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Radhika G
Associate Professor, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, India

Aravindakshan TV
Director, Centre for Advanced Studies in Animal Genetics and Breeding, Mannuthy, KVASU, Kerala, India

Stephy Thomas
Research Assistant, Centre for Advanced Studies in Animal Genetics and Breeding, Mannuthy, KVASU, Kerala, India

Corresponding Author:
Radhika G
Associate Professor, College of Veterinary and Animal Sciences, KVASU, Pookode, Wayanad, Kerala, India

Estimation of genetic diversity in domestic animals using microsatellites

Radhika G, Aravindakshan TV and Stephy Thomas

Abstract
Assessing the variation in domestic animals through genetic diversity analysis, forms the basis for sustainable management and development of Farm Animal Genetic Resources. Microsatellites, which were reported to be highly polymorphic and widely distributed throughout the genome, have proved to be one of the best molecular markers for genetic diversity analysis. International Society for Animal Genetics (ISAG) and FAO advisory group proposed specific microsatellite panels for diversity analysis in livestock species. Microsatellites can be combined with PCR and made more economical, if multiplexing is done along with the usage of fluorescent labelled primers. Usually, 15-25 microsatellites are chosen and data obtained from 30 unrelated individuals from each population. After genotyping, raw data will be obtained in the form of allelic sizes for each microsatellite locus and reliability of data should be checked using appropriate softwares. Genetic variation in the populations can further be assessed by measuring allelic richness, Polymorphic Information Content and private alleles. F-Statistics which describes the statistically expected level of heterozygosity in a population can also be measured from microsatellite data. Genetic distance estimated indicates a measure of genetic divergence between species or between populations within a species and dendrogram can be constructed further. Structure Analysis which detects underlying genetic populations among a set of individuals and Bottleneck analysis which detects genetic signatures of a recent reduction in population size, augments the data on genetic diversity. Though whole genome genotyping arrays with SNP data have advantages, microsatellites are still considered suitable markers to provide complementary information on genetic diversity.

Keywords: genetic diversity estimation, microsatellites, domestic animals

1. Introduction
Genetic diversity within and between populations could be measured and this formed the basis of evolutionary and conservation biology. According to Erikson *et al*. (1993) [17], genetic diversity provided raw material for adoption and evolution, and hence was essential for long term survival of the species and populations. There is worldwide recognition of the need for conservation of livestock diversity and for characterization of breeds and populations including their genetic differentiation and relationships. (Food and Agricultural Organisation (FAO), 1998) [22]
Wang *et al*. (2009) [74] explained genetic diversity as any variation in nucleotides, genes, chromosomes or whole genomes of organisms. According to Mittal and Dubey (2009), genetic diversity could be assessed among different accessions, individuals within same species (intra species), among species (inter species) and/or between genus and families. Effective management of farm animal genetic resources required comprehensive knowledge of not only the breed characteristics, but also data on population size and structure, geographical distribution, production environment and within and between breed genetic diversity (Groeneveld *et al*., 2010) [32]. The genetic diversity of domesticated animals is often overlooked as having a meaningful
contribution to global biodiversity.
Thus, Genetic diversity can be estimated between populations, families and individuals. Since genetic variation forms the basis of all livestock breeding programs, assessing the variation through genetic diversity analysis, forms the basis for sustainable management and development of Farm Animal Genetic Resources. Understanding how to analyse genetic diversity through most appropriate methods, reveals much about the molecular variations and population structure.

2. Role of microsatellites in diversity analysis

Microsatellites were described as tandemly repeated motifs of variable lengths that were distributed throughout the nuclear genome in both coding and noncoding regions (Jarne and Lagoda, 1996) [35]. Microsatellites were di, tri, tetra or penta nucleotide units, repeated in tandem and very useful as genetic markers as they were highly polymorphic, locus specific, co dominant and PCR based (Powell *et al.*, 1996) [59]. Boyce *et al.* (1996) [8] opined that microsatellites showed high variability, high mutation rate, large number, distribution throughout the genome, co dominant inheritance and neutrality with respect to selection. For defining breeds and describing them, microsatellite loci were considered to be best suited (Goldstein and Pollock, 1997) [29]. Luikart *et al.* (1999) [45] reported that mutations showed a much higher rate in microsatellites than in non-neutral markers, which reflected in changes in the number of repeats and thus in the length of fragments.

Gama and Bressan (2011) [26] observed that microsatellite loci had high levels of allelic diversity which were essential in the analysis of genetic diversity. Autosomal microsatellites were reported to be the most popular markers for characterizing the genetic constitution of breeds, establishing breed relationships, describing the history of livestock and the uniqueness at the breed level (Lenstra *et al.*, 2012) [42]. Microsatellites, which were reported to be highly polymorphic and widely distributed throughout the genome, have proved to be one of the best molecular markers for genetic diversity analysis.

Among DNA markers, Fadhil *et al.* (2018) [20] considered polymorphic microsatellite markers, as the most preferable system for diversity analysis in farm animals using PCR applications. Individual identification and certification have gained significant progress in many countries for major livestock species, due to the use of microsatellites (Svishcheva *et al.*, 2020) [67].

2.1 Selection of microsatellites for genetic diversity analysis in domestic animals

Microsatellites were found to be powerful tools to differentiate between breeds and results obtained were in agreement with the breeding history and geographical origin (Saitbekova *et al.*, 1999; Yang *et al.*, 1999) [63, 76, 77]. From 1990s onwards, molecular data became more and more relevant for characterisation of genetic diversity (Groeneveld *et al.*, 2010) [32]. Then came the necessity for providing common guidelines throughout the world for analysis of molecular data, so that global comparison and future development of Animal Genetic Resource was possible.

FAO (2011) [23] issued guidelines advising not to use marker categories such as Major Histocompatibility Complex or protein polymorphisms for diversity studies. They further advised that choice of marker panel was the most critical decision in microsatellite analysis. International Society for Animal Genetics (ISAG) and FAO advisory group proposed panels of 30 microsatellite markers for nine major livestock species (www.globaldiv.eu/docs/ microsatellites%20 markers.pdf). The use of FAO-ISAG panel of microsatellites maximised the utility of the study to the regional or national level to obtain a global view to Animal Genetic Diversity. Many existing datasets from completed characterization studies that have used FAO-ISAG markers allowed new data to be compared with more breeds than any other microsatellite panel, particularly for cattle, sheep and goat (FAO, 2011) [23]. Hale *et al.* (2012) [33] reported that 25 to 30 individuals per population was enough to accurately estimate allele frequencies in microsatellite studies and there appeared to be little benefit when sampling size was increased.

The study of genetic diversity of livestock at the molecular level has developed into an active area of research around the world and hence it is most appropriate to use Microsatellites from FAO-ISAG panel, so that the study maintains a global uniformity. Using SNP chips is a modern alternative for genetic diversity analysis, but demands huge economic commitment.

2.2 Multiplex PCR for Microsatellite Amplification

Multiplex PCR is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. In a multiplexing assay, more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. In a single template PCR reaction, the technique uses a single template DNA along with several pairs of forward and reverse primers to amplify specific regions in a template.

Reports indicated that multiplexing was done for microsatellites as it proved to be less costly in terms of both time and money (Guichoux *et al.*, 2011; Hamoy and Santos, 2012). Researches where multiplexing was done for microsatellite amplification in goats included those performed by Tolone *et al.* (2012) [72] and Agaoglu and Ertugrul (2012) [1]. Lia *et al.* (2018) [43] reported multiplex SSR-PCR as an efficient and economical technology which yielded accurate genetic information under limited resources.

2.3 Tagging PCR Primers with Fluorescent Dyes

For conducting multiplex PCR, while screening large number of individuals, it is possible to fluorescently tag 5` end of PCR primer by making modifications in the primer. The dyes usually used include FAM (Blue), VIC (Green), NED (Yellow) and PET (Red). By carefully standardising the annealing temperature and magnesium chloride concentration or by combining Touchdown PCR with multiplexing, two to four microsatellite loci can be amplified and then genotyped using automated sequencer with software for allele calling.

Another alternative is doing simple PCR with fluorescent labelled single pair of primers and then mixing PCR products obtained from different fluorescent labelled primers, before sending for genotyping. Fluorescent labelled primers were used for microsatellite analysis by several workers (Dixit *et al.*, 2009; Kumar *et al.*, 2009; Dixit *et al.*, 2010; Martinez *et al.*, 2012; Aljumaah *et al.*, 2012, Radhika *et al.* 2016) [14, 40, 15, 47, 3, 62].

One of the major reasons for microsatellites becoming markers of choice in genetic diversity studies is that, it can be combined with PCR or can be made more economical if multiplexing is done along with the usage of fluorescent labelled primers.

2.4 Genotyping

After proper amplification of each microsatellite by PCR, amplified fragments should be checked on agarose gels by electrophoresis and on conformation, sent for genotyping. Since repeat units are small in size, amplification detection methods using capillary electrophoresis on an automated DNA sequencer and subsequent sizing using suitable softwares have to be done. This provided discrimination between two closely sized alleles. After genotyping, raw data

will be obtained in the form of allelic sizes for each microsatellite locus. Binning of alleles can be done using suitable software like Flexibin. Then reliability of data can be checked for null alleles, Linkage Equillibrium and for Hardy Weinberg Equillibrium (HWE). (Amos *et al*., 2007, Kallinowski, 2007 [39] and Excoffier and Lischer, 2010) [5]. A data conversion program considered as starting point for formatting input data files is Convert (Glaubitz, 2004) [28] which can create input files for several other formats. From this point, the file formats obtained maybe used as input files for different computer programs, in order to analyse the following parameters.

3. Estimation of genetic variation

Allelic variation and heterozygosity analysis were reported as the major two approaches which examined genetic variation in populations (Allendorf and Luikart, 2007) [4]. Allelic richness, Polymorphic Information Content (PIC) and private alleles were estimated to determine allelic variation in the data. Counting the total number of alleles at a particular locus in a population is considered as a method to measure genetic variation. But the number of distinct alleles depended heavily on sample size and it became difficult to interpret the result when sample sizes differed across populations. Allelic richness was defined as a measure of allelic diversity that considered the sample size (Mousadik and Petit, 1996) [50] or the number of distinct alleles expected in a random sub-sample drawn from the population (Petit *et al*., 1998) [58].
Barker (1994) [6] suggested that for studies of genetic distance, microsatellite loci should have more than four alleles to reduce the standard error of the distance estimates.

3.1 Private Alleles

Private allele richness or number of unique alleles in a population is a simple measure of genetic distinctiveness. Presence or absence of private alleles was reported to give an idea about the migration rates between populations as Slatkin (1985) [64] proved that there is a linear relationship between actual number of immigrants entering to a subpopulation at each generation (N_m) and average frequency of private alleles at equilibrium. They concluded that if gene flow is small, several private alleles were found in populations that developed by mutations. Lowel and Allendorf (2010) [44] opined that length of time that a new allele stays private was determined by the migration rates, which meant that proportion of alleles that were private decreased as migration rate increased. According to Svishcheva *et al*., (2020) [67], the presence of private alleles in native breeds of cattle indicated unique gene pools, and they further pointed out that the private alleles might be considered as an effective tool for the genetic identification of breeds.
Presence of more private alleles in a population indicated distinctness of that genetic group over others. As the migration rate or gene flow (N_m) increased, the proportion of private alleles started to decrease and in many animal populations, private alleles were observed in very low frequency only.

3.2 Polymorphic Information Content (PIC)

PIC is a measure of polymorphism for a marker locus and is a relative measure of the informativeness of a marker, which depends on the number of alleles of this marker and its relative frequency in the population (Bolstein *et al.*, 1980). Hande (2010) [34] stated that markers were classified as polymorphic and informative, if PIC was greater than 0.5, whereas if it is above 0.75, the locus becomes much more informative.

3.3 Heterozygosity

The measure of genetic diversity in a population is otherwise termed as expected heterozygosity (H_e), which is the expected probability than an individual will be heterozygous at a given locus. Average expected heterozygosity calculated over all loci within a population can be considered as the best general measure of genetic variation as Gorman and Renzi (1979) [30] reported that H_e was not generally affected by sample size and even a few individuals were sufficient for estimating H_e, if a large number of loci were examined. H_e was compared with H_o (observed heterozygosity) to determine whether a population was in Hardy Weinberg Equilibrium.

3.4 F–Statistics

F-Statistics describes the statistically expected level of heterozygosity in a population or expected degree of reduction in heterozygosity when compared to Hardy-Weinberg expectation. F-Statistics include F_{IS}, F_{ST} and F_{IT}. The F-Statistics or the inbreeding coefficients in a population developed by Wright (1965) and extended by Nei (1972) [53] was reported as the oldest and most widely used method to measure the genetic differentiation within and between populations (Allendorf and Luikart, 2007) [4].

3.4.1 F_{IS}

F_{IS} is the inbreeding coefficient of individual relative to subpopulations. It is a measure of departure from Hardy-Weinberg proportions within local subpopulations Wright and Ewens (1969) [75] defined F_{IS} as the proportion of the total inbreeding within a population that was because of inbreeding within subpopulations. It was estimated from heterozygote deficit. Nei (1987) [54] explained a number of factors including inbreeding, locus under selection (genetic hitchhiking), null alleles (non amplifying alleles) and presence of population substructure (Wahlund effect) as possible reasons for lack of heterozygotes in a population. Positive F_{IS} estimates indicated either presence of inbreeding in the population and/or a Wahlund effect as observed by Pariset *et al.* (2003) [56].
Tang (2006) [69] opined that degree of inbreeding was high when F_{IS} was a positive number, whereas there was outbreeding within breeds when F_{IS} was a negative value. Lenstra *et al.* (2012) [42] suggested that for most livestock species, geographical isolation and selective breeding generated multiple subdivisions.

3.4.2 F_{ST}

F_{ST} is a measure of genetic divergence among sub populations and can be used as a distance measure. F_{ST} ranges from zero to one and becomes zero, when populations have equal allele frequencies, during panmixia and F_{ST} is equal to one, when all the genetic variation is explained by population structure, when populations are fixed for different alleles. F_{ST} is otherwise called as fixation index. Genetic structure of population was studied for different species and breed differentiation was reported as 0.099 in dogs (Jordana *et al.*, 1992) [37] 0.1 to 0.2 in humans (Cavilli-Sforza *et al.*, 1994) [11], 0.1 in European cattle breeds (Mac Hugh *et al.*, 1998) [46], 0.17 in goats (Saitbekova *et al.*, 1999) [63] and 0.08 in Spanish horse breeds (Canon *et al.*, 2000) [9]. Generally F_{ST} values

between 0.05 and 0.3 were typical for differentiation of livestock breeds, with a value over 0.15 indicating significant differentiation, although much smaller values could also be significant (Frankham *et al.*, 2002) [25]. F_{ST} calculated for Indian cattle was 0.113 (Mukesh *et al.*, 2004) [51] whereas for Indian sheep breeds, Nalli and Chokla it was 0.083 (Sodhi *et al.*, 2006) and the low level of genetic differentiation between two sheep breeds suggested larger extent of genetic exchange between the breeds through inadvertent mating due to sharing of common breed tract. In an extensive study by Canon *et al.* (2006) [10] where 45 goat breeds from Europe and Middle East were analysed, less than seven per cent of the total genetic variability was attributed to differences among breeds. The results were interpreted as being a consequence of high mobility of goats which have accompanied human migration and commercial routes thus providing the opportunity for admixture among the populations from which the breeds currently recognised have originated (Naderi *et al.*, 2007). Lenstra *et al.* (2012) [42] observed that genetic distances between multiple samples of the same breed or between closely related breeds tend to be smaller than the distance between divergent breeds.

3.5 Gene Migration (N_m)

N_m is the effective number of migrants in a population. In the context of conservation and maintenance of genetic variability, migration values (N_m) could be interpreted as the upper limit of number of migrants per generation, which would allow the maintenance of observed genetic differentiation between breeds (Canon *et al.*, 2000) [9].

Sodhi *et al.* (2006) [66] suggested that high level of gene flow (3.896) was the probable cause of great genetic similarity among neighbouring breeds. Unlike populations in the wild, dispersal ability and hence gene flow among domestic animal populations was believed to be governed more by human intervention than by physical barriers (Gizaw *et al.*, 2007) [27]. Gene flow can be estimated from F_{ST}. High gene flow between breeds is often the reason for less genetic differentiation between them and in case of domestic animals, it is a consequence of human intervention.

3.6 Genetic distance

Genetic distance is a measure of genetic divergence between species or between populations within a species, which gives an indication about the shared common genes between populations. It gives a relative measure of time that has elapsed since the populations have existed as single cohesive unit. Nei (1972) [53] stated that if the rate of genetic change is constant per year or generation, then Nei's standard genetic distance (D_{ST}) increased in proportion to divergence time.

3.6.1 Neighbor-Joining (NJ) and UPGMA trees

In UPGMA described by Sneath and Sokal (1973) [65] a pair of taxa with the smallest distance was combined into one cluster and formed composite taxa. This process was repeated until a rooted tree was made. The branch lengths were calculated so that the sum of branch lengths from taxa to the node connecting the two taxa was half the distance of the two taxa. In UPGMA, the rate constancy (rate of genetic change is constant per generation or year) was assumed.

In NJ method described by Saitou and Nei (1987) [54] all branches were connected to one node and a pair of taxa or population which gave the smallest sum of branch lengths were combined into a cluster and formed a composite taxa. This process was repeated until an unrooted tree was produced.

Takezaki and Nei (1996) [68] opined that if rate constancy approximately holds, UPGMA was efficient in constructing the correct tree topology.

3.6.2 Bootstrap Test

In bootstrap test (Felsenstein, 1985) [24] the loci were resampled with replacement. The phylogenetic tree was constructed with the distance values calculated from the same number of resample loci as that of the original input dataset in each replication. The number of replications in which the branch appeared was counted and the proportion of this number in the total replications was shown in per cent on the branch of the tree.

According to Dadi *et al.* (2008) [13], low bootstrap values implied that the classification observed on the dendrogram were not well supported and did not represent distinct evolutionary units. Zero or negative figures were not to be considered.

3.6.3 Construction of Dendrogram

Phylogenetic reconstruction reduced highly multidimensional data into a two dimensional diagram. Trees of individuals or breeds could be reconstructed on the basis of genetic distances (Lenstra *et al.*, 2012) [42].

Reynold's distance (D_A) was used as an estimator of subdivision parameters and was found to be the most accurate distance measure for recent divergence events measured by microsatellites (Laval *et al.*, 2002) [41]. Lenstra *et al.* (2012) [42] stated that genetic distances between multiple samples of the same breed or between closely related breeds were smaller than the distances between divergent breeds.

3.7 Structure Analysis

Structure Analysis detects underlying genetic populations among a set of individuals genotyped at multiple markers. It computes the proportion of the genome of an individual originating from each inferred population.

Pritchard *et al.* (2000) [61] recognised the need for a procedure to identify genetically differentiated population directly from individual genetic polymorphic data, instead of relying on *a priori* population information. Genotypic clustering models were developed in a Bayesian statistical framework, available in software package Structure V.2.3.4 from Pritchard *et al.* (2000) [61] and modified by Falush *et al.* (2003) [21]. This program offered the prospect of inferring the number of underlying populations, 'k' present in an emperical data set, thus providing an effective way to illustrate the presence of population structure and to distinguish distinct genetic populations.

The most significant factors to determine for structure analysis are the burn in length, ancestry model and estimation of 'k' (number of populations).

Burn in length explained how long to run the simulation before collecting data to make sure that the simulated population reached to drift mutation equilibrium which minimized the starting configuration. Typically, a burn in length of 10,000 to 1,00,000 was reported as more than adequate (Falush *et al.*, 2003) [21]. For the ancestry of individuals, admixture model was preferred as it was flexible for many complexities of real populations as it assumed that individuals had mixed ancestry (Falush *et al.*, 2003) [21].

Parker *et al.* (2004) [57] opined that Structure was designed to

overcome the limitations inherent in phylogenetic tree models and were applied to infer genetic structure in several species. Pritchard and Wen (2004) [60] explained that Structure algorithm did not provide a statistical indication of most likely 'k'. Instead 'k' was identified as a point of inflection on the log likelihood curve that led to a plateau or by the maximum value. One method for estimating 'k' was suggested by Evanno *et al.* (2005) [18], where Ln P(D) equals log likelihood for each k *i.e*. L (k). Therefore when 'k' is approaching a true value, L (k) plateaus and reaches it maximum value. Another method developed by Tapio *et al.* (2010) [71], and widely used, tested similarity between the results of individual runs for each different value of 'k'. At maximum similarity, it is argued that the correct 'k' is obtained.

Structure analysis helps to identify the structure of a population, especially its admixture, directly from individual genetic polymorphic data, instead of relying on prior population grouping.

3.8 Bottleneck Analysis

Bottleneck analysis allowed detecting genetic signatures of a recent reduction in population size based on the assumption that bottlenecks produced a faster reduction of allelic diversity than of heterozygosity (Cornuet and Luikart, 1996) [12]. They computed the distribution of expected heterozygosity by simulating a coalescent process for each locus and population, and under the assumption of mutation drift equilibrium, under three possible mutation models, infinite alleles model (IAM), stepwise mutation model (SMM) and two phase mutation model (TPM). They also stated that distribution obtained by simulation allowed to calculate the significance of the difference between observed and expected heterozygosities under the scope of three statistical tests (Wilcoxon signed-rank, sign and standardized differences tests) implemented in the program.

Tantia *et al.* (2004) [70] reported that any population that experienced a recent bottleneck showed higher than expected heterozygosity for a large majority of loci. Genetic bottleneck occurred when population experienced temporary reduction in size, which influenced distribution of genetic variation within and among populations (Kumar *et al.*, 2009) [40].

For analysisng microsatellite data, R package is recommended. Otherwise, different computer programs have to be chosen for estimating different parameters. In order to estimate the allelic diversity and for calculating F statistics, FSTAT 2.9.3 Package (Goudet, 2001) [31] may be used. Observed and effective number of alleles denoted as n_a and n_e maybe determined by Pop Gene 1.32 (Yeh *et al*., 1999) [77]. Cervus Version 3.0.3 (Kallinowski *et al*., 2007) [39] may be used to determine PIC. To identify genetically differentiated population directly from individual genetic polymorphic data, Structure analysis using software Structure (Pritchard *et al*., 2000) [61] modified by Falush *et al*. (2003) [21] maybe performed. Analysis maybe done using software program Bottleneck V.1.2.02 (Cornuet and Luikart, 1996) [12] to detect genetic signatures of a recent reduction in population size using three possible mutation models IAM, SMM and TPM under the scope of three statistical tests (Sign test, Standardised difference test and Wilcoxon test).

4. Conclusion

The investigation of genetic diversity at molecular level was proposed as a valuable complement and sometimes proxy to phenotypic diversity of local breeds and is presently considered as one of FAO priorities for breed characterization (Ajimone-Marsan *et al.*, 2014). Molecular marker of choice for genetic diversity analysis, the microsatellites, could be successfully utilised for analysing domestic animal populations. This is done by calculating allelic richness, Polymorphic Information Content, private alleles, F-Statistics including F_{IS}, F_{ST} and F_{IT}, Genetic distance, admixture in the populations (Structure analysis) and by detecting genetic signatures of a recent reduction in population size (Bottleneck analysis), using appropriate computer programs. The future of genetic diversity studies lies in the development of SNP panels that assay thousands of markers and whole genome sequencing of livestock species. Yurchenko *et al.* (2017) studied European cattle breeds using whole-genome genotyping arrays and Upadhyay *et al*. (2019) studied Genomic relatedness and diversity of Swedish native cattle breeds. Edea *et al.* (2018) reported that whole genome sequencing in domestic animals, primarily cattle, gained much advance in recent years. Though such arrays with SNP data have advantages, microsatellites are still considered suitable markers to provide complementary information on genetic diversity (Yu *et al*., 2015) and researchers are publishing recent works on microsatellites for genetic characterisation (Mira *et al*. 2021).

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6. References

1. Agaoglu OK, Ertugrul O. Assessment of genetic diversity, genetic relationship and bottleneck using microsatellites in some native Turkish goat breeds. Small Ruminant Res 2012;105:53-60.
2. Ajimone-Marsan P, Colli L, Han JL, Achilli A, Lancioni H, Joost S. The characterization of goat genetic diversity: towards a genomic approach. Small Ruminant Res 2014;4755:1-15.
3. Aljumaah RS, Musthafa MM, Al-Shaikh MA, Badri OM, Hussein MF. Genetic diversity of Ardi goat based on microsatellite analysis. African J Biotechnol 2012;11:16539-16545.
4. Allendorf FW, Luikart G. Conserving Global Biodiversity? Conservation and the Genetics of Populations. Blackwell Publishing, Oxford, UK 2007, 642.
5. Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill AVS. Automated binning of microsatellite alleles: problems and solutions. Mol. Ecol 2007;7:10-14.
6. Barker JSF. A global protocol for determining genetic distances among domestic livestock breed. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production 1994;21:501-508.
7. Bolstein D, White RL, Skolnick M. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J Human Genet 1980;32:314-331.
8. Boyce WM, Hedrick PW, Muggli-Cockett ME, Kalinowski S, Penedo MC, Ramey RR. Genetic variation of major histocompatibility complex and microsatellite loci: a comparison in Bighorn sheep. Genetic

1996;145:421-433.
9. Canon J, Checa ML, Carleos C, Vega-Pla JL, Vallejo M, Dunner S. The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. Anim. Genet 2000;31:39-48.
10. Canon J, Garcia D, Garcia-Atance MA, Obexer-Ruff G, Lenstra JA, Ajmone-Marsan P, Dunner S. Geographical partitioning of goat diversity in Europe and the Middle East. Anim. Genet 2006;37:327-334.
11. Cavilli-Sforza LL, Menozzi P, Piazza A. The History and Geography of Human Genes. Princeton University Press, Princeton, N.J., USA 1994, 1088.
12. Cornuet JM, Luikart G. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 1996;144:2001-2014.
13. Dadi H, Tibbo M, Takahashi Y, Namura K, Hanada H, Amano T. Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations. Anim. Genet 2008;39:425-431.
14. Dixit SP, Verma NK, Aggarwal RAK, Kumar S, Chander R, Vyas MK *et al*. Genetic structure and differentiation of three Indian goat breeds. Asian-Aust. J. Anim. Sci. 2009;22:1234-1240.
15. Dixit SP, Verma NK, Aggarwal RAK, Vyas MK, Rana J, Sharma A *et al*. Genetic diversity and relationship among Southern Indian goat breeds based on microsatellite markers. Small Ruminant Res 2010;91:153-159.
16. Edea Z, Dadi H, Dessie T, Uzzaman MR, Rothschild MF, Kim ES, Sonstegard TS *et al*. Genome-wide scan reveals divergent selection among taurine and zebu cattle populations from different regions. Anim. Genet 2018;49:550-563.
17. Erikson G, Namkoong G, Roberds JH. Dynamic gene conservation for uncertain futures. For. Ecol. Manage. 1993;62:15-37.
18. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol 2005;14:2611-2620.
19. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: A new series of program to perform population genetics analysis under Linus and windows. Mol. Ecol. Resour. 2010;10:564-567.
20. Fadhil M, Zulkadir U, Aytekin I. Genetic Diversity in Farm Animals. Elixir Hor. and Sig 2018;117:50032-50037.
21. Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M. Traces of human migrations in Helicobacter pylori populations. Science 2003;299:1582-1585.
22. FAO. Secondary guidelines for development of National farm animal genetic resources management plans: Management of small populations at site. Food and Agricultural organization, Rome 1998, 215. http://dad:Fao.org/en/refer/library/guidelines/popn.pdf
23. FAO. Molecular Genetic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines. No. 9, Rome. 2011.
24. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-791.
25. Frankham R, Ballou JD, Briscoe DA. An Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK 2002.
26. Gama LT, Bressan MC. Biotechnology applications for the sustainable management of goat genetic resources. Small Ruminant Res 2011;98:133-146.
27. Gizaw S, Van Arendonk JAM, Komen H, Windig JJ, Hanotte O. Population structure, genetic variation and morphological diversity in indigenous sheep of Etiopia. Anim. Gen 2007;38:621-628.
28. Glaubitz JC. Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Mol. Ecol 2004;4:309-310.
29. Goldstein DB, Pollock DD. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. J Hered 1997;88:335-342.
30. Gorman GC, Renzi J. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979;2:242-249.
31. Goudet J. FSTAT. A program to estimate and test gene diversities and fixation indices. Version 2.9.3. 2001. Available: http://www.unil.ch/izea/ softwares/fstat.html.
32. Groeneveld LF, Lenstra JA, Eding H, Toro MA, Scherf B, Pilling D *et al*. Genetic diversity in farm animals – a review. Anim. Genet 2010;41:6-31.
33. Hale ML, Burg TM, Steeves TE. Sampling for microsatellite based population genetic studies: 25-30 individuals per population is enough to accurately estimate allele frequencies. PL0S ONE. 2012;7:e45170. Available http://www.plosone.org/article/ into:doi/10.1371/journal.pone. 0045170.
34. Hande A. Bioinformatic analyses in microsatellite-based genetic diversity of Turkish sheep breeds. M.Sc. thesis. Middle East Technical University, Ankara 2010, 117.
35. Jarne P, Lagoda PJL. Microsatellites from molecules to populations and back. Trends Ecol. Evol 1996;11:424-429.
36. Jordana J, Pares PM, Sanchez A. Analysis of genetic relationships in horse breeds. J. Equine Vet. Sci 1995;15:320-328.
37. Jordana J, Piedrafita J, Sanchez A, Puig P. Comparative F statistics analysis of the genetic structure of ten Spanish dog breeds. J Hered 1992;83:367-374.
38. Jordana J, Ribo O, Pelegrin M. Analysis of genetic relationships from morphological characters in Spanish goat breeds. Small Ruminant Res 1993;12:301-314.
39. Kallinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol 2007;16:1099-1106.
40. Kumar S, Dixit SP, Verma NK, Singh DK, Pande A, Kumar S *et al*. Genetic diversity analysis of the Gohilwari breed of Indian goat (*Capra hircus*) using microsatellite markers. Am. J Anim. Vet Sci 2009;4:49-57.
41. Laval G, Sancristobal M, Chevalet C. Measuring genetic distances between breeds: use of some distances in various short term evolution models. Genet. Sel. Evol. 2002;34:481-507.
42. Lenstra JA, Groeneveld LF, Eding H, Kantanen J, Williams L, Taberlet P *et al*. Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. Anim. Genet 2012;43:4-3-502.
43. Lia D, Wanga S, Shen Y, Meng X, Xu X, Wang R *et al*. A multiplex microsatellite PCR method for evaluating genetic diversity in grass carp (*Ctenopharyngodon*

idellus). Aquaculture and Fisheries 2018;3:238-245
44. Lowel W, Allendorf F. What can genetics tell us about population connectivity? Mol. Ecol 2010;19:3038-3051.
45. Luikart G, Biju-Duval MP, Maudet C. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet. 1999;30:431-438.
46. Mac Hugh DE, Loftus RI, Cunningham P, Bradley DG. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. Anim. Genet. 1998;29:333-340.
47. Martinez A, Ferrando A, Manunza A, Gomez M, Landi V, Jordana J *et al*. Inferring the demographic history of a highly endangered goat breed through the analysis of nuclear and mitochondrial genetic signatures. Small Ruminant Res 2012;104:78-84.
48. Mittal N, Dubey AK. Microsatellite markers – a new practice of DNA based markers in molecular genetics. Pharmacognosy Reviews 2009;3:235-246.
49. Mira T, Alain A, Charbel A, Saab AS. Genetic and morphological characterization of Lebanese mountain local goat population. The Journal of Animal and Plant Sciences 2021;31:409-415.
50. Mousadik A, Petit RJ. High level of genetic differentiation for allelic richness among populations of the Argan tree endemic to Morocco. Theor. Appl. Genet. 1996;92:832-839.
51. Mukesh M, Sodhi M, Bhatia S, Mishra P. Genetic diversity of Indian native cattle breeds as analyzed with 20 microsatellite loci. J Anim. Breed. Genet 2004;121:416-424.
52. Naderi S, Rezaei HR, Taberie P, Zundel S, Rafat SA, Naghash HR *et al*. Large-scale mitochondrial DNA analysis of the domestic goat reveals six haplo groups with high diversity. PLOS One 2007;2:137.
53. Nei M. Genetic distance between populations. Am. Nat. 1972;106:283-292.
54. Nei M. Molecular Evolutionary Genetics. Columbia University Press. New York. 1987, 512.
55. Oosterhout CV, Hutchinson DW, Shipley P. Micro-Checker 2.2.3. 2004. Available http://www.microchecker.hull.ac.uk/.
56. Pariset L, Savarese MC, Cappuccio L, Valentini A. Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of Central Italy. J. Anim. Breed. Genet 2003;120:425-432.
57. Parker MG, Kim LV, Sutter MB, Carlon S, Lorentzen TD, Malek TB *et al*. Genetic structure of the purebred domestic dog. Science 2004;304:1160-1164.
58. Petit RJ, Mousadik A, Pons O. Identifying population for conservation on the basis of genetic markers. Conserv. Biol. 1998;12:844-855.
59. Powell W, Machray GC, Provan J. Polymorphism revealed by simple sequence repeats. Trends in Plant Science 1996;1:215-222
60. Pritchard J, Wen W. Department of Human Genetics, University of Chicago, Chicago, USA 2004.
61. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945-959.
62. Radhika G, Raghavan KC, Aravindakshan TV, Thirupathy V. Genetic diversity and population structure analysis of native and crossbred goat genetic groups of Kerala, India. Small Rumin. Res 2015;131:51-57.
63. Saitbekova N, Gaillard C, Obexer-Ruff G, Dolf G. Genetic diversity in Swiss goat breeds based on microsatellite analysis. Anim. Genet 1999;30:36-41.
64. Slatkin M. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 1985;16:393-430.
65. Sneath PHA, Sokal RR. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W.H. Freeman, San Francisco. 1973, 573.
66. Sodhi M, Mukesh M, Bhatia S. Characterizing Nali and Chokla sheep differentiation with microsatellite markers. 2006;65:185-192.
67. Svishcheva G, Babayan O, Lkhasaranov B, Tsendsuren A, Abdurasulov A, Stolpovsky Y. Microsatellite Diversity and Phylogenetic Relationships among East Eurasian Bos taurus Breeds with an Emphasis on Rare and Ancient Local Cattle Animals 2020;10:1493. doi:10.3390/ani10091493
68. Takezaki N, Nei M. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 1996;144:389-399.
69. Tang C. Analysis of genetic diversity of microsatellite among 13 sheep population in Xinijiang [D]. Master's thesis, Xinijiang Agric. Univ., China 2006.
70. Tantia MS, Behl R, Sheoran N, Singh R, Vijh RK. Genetic variability and bottleneck studies in Zalawadi, Gohilwadi and Surti goat breeds of Gujarat (India) using microsatellites. Indian J Anim. Sci 2004;74:761-767.
71. Tapio I, Tapio M, Li MH, Popov R, Ivanova Z, Kantanen J. Estimation of relatedness among non-pedigreed Yakutian cryo-bank bulls using molecular data: Implications for conservation and breed management. Genet. Sel. Evo. 2010;42:28.
72. Tolone M, Mastrangelo S, Rosa AJM, Portolano B. Genetic diversity and population structure of Sicilian sheep breeds using microsatellite markers. Small Ruminant Res 2012;102:18-25.
73. Upadhyay M, Eriksson S, Mikk S, Strandberg E, Stålhammar H, Groenen MAM *et al*. Genomic relatedness and diversity of Swedish native cattle breeds. Genet. Sel. Evol 2019;51:56.
74. Wang ML, Barkley NA, Jenkins TM. Microsatellite markers in plants and insects. Part I: Applications of biotechnology. Genes Genomes, Genomics 2009;3:54-57.
75. Wright S, Ewens WJ. Evolution and the genetics of populations. The theory of gene frequencies. Science. 1969;168:722-723.
76. Yang L, Zhao SH, Li K, Peng ZZ, Montgomery GW. Determination of genetic relationships among five indigenous Chinese goat breeds with six microsatellite markers. Anim. Genet 1999;30:452-455.
77. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. POPGENE Version 1.32, the user-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. 1999. http://www.ualberta.ca/-fyeh/fyeh.
78. Yurchenko AA, Yudin N, Aitnazarov R, Plyusnina A, Brukhin V, Soloshenko V *et al*. Genome-wide genotyping uncovers genetic profiles and history of the Russian cattle breeds. Hereditary 2017;120:125-137.