genetic or molecular markers for example restriction fragment length polymorphisms (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeats (SSRs) and mitochondrial DNA (mtDNA).

#### 3.1. Restriction Fragment Length Polymorphisms

This type of non PCR based or hybridization based DNA marker used as probe of cDNA, synthetic oligonucleotides or DNA cloned elements. The probe is radioisotopically labeled or with the enzymes which catalyzed the reaction. Then cleavage of DNA is occurred with restriction enzymes. RFLP is old technique and was developed in 1980 to understand the difference between the DNA structures based on the bacterial restriction enzymes (Mburu and Hanotte, 2005).

DNA sequence is digested with the help of known restriction enzyme for the analysis of patterns. Different fragment length patterns are obtained which indicated that enzyme cut the DNA at unrelated portions. If there are mutations in the DNA than these mutations either removes the existing restriction sites or create new restriction sites which results in restriction polymorphisms and these changes can be detected by hybridization probe (Yadav et al., 2017). RFLP is stable technique and provide constant data over the location and time for the detection of genetic diversity but RFLP needs good and large quantity of DNA also RFLP cannot detect the variation in whole genome sequences in sheep. RFLP does not work in case of inbreeding in sheep due to less variability.

#### 3.2. Random Amplification of Polymorphic DNA

Random amplification of polymorphic DNA (RAPD) is the PCR based technique or molecular marker to detect genetic diversity in sheep. This technique was developed in 1990 and new as compared to RFLP (Williams et al., 1990; Welsh et al., 1990). This technique is also called arbitrarily primed PCR (AP-PCR). As the name indicates in this technique DNA segments are randomly amplified. RAPD is most commonly used marker or technique to determine the genetic diversity (Williams et al., 1990) as there is no need to determine the DNA sequence for the targeted gene because primers can bind anywhere in the DNA sequence. This technique is used to determine specific sequences relationship among the species as well as it was also used in many species of animals like buffalo, dog, cat, pig, horse, kangaroo and rabbit (Koh et al., 1998).

As a comparison with RFLP it is cost effective, simple and less amount of DNA is required (Demek et al., 1992; Koller et al., 1993). Although RAPD has also disadvantages as there is occurrence of primers binding which are non-specific and non-reproducible. During the studies of F2 generations RAPD cannot be used as this technique is not able to differentiate homozygote from the heterozygote genotypes. This technique is also poor in the sense of reability and repeatability (Meunier et al., 1993).

#### 3.3. Amplified Fragment Length Polymorphisms

Amplified fragment length polymorphisms also abbreviated as AFLP is actually a combination of PCR and RFLP techniques (Zabeau et al., 1993). In this technique like RFLP with restrictions enzymes DNA is digested. The next step is ligation in which DNA fragments are ligated to adaptors and in amplification these fragments amplified with specific primers (Blears et al., 1998). AFLP is better as compared to RFLP and RAPD as it less time consuming and absolute as well as reliable because of specific amplification respectively. Genetic diversity of sheep breeds can be determined by AFLP by using different breed markers (Hoda et al., 2010). AFLP is genetically stable technique to determine the large number of polymorphic markers which are automatically genotyped (Vos et al., 1995) and is effective to study the population genetics and to understand the animal genetic resources. This technique also has disadvantages as the markers used cannot be able to differentiate the homozygous individuals which are dominant from the dominant heterozygous individuals (Paglia et al., 1998).

#### 3.4. Microsatellites

Genetic diversity is the most important parameter for the analysis of population growth, future population growth and sustainability of animals (Soule, 1987). For population of any breed maintenance, genetic diversity is very important (Hall and Bardley, 1995). Microsatellites also known as simple sequences repeats or SSRs are the markers which are used to determine the genetic diversity both within and between the sheep breeds. Actually microsatellites are repeated sequences within
the genome of the animals. Now a day microsatellites are commonly used markers for animal genetic studies (Civanova et al., 2006). This technique is used for many purposes such as the investigation of diseases, to evaluate the genetic diversity between and among the sheep breeds, determination of genetic resources, understand population genetic, to determine the history and domestication process of sheep, percentage determination and migration of animals. Microsatellites markers are easy to detect as well as there is high level of polymorphisms and availability in the genome of the animals (Ritz et al, 2000).

Microsatellites normally consist of 1-6 bp which are tandemly repeats (Litt and Luty, 1989) and their flanking regions are conservative at the loci whereas high variability is present for repetitive units between and among the species as well as different animals of the same species. Primer designing is very important in this technique based on conserved sequences and then via electrophoresis polymorphisms can be observed (Tautz, 1989).

As compared to RAPD, microsatellites are more stable and highly repeatable technique also this technique is much better than AFLP as it can detect the homozygous individuals from the heterozygous individuals because of its co-dominant markers. Microsatellites are used to investigate different sheep breeds all over the world. High level of genetic variations using microsatellites markers were observed among the Turkish sheep breeds (Gutierrez-Gil et al., 2006). Using different population parameters genetic relationship of Spanish sheep breeds using microsatellites have been studied which provides useful information about the identification and differentiation of breeds (Arranz et al., 1998; 2001). Also Arora and Bhatia, 2004 studied on Indian sheep using microsatellites and Diez-tascon et al. 2000 worked on Merino sheep breeds using microsatellites.

Besides the many advantages of microsatellites these markers have some drawbacks as well. First one is high cost as compared to other techniques. Due to mutation in annealing sites of primer in the presence of null alleles than heterozygote individuals falsely classified as homozygotes. Homoplasy is another problem due to mutations and microsatellites cannot provide information about the biodiversity on functional traits.

4. Mitochondrial DNA Diversity

Mitochondrial DNA (mtDNA) is an important tool of animal phylogenetic study. mtDNA is inherited maternally and within the species its variability is very high. In the mitochondrial genome D loop (control region sequence) rapidly evolves as compared to nuclear DNA. mtDNA also play important role in recognition of population history and demographic expansion (Bruford et al., 2003).

There are three major genomic variations which can be used to determine the history of sheep: mitochondrial genome, autosomes and Y chromosome. Autosomal studies include autosomal microsatellites which can be used globally to detect relationship of sheep breeds across the continents. By using microsatellites animals have been collected from northern and southern parts of Europe (Handley et al., 2007) or Europe and the Middle East (Peter et al., 2007) and performed genetic analysis at continental scale. There is more genetic diversity but less genetic differentiation in South European sheep breeds in contrast to Northern European breeds (Pariset et al., 2011) as genetic diversity will be high in the populations close to domestication center and decline away from it. Non recombining regions of Y chromosome showed the male mediated introgression patterns in the process of breed development (Meadows et al., 2006, 2009).

Male specific region of Y chromosome has now much focus to study the migration of sheep but very less work has been done on it. Male lineages in the form of haplotypes are identified by observing the alleles in the haploid state without X-Y combination pattern. Study of male lineage is very important in livestock because by control mating resulted in limited number of males which contributed to the large progeny in the future generations. Very less nucleotide diversity have been reported in male specific Y chromosome in sheep (Meadows et al., 2004). Y specific probe has been developed by Bradley et al., 1994 to distinguish between two cattle breeds Bos taurus and Bos indicus. Meadows et al., 2006 revealed that there is no direct relationship of mountain sheep both Ovis canadensis and Ovis dalli in the process of domestication of sheep and Mouflon contributed well in the domestic process of sheep. SNP analysis of sheep genome indicated that within individual breed SNPs can identified population substructures (Kijas et al., 2009).

Most information about history and domestication of species generated by using mtDNA. Due to many domestication events and human selection as well as introgression by species results in multiple lineages and their admixtures of mtDNA within the breeds (Pireira et al., 2006, Meadows et al., 2007). As compared to relationship of many other species with their ancestors on the basis of mtDNA, two main haplogroups A and B were found in sheep which were different in their sequences (Hendleder et al., 2002). Haplogroup A and B both found in Asia but haplogroup B mostly found in Europe. Haplogroup C was identified in China, Caucasus, Portugal and Turkey (Tapio et al., 2006). Haplogroup found in species of Sheep hardly correlate geographically. Independent domestication events were lower than the distinct lineages because the wild populations of sheep may be polymorphic or many wild populations introduced new maternal lineages to the domestic sheep (Zeder et al., 2006). Haplogroup D was found in Caucasian and Rumanian Karachai sheep while E is rarely found in Turkish sheep (Groeneveld et al., 2009).
5. Rapid Mutation of mtDNA

Restriction endonuclease enzyme and thermostability analysis shows that mitochondrial DNA mutates unusually faster. Thermostability analysis is actually the difference between the tm values of heteroduplex and homoduplex DNAs. The estimation of sequence differences between the related DNAs can be calculated by comparison of melting temperature of the heteroduplex DNA and the melting temperature of homoduplex DNA. The difference of mtDNA is 5 fold more as compared to nuclear DNA difference on the basis of m (minimum number of base substitution per base pair) by which the two species differ in the sites of cleavage of the nucleotides or sequence difference at the site) and 10 fold on the basis of p (at the cleavage site in comparison of two species the estimated number of base substitution per base pair) which shows that mtDNA usually evolves 5 to 10 times faster than nuclear DNA (Brown et al., 1979). Although high evolutionary rate of mtDNA, made it very useful molecule for evolutionary biologists for the study of species and population relationships. Mechanism of speciation can be thoroughly studied with the help of mtDNA.

6. High Level of Gene Flow from Asia and Europe

Sheep is highly versatile domestic species. Phenotypic differences occur between the breeds on the basis of characteristics such as milk and meat production, wool, tolerance in environment and colour of coat. Molecular genetics revealed the relationship between population of sheep and genetic variation. Autosomal microsatellites can be used to understand the history of sheep population (Forbes et al., 1995; Walling et al., 2004) as well as breed relationship from Europe (Arranz et al., 1998; Diez-Tascon et al., 2000; Tapio et al., 2003) and Asia (Chu et al., 2003).

Molecular evidences revealed that sheep has been transported between geographical areas. Haplotype distribution indicates that sheep has weakest population structure and most dispersed domestic animal between continents. As compared to other domestic animals such as goat (10%), cattle (50%) and sheep has only 2.7% sequence variation between the continents (Meadows et al., 2005). The microsatellite SRYM18 which defines the other haplotypes (Meadows et al., 2006) but major haplotypes excluded these present very less frequency and distributed over different continents.

Genetic variations play important role in the future breeding programmes. Within species individual, group and population genetic differences represent the variation. Genetic resources can be saved by the efforts of conservation. Mitochondrial genome is a very useful tool for the investigation of origin of species. Divergent groups of mtDNA sequence appeared shows that species of domestic animals have multiple maternal origins (Bradley et al., 1996; Luikart et al., 2001; Hiendleder et al., 2002). Events of the domestication occurred for Buffalo and pigs in Asia, for sheep, goat and cattle in Near East and for llama and alpaca in Americas (Bruford et al., 2003).

7. Conclusion

There are many techniques or markers used to investigate the relationship between and among different sheep populations. RFLP is old and hybridization technique in which different restrictions enzymes is used to cut the DNA sequences at various sites. This technique cannot be used while studying whole genome of the animals. RAPD is new technique as compared to RFLP and is used for the study of genetic diversity of sheep. RAPD is actually a PCR based method in which primers can bind anywhere in the genome so no need to determine targeted sequence in the DNA. AFLP is also PCR based technique and is better than both RFLP and RAPD because it is more stable and is used to study wide populations of animals.

Microsatellites are the most used markers or technique used to detect history and relationship between and among the sheep breeds in all over the world. It is used to detect future population growth and parameters, disease investigations and neutral biodiversity. However this technique is not able to detect functional traits of the species. Sheep mainly have two lineages which were arisen from the geographically different domestication events. The other analysis which reveals that domestication occurred only once from the ancestral population which has two mtDNA divergent cannot be neglected but divergence level which isolating the major lineages remarkably anticipate the extent of history of domestication both for sheep (Hiendleder et al., 2002) and other animals (MacHugh and Bradley, 2001) showing strong population structure.

Mitochondrial DNA play important role in phylogenetic study of sheep as mtDNA follow the maternal inheritance and high variable within the species of animals. Mitochondrial DNA (mtDNA) has high rate of evolution as compared to nuclear DNA and reveals the demographic process or complexity the history of sheep (Bruford et al., 2003). According to mtDNA there are two major haplotypes in domestic sheep, mtDNA analysis deny the Argali (Ovis ammon) and Urial as supposed common ancestors of sheep (Hiendleder et al., 2002; Wu et al., 2003) so it is generally agreed Moufflon (Ovis musimon) is the common ancestor of sheep. Haplotypes A and B both present in Asia and haplotype B dominates in Europe (Bruford et al., 2003; Meadows et al., 2005). Haplotype C is very less but present in Turkey, Caucasus and China (Tapio et al., 2006). Haplotypes D is present in Karachi sheep. Haplotype E is occurred in some Turkish animals.

Evidence suggest that sheep have been transported between major geographic areas or regions which is cleared from the sequenced data set which expressed the presence of Haplotype A in many animals of European
breeds (Meadows et al., 2005). Haplotype A which is also found in New Zealand suggesting the early migration of sheep from Asia to Australia (Hiendleder et al., 2002). All the three Asian breeds of sheep have haplotype A which also indicates the movement of sheep during breed development.

Author Contributions
All authors have equal contribution rates, and the authors have read and approved the article.

Conflict of Interest
The authors declare that there is no conflict of interest.

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