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GENETIC DIVERSITY ANALYSIS OF GOAT BREEDS USING MICROSATELLITE MARKERS

AMRITA SUSAN JACOB

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Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **"GENETIC DIVERSITY ANALYSIS OF GOAT BREEDS USING MICROSATELLITE MARKERS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,
22.08.05

AMRITA SUSAN JACOB

CERTIFICATE

Certified that this thesis, entitled **"GENETIC DIVERSITY ANALYSIS OF GOAT BREEDS USING MICROSATELLITE MARKERS"** is a record of research work done independently by **Dr. Amrita Susan Jacob**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannuthy,
22-08-05.

Dr. T.V. Aravindakshan
(Chairman, Advisory Committee)
Assistant Professor (Sr. Scale)
Department of Animal Genetics and Breeding
College of Veterinary and Animal Sciences
Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Amrita Susan Jacob,** a candidate for the degree of Master of Veterinary Science in Animal Genetics and Breeding, agree that this thesis entitled **"GENETIC DIVERSITY ANALYSIS OF GOAT BREEDS USING MICROSATELLITE MARKERS"** may be submitted by Dr. Amrita Susan Jacob, in partial fulfilment of the requirement for the degree.

22.08.05

Dr. T.V. Aravindakshan

(Chairman, Advisory Committee)
Assistant Professor (Sr. Scale)
Department of Animal Genetics and Breeding
College of Veterinary and Animal sciences
Mannuthy.

22.8.05

Dr. K.V. Raghunandanan,

Director
Centre for Advanced Studies in
Animal Genetics and Breeding
College of Veterinary and
Animal Sciences, Mannuthy.
(Member)

22.8.05

Dr. A.P. Usha

Assistant Professor
Department of Animal
Genetics and Breeding
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

22/8/05

Smt. K.S. Sujatha

Assistant Professor
Department of Statistics
College of Veterinary and
Animal Sciences, Mannuthy.
(Member)

30/9/05

External Examiner

(Dr. C.S. Nagaraja)
Associate Professor
Dept. of ASG&B
Veterinary College
Bangalore-24

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Introduction

1. INTRODUCTION

The domestic goat, *Capra hircus* is an important livestock species in India and other developing countries because of their short generation interval, high prolificacy and easy marketing of goats and their products. Goats have fulfilled agricultural, economic, cultural Pand religious roles from very early times in human civilization. They are the most adaptable and geographically widespread livestock species ranging from the high altitude of Himalayas to the deserts of Rajastan and humid coastal areas of India.

India boasts of 20 recognized breeds and innumerable local or non-descript goats, which constitute about 20 per cent of the global figure. The differences among these populations suggest that there is considerable variability among goats in our country.

Breeds are formed by centuries of human and natural selection. Indigenous breeds often possess gene combinations and special adaptations such as disease resistance, ability to survive harsh environmental conditions or poor quality feed. The genetic diversity found in domestic breeds allows development of new characteristics in response to changes in environment, diseases or market conditions. In spite of the unique characteristics possessed by different populations/breeds there is lack of concern for characterization, conservation and improvement of indigenous livestock.

Existence of a large gene pool is critical for a sustainable animal production system. Hence, conservation of genetic variability is of prime importance. The first step in the conservation and exploitation of domestic animal biodiversity is a comprehensive knowledge of the existing genetic variability and partitioning of this variability among breeds.

Several markers can be made use of for determining the genetic distance, a measure of overall evolutionary divergence or in other words a measure of genetic similarities and dissimilarities between populations. These include morphological, chromosomal and biochemical markers such as blood group and serum protein polymorphisms. Recently DNA based markers are increasingly being used for genetic diversity studies as they offer much great resolution of differences between individuals and populations. These include mitochondrial DNA variation, Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD) markers and Variable Number of Tandem Repeats (VNTR) markers. Based on the length of tandem repeats, the VNTR markers are classified into minisatellites with repeat lengths of 15-70 bp and microsatellites with 2-6 bp.

Microsatellites have several advantages over other DNA markers. Microsatellite loci are found in large numbers and are evenly spaced throughout the genome. Technically, microsatellites are desirable than larger VNTRs because they can be analyzed via the polymerase chain reaction (PCR) and alleles can be unambiguously sized on polyacrylamide gels.

Certain distinct populations of goats have been identified in Kerala and in the neighbouring state of Tamil Nadu. These include the Malabari, the Attappadi Black, the non-descript goats of Thrissur and the Salem Black goats. Malabari is the single accredited goat breed of Kerala, found in the northern parts of the state. Attappadi black goats originated from Attappadi region of Palakkad district and are reared mainly for meat and manure by the tribes of Attappadi. Salem Black animals are found in the Salem district of Tamil Nadu. Most of the local populations and indigenous breeds of animals are under constant threat of loss of unique genetic material, which could prove invaluable in the future, as a result of indiscriminate mating and a lack of organized effort for the conservation of these breeds.

Although several studies have been made on goats on the phenotypic characteristics and by using other marker systems, molecular genetic studies are scarce. Keeping in view, the importance of goats in rural economy and the need for conserving unique goat populations, the present study on the genetic variability of goat breeds was undertaken with the following objectives;

1. To characterize different breeds of goats using microsatellite markers.
2. To estimate allele and genotype frequencies at the polymorphic loci.
3. To estimate the genetic relationship among different breeds of goats.

Review of Literature

2. REVIEW OF LITERATURE

2.1 GENETIC DIVERSITY ANALYSIS

There are more than 3500 breeds and strains of domestic animals in the world, although the true magnitude of the existing diversity is not known. These breeds and strains are referred to as the global animal genetic resources. The improvement of domestic animals to meet human needs is dependent on genetic variation, both the within breed variation and the between breed variation. Genetic variation is the basic material of the animal breeder, which is used to mould our domestic animal species to our needs. Loss of variation will restrict the options available to meet unpredictable future requirements. It is, therefore, essential to determine which breeds are to be conserved, for which genetic diversity studies are inevitable.

Twenty goat breeds of India demonstrate the vast genetic resources of our country in terms of animal biodiversity. Most of the breeds of goats in India have evolved naturally through adaptation to agro-ecological conditions; to a limited extent there has been artificial selection for specific needs. These breeds have generally been named after their place of origin or on the basis of prominent characteristics (Acharya, 1982).

Notter (1999) recommended the importance of global programmes to determine genetic distances among livestock breeds and to establish 'core collections' of diversity within each species to ensure the full range of genetic variation within the species. He suggested that the use of molecular techniques to quantify relationships among breeds is important in the management of farm animal genetic diversity.

Giovambattista *et al.* (2001) studied genetic diversity in the Argentinean Creole cattle, which has been bred since its origin in semi wild conditions and with low levels of artificial selection, and suggested that indigenous domestic breeds may be useful as reservoirs of genetic diversity for commercial domestic species.

Eding *et al.* (2002) defined the genetic diversity of a set of populations as the maximum genetic variance that can be obtained in a random mating population that is bred from that set of populations. They proposed a method of making a quantitative assessment of the contribution of breeds to genetic diversity, which would help decision making in genetic conservation plans.

2.2 ISOLATION OF GENOMIC DNA

The isolation of pure, high molecular weight genomic DNA is the first and foremost requirement to carryout genetic analysis such as DNA polymorphisms, linkage studies and parentage testing. Different workers have described several methods of isolating genomic DNA but the standard phenol-chloroform extraction method is the most commonly followed procedure.

Blin and Stafford (1976) described a method of DNA isolation involving phenol extraction from tissues like calf thymus and human placenta.

Apparao *et al.* (1994) developed a rapid and simple procedure, which was a modification of the standard phenol-chloroform extraction method for DNA isolation for RFLP studies.

Aravindakshan *et al.* (1998) compared three methods of DNA extraction namely, the guanidine hydrochloride method, the high salt method and the phenol-chloroform method and demonstrated that both high salt method and

phenol chloroform method produced good yields of high molecular weight DNA from cattle white blood cells whereas the guanidine hydrochloride method failed to yield clean DNA.

Arranz *et al.* (2001) used Proteinase–K digestion followed by salting out for obtaining genomic DNA from blood and semen.

Barker *et al.* (2001) described the different steps involved in DNA extraction from white blood cells of goats using phenol-chloroform extraction procedure.

The isolation of genomic DNA from whole blood using the standard phenol-chloroform extraction method has been described by several workers (Tantia *et al.*, 2004; Iamartino *et al.*, 2004; Chauhan *et al.*, 2004; Vijh *et al.*, 2004; Preethi, 2004).

2.3 YIELD AND PURITY OF DNA

Apparao *et al.* (1994) extracted genomic DNA from cattle, buffalo, sheep, goat and swine using a modified phenol-chloroform extraction method and obtained yields of 250 to 300 µg DNA from 15 ml of whole blood.

Senthil *et al.* (1996) obtained 615.55 $\pm$ 0.72 µg and 444.58 $\pm$ 21.54 µg DNA using high salt method and phenol chloroform method, respectively, from 15 ml blood with the ratio of optical density at 260 and 280 nm more than 1.7.

Aravindakshan *et al.* (1998) reported that the mean yields of DNA extracted from 10 ml of whole blood of cattle and buffalo by phenol and high salt methods were 394.50/446.16 µg and 344.25/432.83 µg, respectively. The ratio of optical densities at 260 and 280 nm was consistent between 1.75 and 1.91 indicating good deproteinisation.

Chitra (2002) and Mathew (2004) reported yields of 231.097±11.65 µg and 350.056±10.048 µg, respectively from 5 ml of blood from goats.

2.4 MOLECULAR MARKERS

The markers revealing variation at DNA sequence level are called molecular markers or genetic markers. Large numbers of genetic polymorphisms can be detected by new laboratory techniques. There is considerable polymorphism in the mammalian genome with estimates of 5 to10 per cent of structural loci being polymorphic as determined by electrophoretic techniques (Smith and Simpson, 1986).

Several molecular markers like AFLP, RAPD and microsatellites have been used extensively for the analysis of genetic diversity in different livestock species (Saitbekova *et al.*, 1999; Arora *et al.*, 2003; Ajmone-Marsan *et al.*, 2001; Suprabha, 2003; Bhattacharya *et al.*, 2004).

2.4.1 Microsatellite Markers

Microsatellite sequences are stretches of tandem repeated short sequence motifs, two to six nucleotides in length (Litt and Luty, 1989) and they can be analysed using the polymerase chain reaction to detect genetic variations.

Microsatellites have been found in all eukaryotic genomes where they occur on an average once in every 10 kb of DNA sequence. They usually have a length of less than 100 bp and are found embedded in unique DNA sequences (Tautz, 1989).

Stallings *et al.* (1991) examined the distribution and conservation of position of GT repetitive sequences in several mammalian genomes. They found that in the human genome, on an average, a GT repetitive sequence occurred in

every 30 kb in DNA of euchromatic regions and that the position of these sequences has been conserved between closely related species such as humans and other primates.

Arranz *et al.* (1996) compared protein markers and microsatellite loci as tools for studying genetic differentiation among cattle populations and found that the heterozygosity and genetic distances were greater with microsatellites allowing a clearer differentiation among closely related species. They suggested that due to the high level of polymorphism displayed by microsatellites it was possible to obtain a better resolution in genetic analysis using a lower number of markers.

Microsatellites, used for genetic diversity analysis among humans, revealed a significant increase in the diversity of African populations, which was consistent with an African origin of modern humans. The differences in the genetic diversity among different populations could be effectively demonstrated by a modest number of loci, which implied that microsatellite systems contained a large amount of information about evolutionary history (Jorde *et al.*, 1997).

2.4.1.1 Cause of Variations at Microsatellite Loci

The alleles at microsatellite loci are distinguished by their size. Mutations may alter the size of an allele by adding or deleting one or more repeats. The mutation rate was found to be exceptionally high implying a high degree of polymorphisms at these loci.

Tautz (1989) studied simple sequence repeats in drosophila, human and whale DNA and found that they were hypervariable in length owing to the difference in number of repeats which probably arose due to slippage mechanism. He also suggested that it could be used for determining individual identity, genome mapping and linkage studies.

Microsatellites with 3 to 5 bp repeats evolved predominantly under the single step stepwise mutation model (Shriver *et al.*, 1993) and for those microsatellites with shorter repeats, the model suggested included multistep mutation events (Di Rienzo *et al.*, 1994).

Chakraborty *et al.* (1997) evaluated the relative mutation rates of microsatellite loci under the stepwise mutation model. They found that the dinucleotide repeats had higher mutation rates than tetranucleotides and non-disease causing trinucleotides had mutation rates intermediate between di- and tetranucleotide repeats.

Pandey *et al.* (2003) suggested that the use of a mixture of highly variable and less variable microsatellites reduce the risk of over estimating genetic variability which occur if only highly variable loci are used for genetic diversity studies.

2.4.1.2 Polymorphism at Microsatellite Loci

Buchanan *et al.* (1994) analysed polymorphism at eight ovine microsatellite loci in six sheep populations representing five breeds and observed that the allele frequencies varied significantly between populations for all the loci studied. They reported that the allele frequencies generated could be used to determine the breed of an individual, to a high degree of accuracy, if it belongs to any of the five breeds tested. It was also observed that some alleles were found only in one breed in very low frequencies and was therefore unlikely to be useful as markers for a breed.

Kim *et al.* (2002) studied microsatellite genetic diversity among Korean goats and found a significant deficit of heterozygotes; a departure from the Hardy-Weinberg equilibrium. They attributed the reason to the limited sample size, genetic drift or non-random mating.

Tomasco *et al.* (2002) from their study on Uruguayan sheep using microsatellites observed that the estimated allele frequencies differed from those reported in literature for most microsatellite loci analysed and suggested that independent estimates were essential for different regions and races.

The mean number of alleles in goats of Sub-Saharan Africa was compared with those from other regions and it was evident that African and Asian goat populations had slightly higher values than European breeds (Chenyambuga *et al.*, 2004).

Tantia *et al.* (2004) used microsatellite allele frequency data for finding out population bottle necks in two goat populations Black Bengal and Chegu, using several statistical tests which revealed population bottle necks in both populations about 200 generations back.

According to Jandurova *et al.* (2004) the high number of detected alleles and high level of heterozygosity in the majority of loci demonstrated the effectiveness of the microsatellite loci selected for estimation of genetic variation within small reproductively isolated populations.

Kumar *et al.* (2005) investigated the population structure and genetic variation among Marwari goats using microsatellite markers and attributed the low genetic variation and heterozygote deficiency to a high rate of inbreeding in the population.

2.4.1.3 Applications of Microsatellite Markers

The high degree of polymorphism, uniform distribution and the abundance exhibited by microsatellites make them the most widely used marker in many genetic studies.

Microsatellites find application in construction of genetic linkage maps in different species of animals (Dietrich *et al.*, 1992; Crawford *et al.*, 1995; Vaiman *et al.*, 1996) and also in the mapping of genes (Georges *et al.*, 1990; Georges *et al.*, 1993).

Buchanan *et al.* (1994) used eight microsatellites for determining evolutionary relationship among sheep breeds. They could demonstrate that the combined allele frequency differences could be used to identify an individual's breed with a high degree of certainty. They suggested that the divergence time between breeds found out using microsatellites was more realistic than that obtained using protein polymorphisms.

Usha *et al.* (1995) used microsatellite polymorphism for finding out the parentage exclusion probability in cattle. They found that using five markers together the probability of exclusion of wrong parentage could be as high as 0.99 across breeds.

In a study on four goat breeds, Luikart *et al.* (1999) found that microsatellite markers in multiplex systems could provide very high power for individual identification and parentage verification. They suggested that the multiplexes would also be useful for rapid screening of breeds for assessing population history, structure and diversity and for reconstructing relationships among breeds.

Canon *et al.* (2001) estimated the genetic diversity among European beef cattle breeds and concluded that hyper-variable microsatellites, with high levels of heterozygosities and large number of alleles, provide an efficient way of evaluating genetic diversity among bovine breeds.

Kaul *et al.* (2001), in their study on genetic variability of Indian pigs using 13 microatellite markers, observed that results from a panel of microsatellites

showed very low probability for genetic similarity between two individuals belonging to two different populations.

Vas concellos *et al.* (2003) used microsatellite markers along with RFLPs at κ-casein, β-lactoglobulin and growth hormone for characterization of Aberdeen Angus breed of cattle. They estimated the genetic distance of this breed with eight other Brazilian breeds and the dendrogram showed Aberdeen Angus in an isolated group.

Chu *et al.* (2003) analysed the association between microsatellite loci linked to Fec^B genes and litter size in Small Tail Han sheep and found that the microsatellite loci located in the 10 cM region covering the Fec^B gene had significant effect on litter size in second parity. They opined that this kind of study was important for improving such low heritability traits as litter size by marker assisted selection.

Kotze *et al.* (2004) devised four microsatellite multiplexes for characterisation of the indigenous Kalahari Red goat breed of southern Africa.

Mukesh *et al.* (2004) observed high polymorphism at 25 microsatellite loci typed in Sahiwal cattle and suggested that the substantial amount of genetic variability within this breed could prove useful in planning future breeding strategies.

2.4.1.4 Conservation of Microsatellite Loci across Species

DNA sequence database search revealed that the locations of dinucleotide microsatellites are often conserved among mammalian species enabling the prediction of the presence of DNA microsatellites using comparative genetic data.

It was found that in closely related species the conservation was close enough to allow PCR primers designed for use in one species to be used to analyse microsatellite length polymorphisms in the other. In addition, the use of heterologous primers will significantly reduce the cost of developing separate markers for different species (Moore *et al.,* 1991).

According to Pepin *et al.* (1995) 40 percent of the microsatellites isolated from cattle could be used to study the caprine genome and characterise economically important gene loci in goats.

Saitbekova *et al.* (1999) used 20 bovine microsatellites for genetic diversity studies in goat breeds and all were found to amplify well in goats.

Yang *et al.* (1999) demonstrated that bovine and ovine microsatellites were effective for detection of polymorphisms in goat breeds.

Arora *et al.* (2003) reported that bovine microsatellite markers were found to be polymorphic in genomes of Bhadawari and Tarai buffalo populations, though the number of alleles obtained and allele size were smaller than that observed in cattle.

2.5 USE OF MICROSATELLITES IN GENETIC DIVERSITY ANALYSIS

Weber and May (1989) characterized 10 (TG)n microsatellites and found them all to be polymorphic in humans. These markers were moderately to highly informative with average polymorphic information content of 0.55 and were inherited in normal codominant fashion.

It was recommended that microsatellite loci identified in mapping studies only be used in genetic diversity analysis. Those markers selected should also be

shown to exhibit Mendelian inheritance and possess at least four alleles per locus (Barker, 1994).

The usefulness of microsatellites for the estimation of genetic distances among closely related populations in different species has been documented by numerous studies (Buchanan *et al.*, 1994; Usha, 1995; Barker *et al.*, 1997; Saitbekova *et al.*, 1999; Kim *et al.*, 2002; Pandey *et al.*, 2003; Maudet *et al.*, 2002; Yang *et al.*, 2003).

Nauta and Weissing (1996) observed that owing to the limited range of allele sizes and high mutation rate, genetic differentiation at microsatellite loci was likely to occur only in small populations and in a short-term perspective.

Microsatellites were found to be powerful tools to differentiate between breeds of goats and the results obtained were in perfect agreement with the breeding histories and geographic origins (Saitbekova *et al.*, 1999; Yang *et al.*, 1999).

Ganai *et al.* (2001) used cattle microsatellite markers to study genetic variation within and among three Indian goat breeds namely, Sirohi, Jamnapari and Barbari. The phylogenetic tree constructed using inter-individual distances showed that the individuals clustered according to the breed to which they belonged.

Tadlaoui-Ouafi *et al.* (2002) estimated the genetic relationship among Moroccan goat breeds using five microsatellite markers. They found that the two breeds studied, though phenotypically distinct, appeared to be closely related and clustered from the four French breeds included in the study.

Dorji *et al.* (2003) quantified the breed diversity and differentiation in Siri cattle of Bhutan using 20 microsatellite markers and also investigated its relationship with other local cattle.

Visser *et al.* (2004) used ten microsatellite markers to investigate the genetic variation among South African goat populations. The number of alleles ranged from 7 to 14 per marker and the average heterozygosity between 0.63 and 0.69 except for Boer goats, which showed the lowest value of 0.49.

Yang *et al.* (2004) employed 14 structural loci and seven microsatellite markers for genetic diversity analysis in sheep and goat populations and reported that all diversity measures calculated were higher for microsatellite loci than structural loci indicating the superiority of microsatellites for population genetic studies.

2.5.1 INRA63

Vaiman *et al.* (1994) assigned this dinucleotide microsatellite marker, INRA63, to goat chromosome number 18 by linkage mapping. They reported seven alleles at this locus with an average size of 180 bp and a PIC value of 0.46.

This marker has been used by several workers for genetic characterisation and variability studies in different species and reported allele numbers ranging from 4 to 13 with a size range of 141to 186 bp. (Russel *et al.*, 2000; Barker *et al.*, 2001; Maudet *et al.*, 2002; Tadlaoui-Ouafi *et al.*, 2002; Mukesh *et al.*, 2004; Chenyambuga *et al.*, 2004)

2.5.2 ILSTS030

ILSTS030 was found to be a polymorphic bovine microsatellite marker composed of 13 CA repeats located on chromosome number two. This marker, with an allele size of 148 bp, was found to be polymorphic in sheep as well as goat (Kemp *et al.*, 1995).

Saitbekova *et al.* (1999) reported 19 alleles in eight Swiss goat breeds for this locus.

Mukesh *et al.* (2004) reported four alleles at this locus within a size range of 158 to 184 bp in Sahiwal cattle.

Kumar *et al.* (2005) observed six alleles ranging in size from 164 to 174 bp among Marwari goats at this locus.

2.5.3 HUJII77

Shalom *et al.* (1994) reported a bovine dinucleotide repeat polymorphism at the HUJII77 locus by screening an NdeII size selected pBS colony. The repeat was found to be $(GT)_2$ TT$(GT)_{15}$ and had a size of 211 bp with a heterozygosity of 86 percent. The marker has been mapped to chromosome number three.

van Hooft *et al.* (1999) used the bovine marker HUJII77 for population genetic studies in African buffaloes and reported it to be polymorphic with five alleles ranging in size from 190 to 212 bp.

Vallejo *et al.* (2003) detected six alleles at the HUJII77 locus while assessing the genetic diversity and background linkage disequilibrium in North American Holstein cattle population.

2.5.4 BM6121

Bishop *et al.* (1994) mapped the bovine dinucleotide microsatellite locus BM6121 to chromosome 16 and reported 11 alleles ranging from 138 to 164 bp at this locus.

2.6 POLYMERASE CHAIN REACTION

The polymerase chain reaction, which involves the in vitro enzymatic synthesis of millions of copies of specific DNA segments, has transformed the way DNA analysis is carried out in molecular studies. Microsatellite markers have become the markers of choice for population genetic studies mainly because of the possibility of combining their analysis with the PCR.

Mullis *et al.* (1986) demonstrated the exponential increase in the concentration of a specific DNA sequence which involved the reciprocal interaction of two oligonucleotides and the DNA polymerase extension products whose synthesis they prime, in repetitive cycles of denaturation, hybridization and polymerase extension.

Saiki *et al.* (1988) reported the highly specific nature of Taq polymerase mediated PCR by finding out that the amplification of DNA and RNA targets were readily accomplished with yields and purities comparable to fragments prepared from clonally isolated recombinants.

Eckert and Kunkel (1990) demonstrated that despite lacking a 3′-5′ proof reading exonuclease, the *Thermus aquatics* (Taq) DNA polymerase could catalyse highly accurate DNA synthesis *in vitro*. They showed that the error rate per nucleotide polymerized at 70°C was as low as 10^{-5} for base substitution and 10^{-6} for frame shift errors. They reported that the frequency of mutation responded to changes in dNTP concentration, pH and $MgCl_2$ concentration.

Lowe *et al.* (1990) designed a computer program, which could rapidly scan nucleic acid sequences to select all possible pairs of oligonucleotides suitable for use as primers to direct efficient amplification of DNA by PCR based on several criteria to guarantee the uniqueness of primer target hybridization.

Erlich *et al.* (1991) reviewed the spectrum of advances made possible by the PCR technology and enumerated several applications including construction of genetic maps, reconstruction of evolutionary history of species, foot printing, gene expression studies and diagnosis of diseases.

Don *et al.* (1991) described a 'touchdown' PCR strategy that was found to conveniently bypass spurious amplifications, which is a frequently encountered problem in PCR, without lengthy optimisation procedures.

Optimisation of PCR, especially the annealing temperature is essential for each primer pair. The annealing temperature is usually calculated as the temperature of melting (Tm) of the primers ±4°C but must be determined empirically. Another important condition to be optimized is the concentration of $MgCl_2$ in the reaction mix (Oh and Mao, 1999).

2.7 DETECTION OF MICROSATELLITE POLYMORPHISMS

Microsatellites can be difficult to type because being short tandem repeats, alleles often differ in size by two or three base pairs, and additional shadow bands arise due to slippage during PCR amplification and to the different mobilities of CA-rich and GT-rich strands (Hughes, 1993). The PCR products can be resolved on polyacrylamide sequencing gels (Yang *et al.*, 1999; Mukesh *et al.*, 2004; Kumar *et al.*, 2005) and sized using appropriate dideoxy sequence ladders. The PCR products separated can be visualized by silver staining, autoradiography or by means of an automated sequencer.

2.7.1 Autoradiography

Visualisation of PCR products by autoradiography can be done either by the direct incorporation of a radiolabeled nucleotide or by the end labeling of one of the primers used in the PCR mix.

Analytical polyacrylamide gels containing radioactive DNA may be fixed or dried before autoradiography. Drying of gels can be accomplished by using a commercial gel dryer after blotting the gel onto a piece of Whatman 3MM paper. Drying of gels is necessary when gels contain DNA labeled with weak β emitting isotopes like ^{35}S or small amounts of ^{32}P which require long exposures to obtain an adequate radiographic image (Sambrook *et al.*, 1989).

2.7.1.1 End-labeling of Primers

End labeling is a rapid and sensitive method for radio active labeling of DNA fragments. Two commonly used methods for end labeling are the "fill-in" method which uses the Klenow fragment of *E. coli* DNA polymerase and the kinase reaction which uses the T_4 polynucleotide kinase to transfer labeled phosphate to the 5' end of the DNA molecule (Harwood, 1996).

Hughes *et al.* (1993) described a technique of end labeling of a single primer in 2.5 µl reaction volume and 6 pM primer with 10 µCi of γ^{32}PATP using T_4 polynucleotide kinase and incubating at 37°C for 30 min. The authors suggested that the use of a single end labeled primer was preferable for incorporating either ^{32}P or ^{35}S labeled nucleotide in the reaction as it resulted in a simpler allele pattern because only the CA or GT strand is labeled and these two had slightly different mobilities.

Buchanan *et al.* (1994) labeled primers using [γ-^{32}P] ATP or [γ-^{33}P] ATP and T_4 Polynucleotide kinase or employed direct incorporation of [α-^{32}P] dATP in PCR product using both unlabelled primers for microsatellite analysis.

Kemp *et al.* (1995) used ^{33}P or ^{32}P for labeling one of the primers of each primer pair to amplify genomic DNA in 5 µl volumes of PCR amplifications. They also ranked the markers by judging the autoradiographs obtained on the basis of the ease of interpretation.

Arranz *et al.* (1996) used ^{35}S dATP in the amplification reaction and the genetic variants were visualised by autoradiography using the sequence of phage M13mp18 as a size standard.

Yang *et al.* (1999) used silver staining of gels for visualisation and analysis of amplified DNA fragments.

Several other workers also accomplished labeling of PCR products with radioisotopes either by end labeling one of the primers with γ^{32}P ATP with polynucleotide kinase enzyme and by using the labeled primer in the PCR assay or by the direct incorporation of radio labeled nucleotides into the reaction mix (Russel *et al.*, 2000; Arranz *et al.*, 2001).

2.7.2 Sequencing

Sanger *et al.* (1977) described the chain termination method of DNA sequencing where dideoxynucleotides were used for specific termination of the growing chain. One of the nucleotides was radiolabeled so that the mixture fractionated by electrophoresis could be subjected to autoradiography from which the required sequence could be read.

The use of α^{35}SdATP in DNA sequencing reaction produced very sharp definition of bands on a sequencing gel autoradiograph due to the sharp path length of β particles emitted by ^{35}S (Biggin *et al.*, 1983).

2.8 STATISTICAL ANALYSIS

2.8.1 Heterozygosity

Nei (1978) evaluated the magnitude of systematic bias introduced by small sample sizes when ordinary method of estimation of average heterozygosity is used and gave an unbiased estimate of population heterozygosity for a single locus as;

$$h = \frac{2n}{2n-1}(1-\Sigma X_i^2)$$

According to Ott (1992), at a given marker locus, an individual is either heterozygous or homozygous. In a sample of individuals, heterozygosity can be estimated as the binomial proportion of heterozygotes in the sample.
Heterozygosity is given by;

$$He = 1 - \sum_{i=1}^{k} pi2$$

2.8.2 Polymorphic Information Content

Botstein *et al.* (1980) derived a measure called polymorphic information content (PIC) for finding out how the polymorphism at a given locus influences the probability of detection of linkage of that locus to another. It is given by the formula;

$$PIC = 1-[\sum_{i=1}^{k} p_i^2] - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

2.8.3 Genetic Distance

Genetic distance calculated using allele frequencies is a powerful method for detecting the effects of natural selection among different genetic groups. Measures of genetic distance provide the best available objective description of the genetic differentiation between any two breeds. Genetic distancing helps to identify genetically unique breeds so that they may be prioritized for breed conservation purposes. It also provides insights into the history of animal domestication.

According to Nei (1978), the number of individuals to be used for estimating genetic distance can be very small if genetic distance is large and average heterozygosity of the two species compared is low.

Barker (1994) suggested that the pair wise genetic distances estimated among different breeds of a species when used to construct a single phylogeny, it best represents all the relationships among the breeds. Such a phylogeny would aid in objective and rational decision making in the choice of breeds for preservation, utilisation and evaluation studies to determine comparative genetic merit for production traits.

Takezaki and Nei (1996) compared various measures of genetic distance used for the reconstruction of phylogenetic trees from microsatellite frequency data and showed that the accuracy of Cavalli-Sforza and Edwards chord distance, D_c and Nei's D_A distance were generally higher than the other distances.

Boyce *et al.* (1997) used both allele size based method and frequency based method of genetic distance estimation in bighorn sheep and observed that even with good repeat loci, size based methods may be unduly influenced by one or only a few loci. They also reported that the genetic distance values tended to increase with increasing geographic distance.

Genetic distance is a measure of overall evolutionary divergence and serves as a useful tool for authentication of pedigree, for characterisation of different breeds or strains within a species and for evaluation of the change in variation in species over time (Mitra *et al.,* 1999).

Considerable levels of apparent breed differentiation were noticed among Korean goats using microsatellite analysis. The results showed that 20.2 percent of total genetic variation was due to breed formation, which was higher than what was observed, in other domestic animals including South East Asian goat populations, by other workers (Kim *et al.,* 2002).

Kalinowski (2002) proved from computer simulation studies that an equivalent precision of estimates of genetic distances could be achieved either by examining a few loci with many alleles or many loci with few alleles.

Vijh *et al.* (2004) estimated several genetic distance measures from microsatellite data in indigenous poultry germplasm using infinite allele model and used them for phylogenetic tree construction. They found that the tree obtained using Nei's D_A was the most appropriate as it could be backed by statistical criterion and a historical and demographic perspective.

Kalinowski (2005) analysed the rate at which increasing sample sizes decrease the coefficient of variation of genetic distance estimates. He reported that the rate depends upon the value of F_{ST}, which is a measure of differentiation between the populations. It was noticed that when the F_{ST} was greater than 0.05, samples of less than 20 per population was sufficient, but when F_{ST} was less than 0.01, samples of 100 or more individuals per population would be useful.

2.8.4 Dendrograms or Phylogenetic Trees

Distance estimates are used in clustering analysis involving methods that allow for unique rates of evolution. The level of phylogenetic distinction of each breed is a measure of the evolutionary distance between it and the other breeds and can be represented effectively by dendrograms or phylogenetic trees.

The reliability of the topology of the dendrogram depends on differences in genetic distance among different pairs of species. If the differences are small, genetic distances must be estimated accurately i.e. a considerable number of individuals should be sampled for each locus (Nei, 1978).

Saitou and Nei (1987) proposed the neighbour joining method for reconstructing phylogenetic trees from evolutionary distance data. The method finds pairs of operational taxonomic units (OTUs) or neighbours that minimize the total branch length at each stage of clustering of OTUs starting with a star like tree.

The pair wise genetic distance values between breeds can be used to construct phylogenetic trees that makes possible to readily identify genetically unique breeds (Ruane, 1999).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

A total of 120 animals, belonging to four genetic groups constituted the material for study. Thirty genetically unrelated animals from each group viz; Malabari, Attappadi Black, Salem Black and non-descript goats of Thrissur were sampled. The Malabari goat samples were obtained from the buck centre maintained at Mannuthy under the "AICRP for Genetic Improvement of Malabari goats". Samples from Attappadi Black goats were collected from the Agali panchayat in Attappadi, Palakkad district. Salem Black animals were sampled from the Salem district of Tamil Nadu. The non-descript goats of Thrissur were obtained from Kunnamkulam region of the district.

3.2 COLLECTION OF BLOOD SAMPLES

Blood samples (5 ml each) were collected from the jugular vein aseptically using sterile disposable syringes and transferred immediately into sterile disposable centrifuge tubes containing EDTA (1 mg/ml of blood) as anticoagulant. The samples were brought in ice to the laboratory and stored at 4°C till processed.

3.3 ISOLATION OF DNA FROM WHOLE BLOOD

DNA was extracted from whole blood using the standard phenol-chloroform extraction procedure (Sambrook *et al.*, 1989) with modifications. The procedure was as follows;

1. Five ml of blood collected in a 15 ml centrifuge tube was centrifuged at 4000 rpm for 10 min and the plasma was discarded leaving erythrocytes and leucocytes.

2. Two to three volumes of ice-cold RBC lysis buffer (150 m*M* NH_4Cl, 10 m*M* KCl, 0.1 m*M* EDTA) was added, mixed well and kept in ice with occasional mixing for 10 minutes for complete lysis of erythrocytes.

3. The leucocytes were recovered by centrifuging at 3500 rpm for 15 min. and the supernatant containing lysed erythrocytes was discarded.

4. Steps 2 and 3 were repeated till the cell pellet was clear without any unlysed erythrocytes.

5. The cell pellet was washed twice with 10 ml of Tris buffered saline (TBS-140 m*M* NaCl, 0.5 m*M* KCl, 0.25 m*M* Tris) by vigorous vortexing followed by centrifugation at 3000 rpm for 10 minutes.

6. The washed white cell pellet was resuspended completely by vortexing in 5 ml of saline EDTA buffer (SE-75 m*M* NaCl, 35 m*M* EDTA). To this mixture 25 µl of Proteinse-K and 0.25 ml of 20 per cent SDS were added, mixed well and incubated at 50°C for a minimum of three hours.

7. To the digested sample, 300 µl of 5 *M* NaCl was added and mixed by vortexing. An equal volume of phenol (pH 7.8) saturated with Tris-HCl, was added, mixed thoroughly by inversion of the tubes for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

8. The aqueous phase containing DNA was collected in fresh tubes, to which an equal volume of saturated phenol:chloroform:isoamyl alcohol (25:24:1) was added. The contents were mixed thoroughly by inversion for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

9. To the aqueous phase collected in fresh tubes, equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed and centrifuged at 3500 rpm for 15 minutes.

10. The supernatant was transferred to a sterile 50 ml beaker and 1/10th volume of 3 *M* Sodium acetate (pH 5.5) was added and mixed.

11. An equal volume of chilled isopropyl alcohol was added and the precipitated DNA was spooled out using a fresh micropipette tip, washed in 70 per cent ethanol and air-dried.

12. Dried DNA was resuspended in 0.5 ml of Tris buffer (TE-10 m*M* Tris, 0.1 m*M* EDTA) and stored at –20°C.

3.4 DETERMINATION OF YIELD AND PURITY OF DNA

Twenty microlitres of the DNA stock solution was diluted to 2 ml with sterile distilled water giving a dilution of 100 times. Optical densities (OD) were measured at 260 nm and 280 nm using a 2 ml cuvette in a UV spectrophotometer (Jenway, UK). Sterile distilled water was used as blank. Yield and purity of DNA samples were estimated as follows.

3.4.1 Yield of DNA Samples

An OD of one at 260 nm wavelength corresponds to approximately 50 µg/ml of double stranded DNA. Concentration of DNA stock solution was calculated as follows.

Concentration of DNA stock solution (µg/ml) = OD_{260} x Dilution factor x 50

3.4.2 Purity of DNA Samples

The quality of DNA samples was first assessed electrophoretically on one per cent agarose gel in 1X TAE buffer in a horizontal submarine gel electrophoresis unit. Purity of DNA samples were assessed by estimating the ratio between the readings at 260 and 280 nm. Pure DNA samples have OD_{260}/OD_{280} ratios of 1.8 and above.

3.5 PCR ANALYSIS

3.5.1 Template DNA

Working solutions of DNA samples were prepared from the DNA stock solution by diluting with sterile triple distilled water to get a final concentration of 50 ng/µl. One microlitre of this working solution was used in every 10 µl PCR assay.

3.5.2 Selection of Primers

A set of 13 microsatellite markers were selected from available literature and the primers were custom synthesized. These markers were typed for their polymorphisity. Four markers viz. INRA63, ILSTS030, BM6121 and HUJII77 which exhibited comparatively higher degree of polymorphism among those typed were chosen for the study. The sequences of the forward and reverse primers for each locus are as follows.

Locus	Primer Sequence (5'-3')	
1. BM6121	F	CTGTTTGCTATAATTTTGTGGAGG
	R	TGGCATTCTACGAGACCACA

Locus	Primer Sequence (5'-3')	
2. INRA063	F	ATTTGCACAAGCTAAATCTAACC
	R	AAACCACAGAAATGCTTGGAAG
3. ILSTS030	F	CTGCAGTTCTGCATATGTGG
	R	CTTAGACAACAGGGGTTTGG
4. HUJII77	F	TCCATCAAGTATTTGAGTGCAA
	R	ATAGCCCTACCCACTGTTTCTG

The primers obtained in lyophilized form were reconstituted in sterile triple distilled water to make a stock solution of 200 p*M*/µl concentration. The solutions were incubated at room temperature for one hour and then stored at -20°C. Working solutions of the primers were prepared by dilution from the stocks.

3.5.3 Incorporation of Radioactivity: End-Labeling of Primers

For visualizing the PCR products by autoradiography, radio-labeling of either the forward or the reverse primer can be done. In the present study, the forward primer for each marker was radio-labeled at the 5' end with γ^{32} P-ATP. The reaction was carried out with the DNA End-labeling Kit1 (Bangalore Genei).

The procedure for end-labeling was as follows:

The following components were added to a 0.2 ml microcentrifuge tube in the order;

10X Polynucleotide kinase (PNK) buffer	-	1 µl
Forward Primers (200p*M*/µl)	-	1 µl
T_4 Polynucleotide kinase (5U/µl)	-	0.5µl
γ^{32} P-ATP (10mCi/ml)	-	1 µl
Nuclease free water	-	6.5µl

The mixture was incubated at 37°C for 30 minutes. The final volume was made upto 40 µl. One microliter of the diluted end-labeled primer was used for every 10 µl PCR assay with sterile distilled water.

3.5.4 PCR Conditions

The PCR conditions for each microsatellite loci were standardised separately. Each reaction was carried out in 10 µl volume. PCR reaction was set up with 1µl of 10X PCR buffer (15 m*M* $MgCl_2$, 100 m*M* Tris-pH 8.3, 500 m*M* KCl), 200 µ*M* dNTP, 1µl of end-labeled and diluted forward primer, 5 p*M* of reverse primer and 0.3 U of Taq DNA polymerase. Concentration of $MgCl_2$ used was 1.5 m*M* for all the primers selected. The reaction mixture was mixed well and subjected to amplification in a thermal cycler (Techne Flexigene). The thermal cycling involved 35 cycles each consisting of denaturation at 94°C for one minute, annealing at 60°C for one minute for INRA063 and BM6121, 58°C for one minute for HUJII77 and ILSTS030 and extension at 72°C for one minute. This was followed by a final extension at 72°C for five minutes. The samples were then cooled down to 4°C and stored at -20°C till further analysis.

3.6 SEQUENCING M13 BACTERIOPHAGE DNA

Determination of the exact size of alleles necessitated comparison with a sequencing ladder from M13. Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (Amersham Biosciences Corporation, USA) according to the manufacturer's instructions as follows.

1. Preparation of annealing mixture.

   The composition of the mixture was as follows;

M13 phage DNA (0.2 µg/µl)	-	5µl
5 X Sequenase reaction buffer	-	2µl
Forward primer (5p*M*/µl)	-	1µl

The volume was made upto 10µl with distilled water. The mixture was centrifuged briefly and incubated at 65°C for two minutes. It was then slowly cooled to room temperature over 15-30 min, and chilled on ice.

2. To the four tubes labeled G, A, T and C, 2.5 µl of each dideoxy termination mixtures (ddGTP, ddATP, ddTTP and ddCTP) were added.

3. Dilution of labeling mix.

The labeling mix provided in the kit was diluted five fold as follows:

5 X Labelling mix	-	0.5µl
Water	-	2.0µl

4. Dilution of enzyme.

The enzyme (Sequenase version 2.0) was diluted eight fold with Sequenase enzyme dilution buffer as follows:

Sequenase enzyme (13U/µl)	-	0.5µl
Sequenase dilution buffer	-	3.5µl

5. Labeling reaction

To the ice cold annealed DNA mixture, the following components were added.

Dithiothreitol (DTT 0.1*M*)	-	1µl
Diluted labeling mix	-	2µl
Diluted enzyme	-	2µl
α^{35}S dATP (10µ Ci/µl)	-	2µl

The contents were mixed well and incubated at room temperature for two to five minutes.

6. To each termination tubes (G, A, T, C), 3.5 µl of labeling reaction mixture was transferred mixed well and incubated at 37°C for five minutes.

7. The reaction was stopped by addition of 4 µl of stop solution provided in the sequencing kit and stored at –20°C

3.7 ELECTROPHORESIS

3.7.1 Checking of Amplification of Target DNA

The PCR products were checked in 1 per cent agarose gels in 1X Tris Acetate EDTA (TAE) buffer in a horizontal submarine electrophoresis unit. Appropriately sized gel tray was cleaned and sealed. The tray was placed on a leveled surface and comb kept in proper position in the tray. One per cent agarose in TAE buffer (0.04m*M* Tris acetate, 0.001m*M* EDTA) was heated in a microwave oven until it was a clear solution, cooled to 60°C and 0.5 µg/ml of Ethidium bromide was added and mixed well. The mixture was cooled to hand bearable temperature and carefully poured into the gel tray avoiding air bubbles. After solidification, the comb and sealing were removed gently and the tray was immersed in the buffer tank (Amersham Pharmacia Biotech, USA) containing 1X TAE buffer. One microlitre of the PCR product was mixed with equal volume of 6X gel loading buffer (Bromophenol Blue 0.25 per cent, Xylene cyanol 0.25 per cent, Sucrose 40 per cent) was loaded into the well carefully. HaeIII digested pBR322 was used as the DNA size marker. It was also mixed with gel loading buffer and was loaded into one of the wells.

Electrophoresis was carried out at 2V/cm until the Bromophenol blue dye migrated more than 2/3rd of length of the gel. The gel was visualized under a UV transilluminator (Hoefer, USA) and checked amplification of target DNA. Those samples with amplified PCR products were subjected to polyacrylamide gel electrophoresis.

3.7.2 Denaturing Polyacrylamide Gel Electrophoresis

The radioactively labeled PCR products were subjected to electrophoresis on 6 per cent denaturing polyacrylamide gels for better resolution. Denaturing Polyacrylamide Gel Electrophoresis (PAGE) was performed on the Vertical Sequencer (Consort, Belgium) as described by Biggin *et al.* (1983). The gels were set between two glass plates (41 x 33cm) separated by 0.35 mm thick spacers.

3.7.2.1 Casting the Gel

The glass plates were cleaned thoroughly with soap solution and dried. Traces of grease and oil were removed by repeated wiping with alcohol. One of the plates was gently coated with Dimethyl dicholorosilane solution (BDH) to prevent the gel from adhering to both the plates. The plates were assembled with 0.35 mm thick spacers in between and the sides and bottom sealed with sealing tape.

The gel was prepared by mixing 60 ml of 0.5X TBE gel mix (6 percent Acrylamide, 6 *M* urea, 0.5X TBE) and 125 µl each of 10 per cent Ammonium persulphate solution (APS) and N, N, N', N', Tetra Methyl Ethylene Diamine (TEMED) in a beaker. The mixture was poured between the glass plates avoiding air bubbles. The plates were clamped and the comb (Shark toothed comb) inserted on top with the toothed surface facing upwards. The gel was allowed to set for an hour before electrophoresis. The tapes and clamps were removed, plates cleaned and assembled in the sequencer. The upper and lower electrode tanks were filled with 1X Tris Borate EDTA (pH 8.3) buffer (TBE 0.045 *M* Tris borate, 0.001 *M* EDTA) to the required level. The comb was removed, wells cleaned with buffer solution and comb was then reinserted in opposite direction with the toothed surface now facing downwards to form sample-loading wells.

3.7.2.2 Loading of Samples

The PCR products were mixed with 3.5 µl formamide loading buffer (0.02 per cent Xylene Cyanol, 0.02 per cent Bromophenol Blue, 10 m*M* EDTA, 98 per cent deionised formamide), denatured at 95°C for 5 min and cooled immediately on ice. About 4 µl each of this mixture was loaded into each well with great care to avoid mixing up of the samples from adjacent wells. Sequenced products of M13 DNA which were also denatured at 95°C for 5 minutes were loaded simultaneously in the middle or side wells.

3.7.2.3 Electrophoresis

The gels were electrophoresed at 40W for three hours. The bromophenol dye in the loading buffer acted as indicator of the mobility of DNA fragments and had a mobility equivalent to a 25 base fragment and the Xylene cyanol dye had a mobility approximately equivalent to a 100 base fragment.

3.8 DRYING OF GELS

After electrophoresis the glass plates were removed from the sequencer, and carefully separated. The gel adhering to one of the plates was transferred to a filter paper. The position of the first well was marked by cutting out a small portion of the corresponding corner. The gel was covered with a cling film and dried in a gel drier at 80°C for one and a half hours.

3.9 AUTORADIOGRAPHY

The cling film was removed after drying and the gel was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2cm) in a cassette (Kiran Hypercassette) fitted with an intensifying screen. The X-ray film was developed after 24 to 48 hours depending on the intensity of radioactive signal.

3.10 DEVELOPMENT OF X-RAY FILM

The X-Ray film was developed in the dark room. Developing was done by transferring the film serially into 1X developer solution (Kodak) for three to five minutes, 1 per cent acetic acid for a minute followed by washing in distilled water and finally into fixer solution (Kodak) for six to ten minutes. The developed film was washed thoroughly in running water and dried.

3.11 MICROSATELLITE TYPING

The genotypes of the animals were determined for each microsatellite loci by comparing the sizes of alleles with M 13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in order. The allele sizes were assigned corresponding to the G, A, T, C bands. The frequency at each locus was determined by direct counting.

3.12 STATISTICAL ANALYSIS

Heterozygosity, Polymorphic Information Content (PIC), number of alleles and allele size range were worked out for each locus and for each breed studied. Analysis of variance was performed to detect the effect of breeds in any of these parameters at the different loci typed. Wilcoxon sign rank test for median difference was used to ascertain whether a significant difference existed among the four breeds between the allele distributions at the four loci typed.

3.12.1 Heterozygosity (He)

Heterozygosity is a measure of usefulness of the marker. Heterozygosity was calculated by the method of Ott (1992).

Heterozygosity is given by;

$$He = 1 - \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of i^{th} allele at a locus. Markers with higher heterozygosity values are more useful.

3.12.2 Polymorphic Information Content (PIC)

The polymorphic information content expresses informativeness or usefulness of a marker for linkage studies. The PIC values of the markers were calculated as;

$$PIC = 1 - [\sum_{i=1}^{k} p_i^2] - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of i^{th} and j^{th} alleles, respectively (Botstein *et al.*, 1980).

3.13 GENETIC DISTANCE

Genetic distance measures provide the best description of genetic differentiation among breeds or genetic groups. The standard genetic distance between the four populations studied were computed using Nei's method as given by Nei (1978). The distance measures were derived using the POPGENE version 1.31 program (Yeh *et al.*, 1999).

Nei's standard genetic distance is given by;

$$Ds = -\ln [J_{XY} / \sqrt{J_X J_Y}]$$

where J_X, J_Y and J_{XY} are averages of $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i$ over r loci and x_i and y_i are the sample allele frequencies of the i^{th} allele in populations X and Y, respectively.

3.14 DENDROGRAM

A dendrogam representing the relationship between the animals belonging to the four goat populations was constructed using the POPGENE version 1.31 program (Yeh *et al.,* 1999). The UPGMA method was used for plotting the dendrogram.

Results

4. RESULTS

4.1 ISOLATION OF GENOMIC DNA

A total of 120 DNA samples were isolated from whole blood obtained from the four genetic groups of goats, thirty each from Malabari, Attappadi Black, Salem Black and non-descript goats of Thrissur.

4.1.1 Yield and quality of DNA

The mean concentration and yield of DNA obtained from 5 ml of whole blood was 722.87±21.47 µg/ml and 361.43±10.73 µg/5ml, respectively. The average value of the ratio of optical density at 260 and 280 nm, which indicates the purity of DNA isolated, was found to be 1.54±0.015. The values are presented in Table 4.1. The DNA samples checked for quality by agarose gel electrophoresis appeared as single bands without sheared fragments indicating good quality.

4.2 PCR ANALYSIS

Microsatellite primers were selected from published literature, custom synthesized and used for PCR amplification of the samples. Out of the 13 bovine markers tested, most markers exhibited very low or moderate degree of polymorphism in goats. Four markers, which were found to be highly polymorphic in goats, were selected for the study. These were the bovine dinucleotide repeat microsatellites INRA63, ILSTS030, BM6121 and HUJII77. PCR amplification was performed on all the 120 samples using the primer pairs for the four selected loci.

Table 4.1 Concentration, yield and purity of DNA obtained from the blood samples of the four goat populations.

Parameter	**Mean Value (n=120)**
Concentration (µg/ml)	722.87±21.47
Yield (µg/5ml)	361.43±10.73
OD ratio (260nm/ 280nm)	1.54±0.015

4.2.1 Optimisation of PCR

The composition of the PCR reactions and temperature and time of the cycles were optimised separately for each primer for the efficient amplification and accurate typing of the products. The reaction components and cycle parameters for each primer pair is presented in Tables 4.2 and 4.3.

The PCR products were checked for amplification by electrophoresis on one per cent agarose gels using *Hae*III digested pBR322 as the size standard. Amplified products of the expected size range were observed for all loci.

4.3 NUMBER OF ALLELES, SIZE AND FREQUENCY

The amplified products were fractionated by denaturing polyacrylamide gel electrophoresis. The pattern of bands produced at each locus was visualised by autoradiography. Direct counting was employed for detecting the number of alleles at each locus. The allele sizes were approximated by comparing with sequence of M13 mp18 single standard DNA size standard, which was run along with the samples. Among the 4 loci, ILSTS030 showed the presence of shadow bands. The different genotypes and their frequencies obtained for the selected loci in the four genetic groups analysed are presented in Tables 4.4 to 4.7. The different alleles and their frequencies for the four markers are presented in Tables 4.8 to 4.11. The allele size range, number of alleles, heterozygosity and PIC for the different loci tested is summarised in tables 4.12 to 4.15. The autoradiographs showing polymorphism at the four loci are presented in Plates 1 to 4

Table 4.2 Standardised conditions for the PCR assay

Sl. No.	Parameter	Value
1.	Template DNA (ng)	50
2.	$MgCl_2$ (m*M*)	1.5
3.	10X Reaction Buffer (µl)	1
4.	dNTPs (µ*M*)	200
5.	Forward Primer (p*M*)	5
6.	Reverse Primer (p*M*)	5
7.	Taq DNA Polymerase (U)	0.3
8.	Reaction Volume (µl)	10

Table 4.3 Standardised temperature and cycling conditions for the PCR at different microsatellite loci analysed.

Sl. No.	Parameter	Temperature/Time			
		BM6121	**HUJII77**	**ILSTS030**	**INRA63**
1.	Denaturation (°C/min)	94	94	94	94
2.	Annealing (°C/min)	60	58	58	60
3.	Extension (°C/min)	72	72	72	72
4.	No. of Cycles	35	35	35	35
5.	Final extension (°C/5 min)	72	72	72	72

4.3.1 INRA63

Ten alleles were detected in Salem Black animals with a size ranging from 152-172 bp. Nine alleles with a size range of 154-170 were detected in Malabari, and seven alleles each were detected in Attappadi Black and non-descript goats with a size range of 158-170 and of 152-166 bp, respectively. The allele sizes and their frequencies for this locus are presented in Table 4.8. The genotypes and frequencies at this locus are summarised in Table 4.4.

4.3.2 ILSTS030

This marker was found to be highly polymorphic in all the breeds studied. Fourteen alleles each were detected in Salem Black and non-descript goats within a size range of 150-178 and of 148-174 bp, respectively. In Attappadi Black and Malabari goats 12 alleles each were detected and the sizes ranged from 146-170 and 148-170 bp. Out of the 120 samples tested, two failed to produce detectable product after PCR assay. The genotypes and frequencies at this locus is summarised in Table 4.5. Allele size and frequencies are presented in Table 4.9.

4.3.3 BM6121

Thirteen alleles were detected at the BM6121 locus among all the four populations. Ten alleles were detected in both Salem Black goats and Attappadi black goats with a size range of 153-175 and 155-173 bp, respectively. Malabari and non-descript animals had 8 and 9 alleles within a size range of 153-167 and 151-167 bp, respectively. Five samples failed to amplify at this locus. The genotypes and frequencies at this locus are summarised in Table 4.7. Allele frequencies and size of alleles at this locus are presented in Table 4.11.

4.3.4 HUJII77

This marker was found to be the most polymorphic of the four markers used, detecting a total of 21 alleles in the goat populations analysed. Out of the 120 samples tested one failed to produce a detectable product. The Salem black goats had a wider range and number of alleles compared to other populations with 17 alleles ranging from 189-229 bp in size. Malabari and Attappadi black animals had 13 alleles each with size ranges of 191-221 and 193-225 bp, respectively. Fifteen alleles within a size range of 193-221 bp were detected in non-descript animals. The genotypes and frequencies at this locus is summarised in Table 4.6. The different alleles and their frequencies obtained in the four breeds are presented in Table 4.10.

4.4 HETEROZYGOSITY

The heterozygosity values for each locus was calculated by the method suggested by Ott (1992). The heterozygosity values for each marker in different breeds are presented in Tables 4.12 to 4.15. The mean values obtained were 0.774 for INRA63, 0.878 for ILSTS30, 0.905 for BM6121 and 0.889 for HUJII77. The mean values are presented in Table 4.16.

4.5 POLYMORPHIC INFORMATION CONTENT (PIC)

Polymorphic information content is a measure of the informativeness of a marker and was calculated using the method of Botstein *et al.* (1980). The PIC values for each marker in the different breeds are presented in Tables 4.12 to 4.15. The mean PIC values obtained were 0.744 for INRA63, 0.866 for ILSTS030, 0.880 for HUJII77 and 0.834 for BM6121. The mean values are presented in Table 4.16.

Table 4.4 Genotypes and frequencies at the INRA63 locus in the different goat populations.

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
1	152/158	0.000000	0.000000	0.000000	0.033333
2	152/160	0.033333	0.000000	0.000000	0.000000
3	154/160	0.000000	0.033333	0.000000	0.033333
4	156/158	0.000000	0.000000	0.000000	0.033333
5	156/164	0.033333	0.033333	0.000000	0.000000
6	158/158	0.000000	0.000000	0.000000	0.033333
7	158/162	0.000000	0.000000	0.033333	0.100000
8	158/164	0.033333	0.000000	0.000000	0.033333
9	158/166	0.100000	0.066667	0.000000	0.000000
10	160/162	0.000000	0.033333	0.000000	0.033333
11	160/164	0.033333	0.066667	0.000000	0.166667
12	160/166	0.066667	0.066667	0.000000	0.000000
13	160/168	0.000000	0.033333	0.033333	0.000000
14	160/170	0.033333	0.000000	0.000000	0.000000
15	162/162	0.000000	0.100000	0.000000	0.066667
16	162/164	0.000000	0.033333	0.100000	0.166667
17	162/166	0.000000	0.133333	0.066667	0.033333
18	162/168	0.100000	0.100000	0.033333	0.000000
19	164/164	0.033333	0.066667	0.033333	0.166667
20	164/166	0.033333	0.100000	0.100000	0.100000
21	164/168	0.166667	0.000000	0.266667	0.000000
22	164/170	0.000000	0.033333	0.000000	0.000000
23	166/166	0.100000	0.000000	0.000000	0.000000
24	166/168	0.133333	0.000000	0.100000	0.000000
25	166/170	0.000000	0.033333	0.000000	0.000000
26	168/168	0.066667	0.033333	0.166667	0.000000
27	168/170	0.000000	0.000000	0.033333	0.000000
28	168/172	0.033333	0.000000	0.000000	0.000000
29	170/170	0.000000	0.000000	0.033333	0.000000

Table 4.5 Genotypes and frequencies at the ILSTS030 locus in the different goat populations.

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
1	146/158	0.000000	0.033333	0.000000	0.000000
2	148/158	0.000000	0.000000	0.000000	0.068966
3	148/160	0.000000	0.033333	0.100000	0.000000
4	150/156	0.034483	0.000000	0.000000	0.000000
5	150/158	0.034483	0.000000	0.000000	0.000000
6	150/160	0.000000	0.000000	0.033333	0.034483
7	150/162	0.000000	0.000000	0.033333	0.034483
8	152/154	0.000000	0.000000	0.033333	0.103448
9	152/160	0.034483	0.200000	0.100000	0.000000
10	152/162	0.000000	0.033333	0.133333	0.068966
11	154/162	0.000000	0.200000	0.000000	0.000000
12	154/170	0.000000	0.000000	0.033333	0.000000
13	156/162	0.034483	0.000000	0.033333	0.000000
14	156/164	0.275862	0.033333	0.066667	0.034483
15	156/166	0.000000	0.033333	0.033333	0.034483
16	156/168	0.000000	0.033333	0.000000	0.000000
17	158/160	0.000000	0.000000	0.000000	0.034483
18	158/166	0.172414	0.033333	0.033333	0.000000
19	160/160	0.000000	0.033333	0.033333	0.000000
20	160/162	0.000000	0.000000	0.066667	0.000000
21	160/164	0.000000	0.000000	0.066667	0.000000
22	160/168	0.000000	0.033333	0.000000	0.000000
23	160/170	0.000000	0.000000	0.033333	0.034483
24	160/172	0.000000	0.000000	0.000000	0.034483
25	162/162	0.034483	0.000000	0.000000	0.000000

(Cont'd)

Table 4.5 (continued...) Genotypes and frequencies at the ILSTS030 locus in the different goat populations.

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
26	162/164	0.034483	0.000000	0.033333	0.034483
27	162/166	0.000000	0.066667	0.000000	0.103448
28	162/168	0.034483	0.133333	0.000000	0.000000
29	162/170	0.000000	0.066667	0.066667	0.000000
30	162/172	0.034483	0.000000	0.000000	0.034483
31	162/176	0.034483	0.000000	0.000000	0.000000
32	164/164	0.000000	0.033333	0.000000	0.000000
33	164/170	0.000000	0.000000	0.000000	0.103448
34	164/172	0.034483	0.000000	0.000000	0.103448
35	164/174	0.000000	0.000000	0.000000	0.068966
36	164/178	0.034483	0.000000	0.000000	0.000000
37	166/166	0.000000	0.000000	0.000000	0.034483
38	166/168	0.034483	0.000000	0.000000	0.000000
39	166/170	0.034483	0.000000	0.000000	0.000000
40	166/172	0.034483	0.000000	0.000000	0.000000
41	166/174	0.034483	0.000000	0.000000	0.000000
42	168/168	0.000000	0.000000	0.033333	0.000000
43	168/170	0.000000	0.000000	0.000000	0.034483
44	168/172	0.034483	0.000000	0.000000	0.000000
45	170/170	0.000000	0.000000	0.033333	0.000000

Table 4.6 Genotypes and frequencies at the HUJII77 locus in the different goat populations.

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
1	189/189	0.033333	0.000000	0.000000	0.000000
2	191/191	0.033333	0.000000	0.000000	0.000000
3	191/193	0.000000	0.034483	0.000000	0.000000
4	191/209	0.000000	0.034483	0.000000	0.000000
5	193/193	0.000000	0.068966	0.066667	0.066667
6	193/197	0.033333	0.000000	0.000000	0.000000
7	193/201	0.000000	0.000000	0.066667	0.000000
8	193/211	0.000000	0.034483	0.000000	0.000000
9	195/195	0.000000	0.034483	0.033333	0.033333
10	195/197	0.000000	0.000000	0.000000	0.133333
11	195/205	0.000000	0.000000	0.000000	0.033333
12	195/209	0.000000	0.034483	0.000000	0.000000
13	195/211	0.000000	0.034483	0.000000	0.066667
14	195/213	0.000000	0.034483	0.000000	0.000000
15	195/215	0.000000	0.034483	0.066667	0.000000
16	195/219	0.000000	0.000000	0.000000	0.033333
17	197/197	0.133333	0.137931	0.066667	0.000000
18	197/205	0.000000	0.000000	0.000000	0.033333
19	197/207	0.000000	0.000000	0.000000	0.033333
20	197/211	0.000000	0.034483	0.000000	0.033333
21	197/213	0.000000	0.034483	0.000000	0.033333
22	197/215	0.000000	0.000000	0.000000	0.033333
23	199/199	0.033333	0.000000	0.000000	0.000000
24	199/213	0.033333	0.000000	0.000000	0.033333
25	199/215	0.000000	0.000000	0.033333	0.000000
26	199/219	0.000000	0.034483	0.000000	0.000000
27	201/201	0.000000	0.068966	0.000000	0.033333
28	201/205	0.000000	0.000000	0.000000	0.033333
29	201/207	0.000000	0.000000	0.033333	0.000000
30	201/211	0.033333	0.000000	0.033333	0.000000
31	201/215	0.033333	0.000000	0.000000	0.033333
32	201/217	0.000000	0.000000	0.000000	0.033333
33	201/219	0.000000	0.034483	0.000000	0.000000

(Cont'd)

Table 4.6 (continued...) Genotypes and frequencies at the HUJII77 locus in the different goat populations.

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
34	203/211	0.000000	0.000000	0.000000	0.033333
35	205/205	0.000000	0.000000	0.000000	0.033333
36	205/209	0.033333	0.000000	0.033333	0.000000
37	205/211	0.000000	0.000000	0.066667	0.000000
38	205/217	0.000000	0.000000	0.000000	0.033333
39	207/207	0.000000	0.034483	0.033333	0.000000
40	207/209	0.066667	0.000000	0.000000	0.000000
41	207/211	0.033333	0.000000	0.033333	0.000000
42	207/213	0.000000	0.000000	0.000000	0.033333
43	207/219	0.033333	0.000000	0.000000	0.000000
44	209/211	0.033333	0.000000	0.000000	0.000000
45	209/217	0.033333	0.000000	0.000000	0.000000
46	209/227	0.033333	0.000000	0.000000	0.000000
47	211/211	0.066667	0.034483	0.000000	0.000000
48	211/213	0.033333	0.000000	0.000000	0.000000
49	211/215	0.000000	0.034483	0.000000	0.000000
50	211/217	0.066667	0.000000	0.000000	0.000000
51	211/219	0.033333	0.034483	0.000000	0.000000
52	211/221	0.000000	0.034483	0.000000	0.000000
53	211/223	0.033333	0.000000	0.000000	0.000000
54	213/213	0.000000	0.034483	0.000000	0.000000
55	213/215	0.000000	0.000000	0.000000	0.066667
56	213/217	0.033333	0.000000	0.000000	0.033333
57	213/223	0.033333	0.000000	0.000000	0.000000
58	215/215	0.000000	0.000000	0.133333	0.000000
59	215/217	0.000000	0.000000	0.133333	0.000000
60	217/217	0.000000	0.000000	0.100000	0.000000
61	217/221	0.000000	0.000000	0.033333	0.033333
62	217/229	0.033333	0.000000	0.000000	0.000000
63	219/219	0.000000	0.103448	0.000000	0.000000
64	219/221	0.000000	0.000000	0.000000	0.033333
65	219/229	0.033333	0.000000	0.000000	0.000000
66	225/225	0.000000	0.000000	0.033333	0.000000

Table 4.7 Genotypes and frequencies at the BM6121 locus in the different goat populations

Sl. No.	Genotype	Frequency			
		Salem Black	Malabari	Attappadi Black	Non-descript
1	151/155	0.000000	0.000000	0.000000	0.035714
2	153/153	0.035714	0.034483	0.000000	0.000000
3	153/155	0.000000	0.034483	0.000000	0.035714
4	153/163	0.071429	0.000000	0.000000	0.035714
5	153/167	0.035714	0.000000	0.000000	0.000000
6	155/155	0.071429	0.103448	0.000000	0.000000
7	155/157	0.000000	0.000000	0.000000	0.035714
8	155/159	0.107143	0.000000	0.066667	0.035714
9	155/161	0.142857	0.034483	0.100000	0.107143
10	155/163	0.035714	0.103448	0.000000	0.000000
11	155/165	0.000000	0.068966	0.066667	0.000000
12	155/167	0.000000	0.000000	0.033333	0.000000
13	157/157	0.000000	0.000000	0.000000	0.071429
14	157/159	0.035714	0.000000	0.000000	0.035714
15	157/163	0.035714	0.068966	0.033333	0.071429
16	157/165	0.000000	0.034483	0.066667	0.071429
17	157/167	0.000000	0.034483	0.033333	0.000000
18	159/161	0.000000	0.034483	0.033333	0.000000
19	159/163	0.000000	0.000000	0.033333	0.035714
20	159/165	0.071429	0.000000	0.033333	0.035714
21	159/167	0.000000	0.000000	0.066667	0.000000
22	161/161	0.035714	0.034483	0.033333	0.035714
23	161/163	0.035714	0.068966	0.033333	0.000000
24	161/165	0.035714	0.000000	0.066667	0.000000
25	161/167	0.000000	0.034483	0.066667	0.035714
26	161/169	0.000000	0.000000	0.033333	0.000000
27	163/163	0.035714	0.103448	0.033333	0.000000
28	163/165	0.035714	0.034483	0.000000	0.107143
29	163/167	0.000000	0.000000	0.000000	0.071429
30	165/165	0.035714	0.068966	0.033333	0.035714
31	165/167	0.071429	0.103448	0.033333	0.107143
32	165/171	0.000000	0.000000	0.033333	0.000000
33	167/167	0.035714	0.000000	0.033333	0.000000
34	169/173	0.000000	0.000000	0.033333	0.000000
35	173/175	0.035714	0.000000	0.000000	0.000000

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Table 4.8 Alleles, their size and frequency at the INRA63 locus in the four goat populations investigated.

Allele and Size (bp)	Frequency			
	Salem Black	Malabari	Attappadi Black	Non-descript
1 (152)	0.016667	0.00000	0.000000	0.01667
2 (154)	0.000000	0.01667	0.000000	0.01667
3 (156)	0.016667	0.01667	0.000000	0.01667
4 (158)	0.066667	0.03333	0.016667	0.13333
5 (160)	0.100000	0.11667	0.016667	0.11667
6 (162)	0.050000	0.25000	0.116670	0.20000
7 (164)	0.183333	0.20000	0.266670	0.43333
8 (166)	0.250000	0.21667	0.133330	0.06667
9 (168)	0.283333	0.11667	0.400000	0.00000
10 (170)	0.016667	0.03333	0.050000	0.00000
11 (172)	0.016667	0.00000	0.000000	0.00000

Table 4.9 Alleles, their size and frequency at the ILSTS030 locus in the four goat populations investigated.

Allele and size (bp)	Frequency			
	Salem Black	Malabari	Attappadi Black	Non-descript
1 (146)	0.000000	0.01667	0.00000	0.00000
2 (148)	0.000000	0.01667	0.05000	0.03448
3 (150)	0.034483	0.00000	0.03333	0.03448
4 (152)	0.017241	0.11667	0.06667	0.08621
5 (154)	0.000000	0.10000	0.10000	0.05172
6 (156)	0.172414	0.05000	0.06667	0.03448
7 (158)	0.103448	0.03333	0.01667	0.05172
8 (160)	0.017241	0.16667	0.23333	0.06897
9 (162)	0.120690	0.25000	0.18333	0.13793
10 (164)	0.189655	0.05000	0.08333	0.17241
11 (166)	0.155172	0.06667	0.03333	0.10345
12 (168)	0.051724	0.10000	0.03333	0.01724
13 (170)	0.017241	0.03333	0.10000	0.08621
14 (172)	0.068966	0.00000	0.00000	0.08621
15 (174)	0.017241	0.00000	0.00000	0.03448
16 (176)	0.017241	0.00000	0.00000	0.00000
17 (178)	0.017241	0.00000	0.00000	0.00000

Table 4.10 Alleles, their size and frequency at the HUJII77 locus in the four goat populations investigated.

Allele and Size (bp)	Frequency			
	Salem Black	Malabari	Attappadi Black	Non-descript
1 (189)	0.03333	0.00000	0.00000	0.00000
2 (191)	0.03333	0.03448	0.00000	0.00000
3 (193)	0.01667	0.10345	0.10000	0.06667
4 (195)	0.00000	0.10345	0.06667	0.16667
5 (197)	0.15000	0.17241	0.06667	0.15000
6 (199)	0.05000	0.01724	0.01667	0.01667
7 (201)	0.03333	0.08621	0.06667	0.08333
8 (203)	0.00000	0.00000	0.00000	0.01667
9 (205)	0.01667	0.00000	0.05000	0.10000
10 (207)	0.06667	0.03448	0.06667	0.01667
11 (209)	0.10000	0.03448	0.01667	0.01667
12 (211)	0.20000	0.13793	0.06667	0.06667
13 (213)	0.06667	0.06897	0.00000	0.10000
14 (215)	0.01667	0.03448	0.25000	0.06667
15 (217)	0.08333	0.00000	0.18333	0.06667
16 (219)	0.05000	0.15517	0.00000	0.03333
17 (221)	0.00000	0.01724	0.01667	0.03333
18 (223)	0.03333	0.00000	0.00000	0.00000
19 (225)	0.00000	0.00000	0.03333	0.00000
20 (227)	0.01667	0.00000	0.00000	0.00000
21 (229)	0.03333	0.00000	0.00000	0.00000

Table 4.11 Alleles, their size and frequency at the BM6121 locus in the four goat populations investigated.

Allele and Size (bp)	Frequency			
	Salem Black	Malabari	Attappadi Black	Non-descript
1 (151)	0.00000	0.00000	0.00000	0.01786
2 (153)	0.08929	0.05172	0.00000	0.03571
3 (155)	0.21429	0.18966	0.13333	0.12500
4 (157)	0.01786	0.06897	0.06667	0.17857
5 (159)	0.12500	0.01724	0.10000	0.07143
6 (161)	0.14286	0.13793	0.20000	0.10714
7 (163)	0.14286	0.22414	0.08333	0.16071
8 (165)	0.14286	0.22414	0.18333	0.19643
9 (167)	0.08929	0.08621	0.16667	0.10714
10 (169)	0.00000	0.00000	0.03333	0.00000
11 (171)	0.00000	0.00000	0.01667	0.00000
12 (173)	0.01786	0.00000	0.01667	0.00000
13 (175)	0.01786	0.00000	0.00000	0.00000

Table 4.12 Allele size range, Number of alleles, Heterozygosity and Polymorphic Information Content (PIC) at the INRA63 locus in the four goat populations investigated.

Observation	Salem Black (n =30)	Malabari (n =30)	Attappadi Black (n =30)	Non-descript (n =30)
Allele size range (bp)	152-172	154-170	158-170	152-166
No. of alleles	10	9	7	8
Heterozygosity	0.806	0.821	0.734	0.736
PIC	0.779	0.796	0.695	0.703

Table 4.13 Allele size range, Number of allels, Heterozygosity and Polymorphic Information Content (PIC) at the ILSTS030 locus in the four goat populations investigated.

Observation	Salem Black (n =29)	Malabari (n =30)	Attappadi Black (n =30)	Non-descript (n =29)
Allele size range (bp)	150-178	146-170	148-170	148-174
No. of alleles	14	12	12	14
Heterozygosity	0.876	0.864	0.870	0.903
PIC	0.862	0.851	0.857	0.895

Table 4.14 Allele size range, Number of alleles, Heterozygosity and Polymorphic Information Content (PIC) at the HUJII77 locus in the four goat populations investigated.

Observation	Salem Black (n =30)	Malabari (n =29)	Attappadi Black (n =30)	Non-descript (n =30)
Allele size range (bp)	189-229	191-221	193-225	193-221
No. of alleles	17	13	13	15
Heterozygosity	0.900	0.888	0.867	0.902
PIC	0.892	0.878	0.855	0.894

Table 4.15 Allele size range, Number of alleles, Heterozygosity and Polymorphic Information Content (PIC) at the BM6121 locus in the four goat populations investigated.

Observation	Salem Black (n =28)	Malabari (n =29)	Attappadi Black (n =30)	Non-descript (n =28)
Allele size range (bp)	153-175	153-167	155-173	151-167
No. of alleles	10	8	10	9
Heterozygosity	0.860	0.829	0.858	0.858
PIC	0.845	0.807	0.842	0.842

Table 4.16 Mean Heterozygosity and PIC of the four microsatellite markers tested in the different goat populations.

Parameter	Microsatellite loci			
	INRA63	**ILSTS030**	**HUJII77**	**BM6121**
Mean Heterozygosity	0.774	0.878	0.889	0.852
Mean PIC	0.744	0.866	0.880	0.834

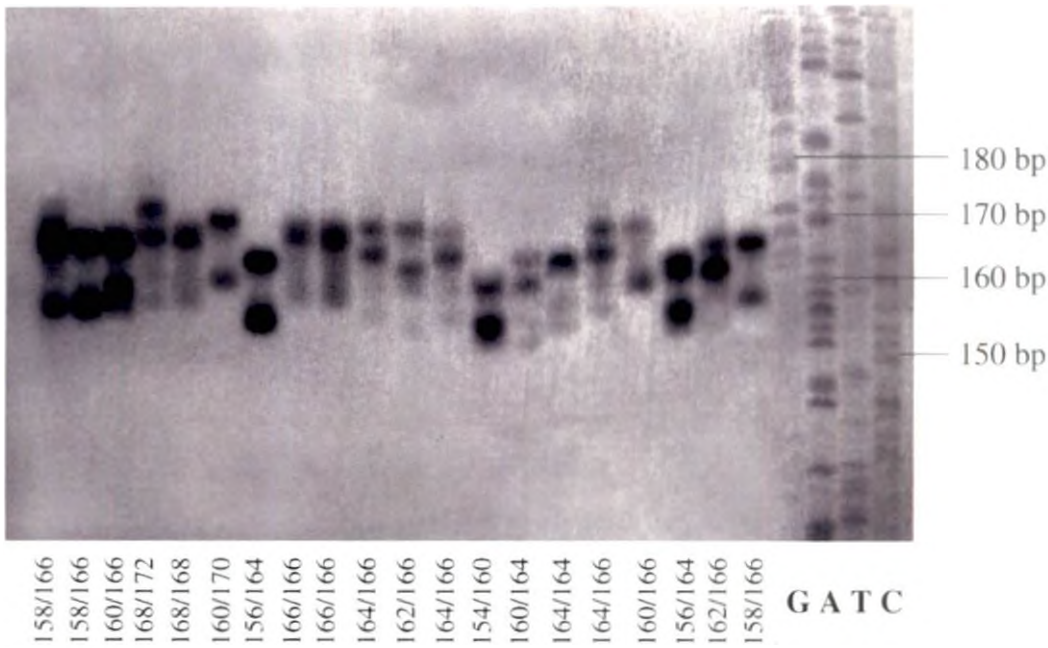

Plate 1. Autoradiograph showing polymorphism at INRA63 locus GATC represents M13 sequence used as marker

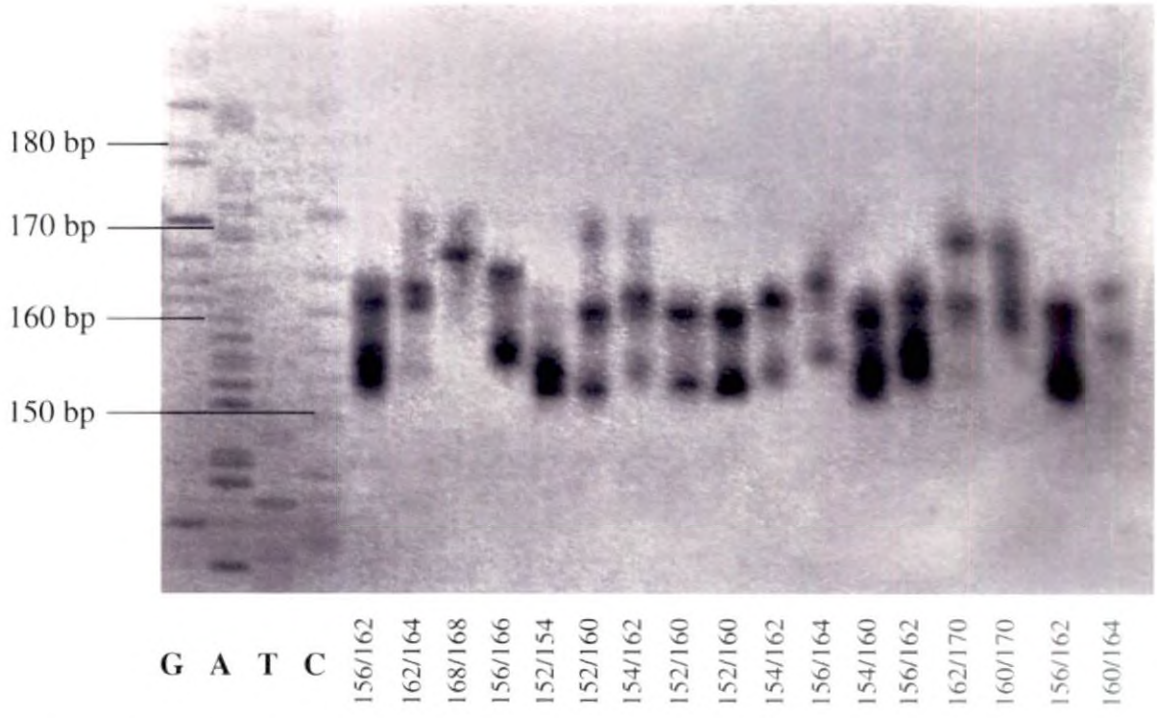

Plate 2. Autoradiograph showing polymorphism at ILSTS030 locus
GATC represents M13 sequence used as marker

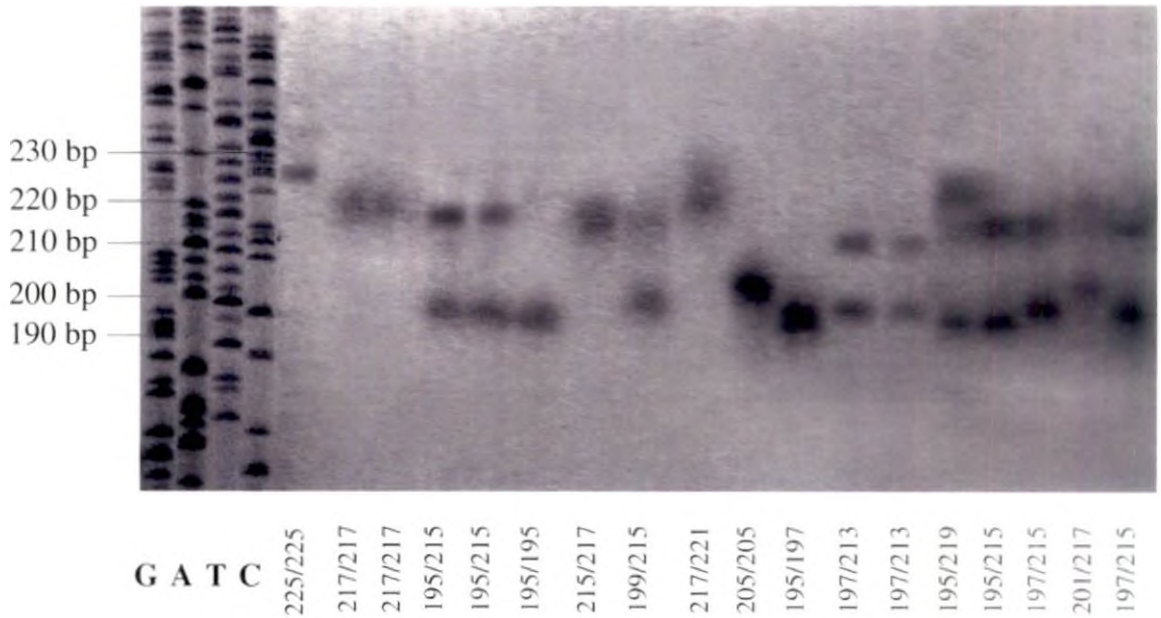

Plate 3. Autoradiograph showing polymorphism at HUJII77 locus GATC represents M13 sequence used as marker

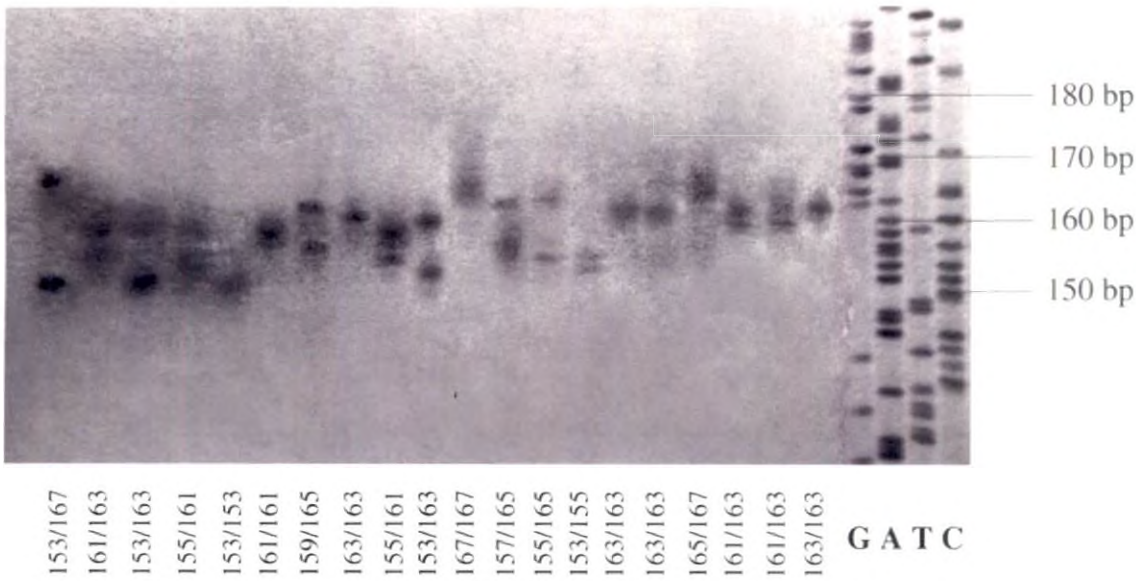

Plate 4. Autoradiograph showing polymorphism at BM6121 locus GATC represents M13 sequence used as marker

4.6 GENETIC DISTANCE

Genetic distances among the four goat populations were calculated according to Nei's formula given by Nei (1978) using the POPGENE version 1.31 program (Yeh *et al.*, 1999). Nei's genetic distance between Salem Black and Malabari populations was 0.2676, between Salem Black and Attappadi Black, 0.3092, between Salem Black and non-descript animals, 0.3880, between Malabari and Attappadi Black, 0.2947, between Malabari and non-descript, 0.2237 and that between Attappadi Black and non-descript was found to be 0.3777. The genetic distance matrix is presented in Table 4.17.

4.7 DENDROGRAM

Dendrogram of relationship between the four populations under study was plotted using the POPGENE program. The dendrogram showed two clusters grouping Salem Black goats and Attappadi Black goats in one cluster and Malabari and non-descript goats in the other. The dendrogram is presented in Plate 5.

4.8 STATISTICAL ANALYSIS

Analysis of variance was performed to detect the effect of breeds in allele numbers, heterozygosity, PIC and allele size range at the different loci typed. No significant difference was observed among breeds for the parameters tested ($p \leq 0.05$) except for the allele size range. It was found that the allele size range observed for the Salem black animals varied significantly from that of the other goat populations . The other three breeds were found to be homogenous for the size range of alleles. The Wilcoxon sign rank test for median difference performed to test breed variation in allele distributions at each of the four loci did not detect a significant variation between breeds ($p \leq 0.05$).

Table 4.17 Nei's Genetic distance matrix for the four goat populations analysed.

	Salem Black	**Malabari**	**Attappadi Black**	**Non-descript**
Salem Black	0.0000			
Malabari	0.2676	0.0000		
Attappadi Black	0.3092	0.2947	0.0000	
Non-descript	0.3880	0.2237	0.3777	0.0000

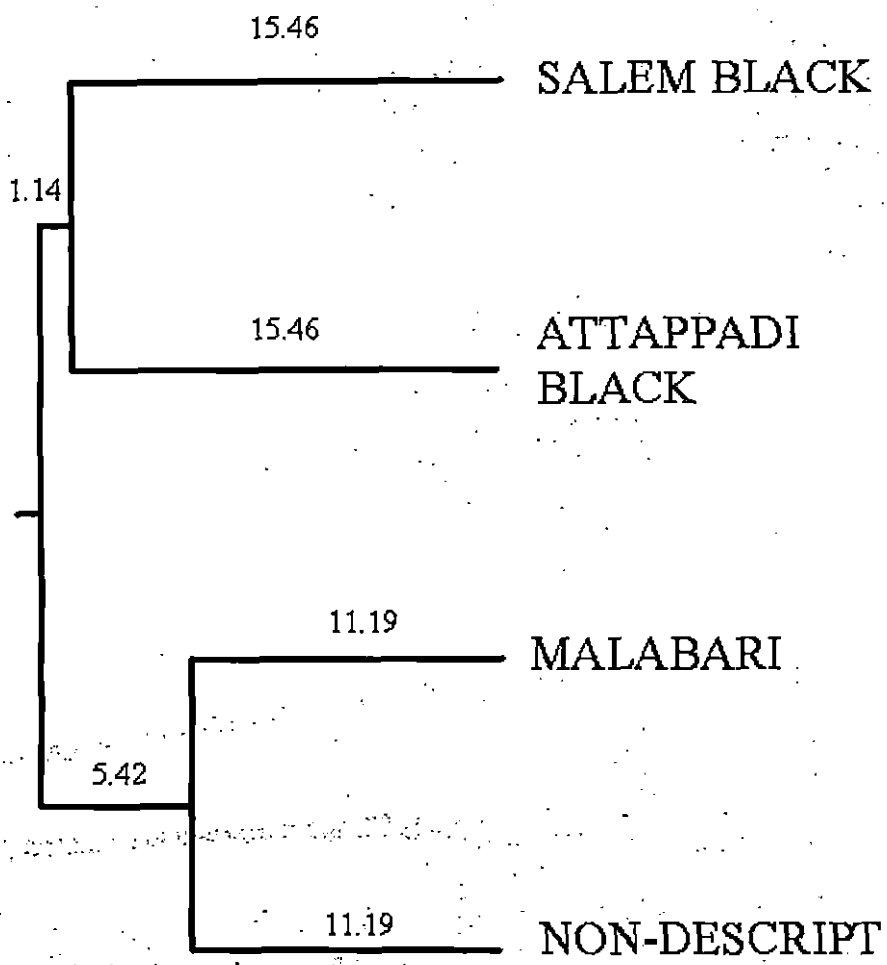

Plate 5. Dendrogram representing relationship between the four goat breeds studied constructed using the UPGMA method. Numbers indicate branch lengths.

Discussion

5. DISCUSSION

The primary unit in animal genetic resources is a breed, strain or a geographically defined population. In the developing countries breeds are not clearly defined, yet there exists strains or geographically separated populations. Conservation plans should ensure the maintenance of maximum genetic diversity in the global gene pool of each species so as to allow for future unforeseen needs in the development of sustainable animal production system. Measures of genetic distance provide the best available objective description of genetic differentiation between breeds. Microsatellites, of all genetic markers have proved to be most useful for the analysis of genetic relationships (Saitbekova *et al.*, 1999; Yang *et al.*, 2004). This study was attempted with the objectives of characterising and establishing relationship, if any, between the four goat breeds, viz; Salem Black, Malabari, Attappadi Black and the non-descript goats of Thrissur.

5.1 DNA ISOLATION AND YIELD OF DNA

DNA isolation from whole blood was carried out using the standard Phenol-Chloroform extraction procedure (Sambrook *et al.*, 1989) with the modification of overnight incubation of the WBC suspension with SDS and Proteinase-K. Phenol-Chloroform extraction procedure is a common and efficient technique for DNA isolation from blood.

The mean yield of DNA obtained from 5 ml goat blood was 361.3±10.7 µg. Senthil *et al.* (1996) and Aravindakshan *et al.* (1998) had reported average yields of 444.58±21.54µg and 394.50±34.26µg, respectively per 10-15 ml cattle blood using the Phenol-Chloroform extraction procedure. Chitra (2002) and Mathew (2004) reported yields of 230.97±11.65 µg and 350.05±10.05µg, respectively, from 5 ml of goat blood using the same procedure. The higher DNA yield obtained from goats when compared to cattle is possibly due to the higher leucocyte count in goats.

5.2 MICROSATELLITE ANALYSIS

5.2.1 PCR Conditions

Polymerase chain reaction involves two oligonucleotide primers that flank the DNA segment to be amplified and repeated cycles of heat denaturation of DNA , annealing of the primers to their complementary sequence and extension of the annealed primers with DNA polymerase. This results in the exponential accumulation of the specific target fragment approximately 2^n times, where n is the number of cycles (Saiki *et al.*, 1988)

PCR conditions were optimised for the primers selected to obtain specific products. Annealing temperature of the primer and magnesium chloride concentration are the two important factors determining the stringency of primer hybridisation. The annealing temperature for the primer pairs was optimised by using a temperature gradient program with a range of 55°C to 65°C. An annealing temperature of 60°C was found optimal for the primers INRA63 and BM6121 and 58°C for ILSTS030 and HUJII77. All the primers yielded specific products at 1.5 m*M* magnesium chloride concentration.

Other conditions affecting the specificity of the polymerase chain reaction include concentration of enzyme and primers as well as the annealing time, extension time and number of cycles. These conditions were kept constant for all the primers used. A concentration of 0.3 U/reaction of *Thermus aquaticus* DNA polymerase and 5 p*M* of primers per reaction gave satisfactory results. Time for denaturation, annealing and extension was one minute with a final extension time of 10 minutes.

5.2.2 Microsatellite Primers

Thirteen bovine microsatellites were included in the study. Two of these failed to yield products on PCR amplification with caprine genomic DNA.

Among the primers which had successful amplification, seven demonstrated low degree of polymorphism. Four markers detecting higher number of alleles were chosen for further analysis. Moore *et al.* (1991) had found that the degree of polymorphism displayed by a particular microsatellite in any species was variable. The template DNA from sheep, horse and humans were amplified using bovine microsatellites. Specific products were obtained in 56 per cent samples of ovine DNA out of which 42 per cent showed polymorphisms. With equine DNA, 6.2 per cent gave specific but monomorphic products while no specific product was obtained using human DNA. The close homology between cattle, sheep and goats makes it possible to devise a single panel of markers for genetic distance characterisation in these species.

Saitbekova *et al.* (1999) and Tadlaoui Ouafi *et al.* (2002) successfully used bovine primers for genetic diversity studies in goats. Yang *et al.* (2004) also reported using sheep microsatellites for similar studies in goat populations.

5.2.2.1 INRA63

Eleven alleles were observed at this locus considering all the four goat populations. The highest number of ten alleles was observed in Salem Black goats followed by nine in Malabari, eight in non-descript goats and seven in Attappadi Black. The size range of alleles varied between 152 and 172 bp. These observations were in concordance with that reported by other workers who used the same marker. Pepin *et al.* (1995) reported that this bovine primer could produce strong amplification with only one band (in the case of homozygous individuals) or two bands (in the case of heterozygous individuals) of the expected size and absence of non-specific bands with goat DNA. They reported six alleles with an average size of 170 bp in goats when compared to seven alleles of average size 180 bp in cattle. Mukesh *et al.* (2004) reported three alleles with a size range of 170-188 bp in Sahiwal cattle. Chenyambuga *et al.* (2004) reported 10 alleles with a size range of 141-179 bp in the goats of sub Saharan Africa. .

5.2.2.2 ILSTS030

A total of seventeen alleles were detected at this locus 14 each in Salem Black and non-descript goats and 12 each in Attappadi black and Malabari. The size of alleles ranged from146-178 bp. Nineteen alleles were detected at this locus in Swiss goats by Saitbekova *et al.* (1999). Allele size range noticed at this locus in Marwari goat breed was reported to be 164-174 bp (Kumar *et al.*, 2005).

The presence of shadow bands was a feature noticed with this primer. According to Murray *et al.* (1993) shadow bands were produced on PCR of DNA sequences containing a CA dinucleotide repeat. They found that shadow bands appeared due to two base pair deletions occurring randomly in the CA repeat region during PCR. They attributed the reason for the production of shadow bands to slippage synthesis or recombination events.

5.2.2.3 BM6121

This bovine primer was found to produce weak products when amplified with goat DNA. Bands were less clear and genotyping was difficult compared to the other three markers used. It also showed the presence of non-specific products in the autoradiograph even after repeated adjustments made on the temperature of annealing and concentration of magnesium chloride. Hence, the cause of weak amplification and non-specificity may be attributed to a lower degree of homology between this particular bovine microsatellite and the corresponding caprine sequence. An increased quantity of product was used in the electrophoresis or the exposure time during autoradiography was increased so as to obtain readable bands. In spite of these difficulties a total of 13 alleles could be detected at this locus, ten each in Salem black and Attappadi Black, nine in non-descript goats and eight in Malabari. The size range of alleles was found to be 151-175 bp. No other reports were available on the use of this marker in diversity studies.

5.2.2.4 HUJII77

This marker was found to be the most polymorphic of all the four markers typed detecting 21 alleles with a size range between 189-229 bp. Seventeen alleles were observed in Salem Black goats, fifteen in non-descript and thirteen each in Malabari and Attappadi Black. An average allele size of 211 bp was reported in cattle by Shalom *et al.* (1994). Vallejo *et al.* (2003) observed the presence of six alleles among 23 Holstein Friesian bulls typed at this locus.

5.3 ALLELE FREQUENCY

The number of alleles at different marker loci and their frequencies are indicators of genetic variability and also form the basis of all diversity indices for estimation of genetic distances and construction of phylogenetic trees.

The number of alleles ranged from seven to seventeen in the whole population. The highest mean number of alleles per locus was noticed in Salem black goats. The allele size range was also higher in this group. Considering the fact that the sample size was constant for all the four groups of animals, Salem Black goats can be considered to be more genetically diverse than the other groups based on the number of alleles and size range.

The high mean number of alleles per locus is an indication of high genetic variability within breeds (Sodhi *et al.*, 2003). The mean number of alleles is dependent on sample size because of the presence of unique alleles in the population which occur in low frequencies and also because the number of observed alleles tends to increase with increase in population size (Kotze *et al.*, 2004).

No significant difference could be detected among the breeds for the allele distributions at the four loci studied. It was observed that a few alleles were predominant, i.e., a few alleles were present at a higher frequency, and this

differed with loci and with breed. For the ILSTS030 locus the common alleles occurred in the medium range of size and for INRA63 it occurred towards the upper extreme of size in all the four breeds. No such clear-cut distribution could be outlined for the HUJII77 locus except in Attappadi Black goats where the alleles 215 bp and 217 bp were the more frequent ones and in Salem Black in which the allele 211 bp was more frequent. At the INRA63 locus, alleles 166 bp and 168 bp were predominant in Salem Black, 164 bp and 166 bp in Malabari, 164 bp and 168 bp in Attappadi Black and 162 bp and 164 bp in Non descript goats. At the ILSTS030 locus, the allele 164 bp was more frequent in Salem Black and Non descript goats, allele 162 bp in Malabari and allele 160 bp in Attappadi Black. At the BM 6121 locus, the allele 155 bp was present at a frequency of 0.2 in Salem Black goats. Malabari animals had a predominance of three alleles, viz; 155 bp, 163 bp and 165 bp. Alleles 161 bp and 165 bp were more frequent in Attappadi Black, and alleles 157 bp and 165 bp in the non-descript goats. In most cases, the frequency distribution of alleles did not follow a consistent pattern. These results are in accordance with data previously reported for microsatellites by other authors (Forbes *et al.*, 1995; Arranz *et al.*, 2001).

The occurrence of a few highly frequent alleles in the different populations could be suggestive of probable linkage of these loci to traits of economic importance and selection for those traits. However, to obtain more consistent results and to be able to draw definite conclusions, the study should be undertaken incorporating a higher number of animals representing each breed. Another probable reason for the predominance of certain alleles in a population is the higher rates of inbreeding within the populations. This is even more likely since the numbers of males in most domestic animal populations are low.

The non-uniform pattern of allele frequency observed for microsatellites is due to the high variability these markers display. High mutation rates account for the large variability displayed by the microsatellite. This instability is partially explained by polymerase slippage mechanisms that tend to produce non identical copies of repeated DNA sequences (Schlotterer and Tautz, 1992).

Moreover unequal recombination exchange between homologous chromosomes during meiosis also contributes to the high microsatellite mutation rate.

Irregularity of microsatellite distributions can be attributed to the complex evolution at these loci. Single step mutation events account for about 90 per cent of microsatellite mutation events, followed by double step mutations and a limited number of multi step mutations (Weber and Wong, 1993; Di Rienzo *et al.*, 1994). These authors suggested that this extended single step mutation produces a much larger variance of allele size when compared to the simple single step mutation model.

5.4 HETEROZYGOSITY AND POLYMORPHIC INFORMATION CONTENT

Heterozygosity is a measure for assessing the genetic variability within a population. The heterozygosity values were found high for all the breeds at all loci and ranged from 0.734 to 0.9. These high values indicate the suitability of the markers for diversity studies. Heterozygosity values were not significantly different between breeds. This is possibly due to the weak genetic structuring in domestic goats (Luikart *et al.*, 2001). All the four breeds demonstrated high heterozygosity values, which was in accordance with the expectation for microsatellite loci. Similar values for heterozygosity were reported in different goat populations by other workers. (Tadlaoui-Ouafi *et al.*, 2002, Yang *et al.*, 2004, Kumar *et al.*, 2005)

Polymorphic information content is a measure of the informativeness of the marker. Microsatellite markers have an average PIC value pf 0.6 (Vaiman *et al.*, 1994). The PIC values were high at all loci and in all the breeds and ranged from 0.69 to 0.89. The values showed no significant difference among the different breeds investigated.

5.5 GENETIC DISTANCE

The allele frequencies at each locus for each breed were used for computing the genetic distance between each pair of breeds. The Nei's genetic distance between the breed ranged from 0.224 to 0.388. The genetic distance measures the time that has elapsed since the populations were genetically equivalent. In other words, it indicates the time of divergence of the breeds from each other. According to the results obtained, the highest value of genetic distance was found to be between the Salem Black and non-descript breeds. This finding is in agreement with the geographic distance between the breeds. The lowest value of genetic distance was found between Malabari goats and non-descript goats of Thrissur, which can be substantiated by the fact that cross breeding of non-descript goats and Malabari is practiced by the goat farmers in Thrissur.

Barker (1994) recommended that since distance measures cannot account for consequences of artificial selection on morphological or economic traits or for natural selection for fitness and cannot accurately measure the time since divergence from a common ancestral population, they should only be used as an initial guide to population structure and breed differentiation.

Arranz *et al.* (1996) observed that genetic distances calculated from microsatellite allele frequencies are usually higher when compared to other marker systems because of the greater variation at these loci.

5.6 DENDROGRAM

The dendrogram constructed from the genetic distance data using the POPGENE program grouped Salem Black and Attappadi Black animals in one cluster and Malabari and non descript goats in the other. This suggests a closer relationship between the breeds grouped in a single cluster. The pattern observed is in agreement with the geographic distance between these populations. Similar

results were obtained in several studies by other workers (Yang *et al.*, 1999; Saitbekova *et al.*, 1999; Vasconcellos *et al.*, 2003). The non-descript goats of Thrissur may have inherited Malabari genes due to cross breeding since transportation of Malabari goats from their breeding tract to Thrissur is not an uncommon practice.

The results of the study indicate a high genetic variation within all the four populations. There was no significant difference between the breeds for the different parameters tested like number of alleles, allele distribution, and heterozygosity. This can be attributed to the lower sample size since the populations studied were phenotypically distinct. It was found that the set of microsatellite markers tested in this study could be used satisfactorily for molecular characterisation and genetic diversity studies in South Indian goat breeds. However, use of more number of markers and increasing the sample size would allow a better differentiation between populations that are closely related.

This was a pilot study using microsatellite markers for characterisation and genetic distancing in above mentioned goat populations. The information would prove useful for meeting the demands of future breeding programmes and formulating effective conservation strategies for genetic diversity within breeds. Although only four microsatellite markers were used in the present study, the findings will definitely form a foundation for related investigations in the future.

Summary

6. SUMMARY

Awareness of the value of genetic resources has stimulated the study of genetic diversity of native breeds of animals. Population genetic structure within domestic breeds is an essential component of genetic diversity. Microsatellites have been proved to be successful in defining genetic structure and genetic relationships among different breeds. Microsatellites display high levels of variation and consequently enable population differentiation to be found more effectively. In the present study, an attempt is made to study the genetic variability and relationship among four breeds of goats, namely, Salem Black, Malabari, Attappadi Black and the non-descript goats of Thrissur.

DNA was isolated from 30 genetically unrelated animals of each breed using the phenol-chloroform extraction procedure. The mean yield obtained per five ml of blood was 361.43±10.73 µg. The average value for the ratio of optical density (260 nm/280 nm) was 1.54±0.015.

A set of microsatellite primer pairs were selected from the published literature and custom synthesised and used for PCR analysis. Out of this chosen panel, four were selected based on polymorphic patterns and amplification efficiency and used for further analysis. PCR conditions were optimised separately for each primer. Each reaction was carried out in 10 µl volume and contained 1 µl of 10X PCR reaction buffer, 200 µ*M* dNTP, 5 p*M* each of forward and reverse primers and 0.3 U of the *Thermus aquaticus* DNA polymerase. A $MgCl_2$ concentration of 1.5 m*M* was found to yield satisfactory results for all the primers. Since autoradiography was used for the detection of the PCR products, the forward primer of each primer pair was end labeled with γ^{32}P-ATP and incorporated in the PCR reaction mix.

The sequenced M13 phage DNA was used as the marker for scoring alleles. The sequencing was done by the dideoxy method following

manufacturer's instructions in the Sequenase Version 2.0 (Amersham Biosciences, USA) sequencing kit.

Denaturing polyacrylamide gel electrophoresis was used to fractionate the amplified products and bands produced were visualized by autoradiography. The gel after electrophoresis and drying was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2 cm) in a cassette (Kiran Hypercassette) fitted with an intensifying screen. The X-ray film was developed after 24 to 48 hours or more depending on the intensity of radioactive signal. The number of alleles at each locus for each breed was scored and sizes assessed by comparing with the sequence of M13 single stranded DNA size standard.

A total of eleven alleles were detected at the INRA63 locus. The mean heterozygosity and PIC values obtained were 0.774 and 0.743, respectively. Seventeen alleles were detected at the ILSTS030 locus. Then mean values of heterozygosity and PIC were 0.878 and 0.866, respectively. Amplification at the BM6121 locus was not optimal and yielded only weak products. Nevertheless, 13 alleles were detected and the mean heterozygosity and PIC values were 0.851 and 0.833, respectively. The HUJII77 locus was the most polymorphic of all the four loci detecting 21 alleles with a mean heterozygosity and PIC values of 0.899 and 0.88, respectively.

Allele frequencies at each locus for each breed were used to calculate the genetic distances among the four breeds. Nei's genetic distance was worked out using the using the POPGENE version 1.31 program. The distance measure between Salem Black and non-descript goats was 0.388 which was the highest value, followed by that between Salem Black and Attappadi Black which was 0.309. Distance measures between Malabari and Attappadi Black was 0.295, that between Salem Black and Malabari, 0.268 and that between Attappadi Black and non-descript goats was 0.377. Malabari and non-descript goats had the lowest genetic distance of 0.224 indicating that

these two breeds diverged recently when compared to Malabari and Salem Black or Malabari and Attappady Black.

The dendrogram constructed using the POPGENE program grouped the Salem Black and Attappadi Black goats in one cluster and Malabari and non-descript goats of Thrissur in another which was in agreement with the geographic distance between the breeds.

The results in the study indicated no significant difference among the breeds for the different measures of genetic diversity like number of alleles at each locus and heterozygosity. This can be attributed to the lower sample size since the populations studied were phenotypically distinct. The Salem Black animals showed a higher range of allele sizes and more number of alleles at the different loci typed. It was also found that the allele frequency distribution at the different loci did not follow a definite pattern and was more or less skewed though a few alleles were predominant at each locus and in each breed.

It was found that the set of microsatellite markers tested in this study could be used satisfactorily for molecular characterisation and genetic diversity studies in South Indian goat breeds. However, incorporation of more number of markers and increasing the sample size is recommended so as to enable a clearer differentiation among closely related populations.

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Annexures

ANNEXURE – 1

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

Acrylamide (40 %)

Acrylamide	380 g
N_1N – Methylene bisacrylamide	20 g
Water to	1000 ml

Agarose (1 %)

Weighed 0.6 g of agarose powder and mixed with 60 ml of 1 X TAE buffer in a conical flask. Solution heated in a microwave oven until boiling and cooled slowly.

Ammonium persulphate (10%)

Ammonium persulphate	100 mg
Water to	1 ml

Denaturing Polyacrylamide Gel

0.5 X TBE Gel mix	60 ml
TEMED	0.125 ml
Ammonium per sulphate (10 %)	0.125 ml

Mixed well without air bubbles

EDTA (0.5 *M*, pH 8.3)

Dissolved 18.61g of EDTA (disodium, dihydrate) in 80 ml of distilled water by bringing the pH to 8.3 with NaOH solution and volume made upto 100 ml. Stored at room temperature after filtration and autoclaving.

Ethidium Bromide (10 mg/ml)

Dissolved 100 mg ethidium bromide in10 ml of distilled water. Solution stored at 4 °C in a dark coloured bottle.

Formamide dye/ Stop buffer

Deionised formamide	98 %
Xylene cyanol	0.025 %
Bromophenol blue	0.025 %
0.5 M EDTA	10 m*M*

Gel loading buffer

Bromophenol Blue	0.25 %	50 mg
Xylene Cyanol	0.25 %	50 mg
Sucrose	40 %	8 g

Components stirred well in 20ml distilled water and stored at 4°C.

Phenol (Saturated, pH 7.8)

Commercially available crystalline phenol melted at 65 °C in a water bath. Hydroxyquinoline added to a final concentration of 0.1 percent. To the molten phenol, 0.5 M Tris HCl (pH 8.0) was added in equal volume and stirred for 30 min. on a magnetic stirrer. The contents were transferred into a separating funnel. Lower phenolic phase was collected and mixed with equal volume of 0.1 M Tris HCl (pH 8.0) and stirred again for 30 min. The phenolic phase was collected and extraction repeated with 0.1 M Tris HCl (pH 8.0) until the pH of phenolic phase was more than 7.8. Finally 0.1 volume of 0.01 M Tris HCl (pH 8.0) added and stored in a dark bottle at 4 °C.

RBC lysis buffer

Ammonium chloride	150 mM	8.0235 g
Potassium chloride	10 mM	0.7455 g
EDTA	0.1 mM	0.0372 g

Dissolved the contents in distilled water and volume made up to 1000 ml. Stored at 4 °C after filtration and autoclaving.

Sodium acetate (3 M, pH 5.5)

Dissolved 40.824 g of sodium acetate in 70 ml of distilled water and pH adjusted to 5.5 with glacial acetic acid. Volume made up to 100 ml, autoclaved and stored at room temperature.

Sodium chloride (5 M)

Dissolved 29.22 g of sodium chloride in 80 ml distilled water and volume made up to 100 ml. Solution filtered and stored at room temperature.

Sodium chloride – EDTA (SE) buffer (pH, 8.0)

Sodium chloride	75 mM	4.383 g
EDTA	35 mM	9.306 g

Dissolved the contents in 900 ml distilled water and pH adjusted to 8.0. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4°C.

Sodium dodecyl sulphate (SDS) 20 %

SDS	20 g
Distilled water to make up to	100 ml

Stirred, filtered and stored at room temperature.

Tris Acetate EDTA (TAE) buffer (50X)

Tris base	48.4 g
Glacial acetic acid	11.42 ml
0.5 M EDTA (P^H 8.0)	20 ml
Distilled water up to 1000 ml	

Autoclaved and stored at room temperature.

Tris-Borate EDTA (TBE) buffer (pH 8.3) 10X

Tris base	108.0 g
Boric acid	55.0 g
EDTA	9.3 g

Dissolved in 700 ml of distilled water and pH adjusted to 8.3. Volume made up to 1000 ml, autoclaved and stored at room temperature.

TBE Gel mix (0.5X)

40 % Acrylamide	150 ml
10X TBE buffer	50 ml
Urea	450 g

Mixed well in 700 ml distilled water, volume made up to 1000ml and stored at 4 °C.

Tris Buffered Saline (TBS) pH 7.4

Sodium chloride	140 mM	8.18 g
Potassium chloride	0.5 mM	0.0373 g
Tris base	0.25 mM	0.0303 g

Dissolved in 900 ml of distilled water and p^H adjusted to 7.4. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4 °C.

ANNEXURE – II

SOURCES OF IMPORTANT CHEMICALS AND ENZYMES USED IN THIS STUDY

(A) CHEMICALS

Acrylamide (Molecular Biology grade)	-	SRL, Bombay
Agarose (Low EED)	-	Bangalore Genei Pvt. Ltd.
Ammonium chloride	-	SRL, Bombay
Ammonium per sulphate	-	SRL, Bombay
N-N-Methylene Bis acrylamide	-	SRL, Bombay
Boric acid	-	SRL, Bombay
Chloroform	-	Merck
Crystalline phenol	-	Merck
Di-sodium hydrogen orthophosphate	-	SRL, Bombay
dNTPs.	-	Finn Enzymes
EDTA	-	SRL, Bombay
Ethanol	-	Merck
Ethidum bromide	-	BDH lab, England
6 X gel loading buffer	-	Bangalore Genei Pvt. Ltd.
Glacial acetic acid	-	BDH-E, Merck (India) Ltd.
Hydroxy quinolone	-	Qualigens Chemicals, Mumbai
Isoamyl alcohol	-	Merck
Methanol	-	SRL, Bombay
Potassium chloride	-	SRL, Bombay
Sodium acetate	-	SRL, Bombay
Sodium chloride	-	SRL, Bombay
Sodium dodecyl sulphate (SDS)	-	SRL, Bombay
TEMED	-	SRL, Bombay
Tris base	-	SRL, Bombay
Urea	-	SRL, Bombay

(B) PRIMERS

Invitrogen (India) Pvt. Ltd.

(C) MOLECULAR MARKERS

pBR322 DNA /*Hae*III digest	- Bangalore Genei Pvt. Ltd.
M13 sequencing ladder	- Amersham Pharmacia Biotech, USA.

(D) ENZYMES

Taq DNA polymerase	- Bangalore Genei Pvt. Ltd.
Proteinase-K	- Bangalore Genei Pvt. Ltd.
Polynucleotide kinase	- Bangalore Genei Pvt. Ltd.

(E) KITS

DNA-End-labelling kit	- Bangalore Genei Pvt. Ltd.
Sequenase version 2.0 DNA sequencing kit	- Amersham Pharmacia Biotech, USA.

(F) ISOTOPES

γ^{32} P-ATP	- BRIT, Bombay
α^{35} S-dATP	- BRIT (Jonaki), Hyderabad.

ANNEXURE – III

ABBREVIATIONS

RFLP	Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
VNTR	Variable Number of Tandem Repeat
RAPD	Random Amplified Polymorphic DNA
DNA	Deoxyribo Nucleic Acid
PAGE	Polyacrylamide Gel Electrophoresis
PIC	Polymorphic Information Content
EDTA	Ethylene Diamine Tetraacetic Acid
DTT	Dithiothretol
TEMED	N, N, N, N Tetramethylethylenediamine
APS	Ammonium Persulphate
PNK	Polynucleotide Kinase
He	Heterozygosity
cM.	Centimorgan
µl	microlitres
µg	microgram
mg	milligram
mM.	millimolar
mCi	millicurie
bp	basepair
rpm	revolutions per minute
SDS	Sodium Dodecyl Sulphate
dNTP.	Deoxy Nucleotide Triphosphate
ddATP.	Dideoxy Adenosine Triphosphate
ddCTP	Dideoxy Cytosine Triphosphate.
ddGTP.	Dideoxy Guanosine Triphosphate
ddTTP.	Dideoxy Thymidine Triphosphate

GENETIC DIVERSITY ANALYSIS OF GOAT BREEDS USING MICROSATELLITE MARKERS

AMRITA SUSAN JACOB

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

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Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

The study was undertaken to assess the genetic variability among four goat breeds of South India using microsatellite markers. Three breeds studied were native to Kerala. These were Malabari, Attappadi Black and non-descript goats of Thrissur. The fourth breed, Salem Black, originated in the Salem district of Tamil Nadu. Microsatellite analysis was carried out using four highly polymorphic bovine markers.

Blood samples from 30 genetically unrelated animals of each breed were collected and used as a source of DNA. The phenol-chloroform extraction procedure was used and the mean yield of DNA obtained was 361.43±10.73 μg/five ml blood. The four markers selected for the study were, INRA63, ILSTS030, HUJII77 and BM6121. PCR conditions were standardised for all the primers. The forward primer of each primer pair used in the PCR assay was end labeled with γ^{32}P-ATP prior to setting up of the PCR. M13 DNA was sequenced and used as the size standard.

The PCR products were separated by denaturing polyacrylamide gel electrophoresis. Detection of the products was done by autoradiography. Gels after electrophoresis were dried and was set for autoradiography with X-ray film in a cassette fitted with intensifying screen. Allele sizes were obtained by comparing with the sequence of M13 single stranded DNA size standard.

A total of eleven alleles were detected at the INRA63 locus. The mean heterozygosity and PIC values obtained were 0.774 and 0.743, respectively. Seventeen alleles were detected at the ILSTS30 locus. The mean values of heterozygosity and PIC were 0.878 and 0.866, respectively. Thirteen alleles were detected at the BM6121 locus with mean heterozygosity and PIC values of 0.851 and 0.833, respectively. The HUJII77 locus was the most polymorphic of all the four loci detecting 21 alleles. The mean heterozygosity and PIC values were 0.899 and 0.88, respectively.

The allele frequency measures were used to estimate the Nei's standard genetic distance among the populations using the POPGENE program. The distance measures ranged from 0.388 to 0.224, with the highest value noticed between Salem Black and non-descript goats of Thrissur and the lowest between Malabari and non-descript animals.

A dendrogram was constructed using the POPGENE program which grouped the Salem Black and Attappadi Black goats in one cluster and Malabari and the non-descript goats of Thrissur in another.