GENETIC AND PHENOTYPIC VARIATIONS OF GEOGRAPHICALLY DIFFERENT GOAT POPULATIONS OF KERALA

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Thesis submitted in partial fulfilment of the requirement for the degree of

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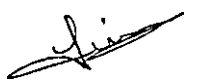
DECLARATION

I hereby declare that this thesis, entitled "**GENETIC AND PHENOTYPIC VARIATIONS OF GEOGRAPHICALLY DIFFERENT GOAT POPULATIONS OF KERALA**" 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.



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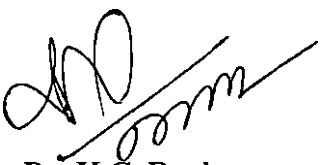
CERTIFICATE

Certified that the thesis entitled **"GENETIC AND PHENOTYPIC VARIATIONS OF GEOGRAPHICALLY DIFFERENT GOAT POPUALTIONS OF KERALA"** is a record of research work done independently by Dr. Jimcy Joseph under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship, other similar title to her of any other University or society.



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EXTERNAL EXAMINER

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Introduction

1. INTRODUCTION

Goats (*Capra hircus*) play an important role in modern agriculture. They are a major source of much needed protein through meat and milk. As they possess many heritable traits that are of economic importance, they are a source of livelihood for the millions of rural poor in the country.

India is a rich repository of goat germplasm being home to 20 recognized breeds of goat with a total population of 120.10 million (Livestock Census, 2003). Indian goat breeds exhibit enormous variations in growth, fecundity, production of meat, milk and fiber, disease resistance and heat tolerance. Goat production has witnessed excellent growth over the years despite a negative campaign against it for its perceived adverse impact on vegetation, forest and gazing lands. However, goat population in Kerala has been showing a declining trend after 1996, mainly due to indiscriminate slaughter, shrinking of grazing lands and urbanization. Of late goat production programmes are getting a momentum, possibly because of the efforts of local self governments. The present goat population in Kerala is 12.13 lakhs (Livestock Census, 2003), which constitutes 1.01 percentage of the total goat population in India.

Goat production in Kerala is mainly dependent on its native breed: Malabari or Tellichery, which is supposed to have originated centuries ago by mixing of native feral goats with Arab, Surti and Mesopotamian goats along with the native goats of Western Coast (Kaura, 1952). The breed is highly variable in both physical and biometric characters, owing to its origin. It is well known for high prolificacy, milk yield and adaptability to the hot humid conditions prevalent in the state.

However, not much information is available on the genetic and phenotypic variability of local goat populations in other parts of the state, since most of the studies have concentrated on Malabari goats in Northern Kerala. Although these local populations appeared to provide optimal material for

improvement, because of their rusticity and their phenotypic and assumed genetic variability, no concerted efforts have been made to study and describe these local populations despite their roles in rural people's livelihood. Selective breeding utilizing this variability within a population to upgrade that population or other populations is less often a part of our breeding programs, which could be attributed in part to the failure of agricultural development policies in Kerala. Since the genetic resources required for the future are difficult to predict, studying the genetic status of these local goat populations can make a major contribution to an effective long-term management of the species.

Important advances to some of the economically important characters in several species of livestock have been achieved based on phenotypic performance. However, the main problem with monitoring quantitative variation is that, the results may be complicated by variation from environment and non-additive genetic sources (Falconer and Mackay, 1996). To determine ecological correlates of fitness within populations and to determine the extent of divergence among populations, one often needs access to independently segregating genetic markers that do not influence the organism's phenotype (Parker *et al.*, 1998)). The hope of obtaining highly informative genetic markers for tracking individuals or their genes under field conditions has led many population geneticists to consider switching to DNA based techniques. One of the recent DNA markers is microsatellites. The analysis of microsatellites is currently regarded as the most useful technique in the study of genetic diversity of closely related populations. The usefulness of microsatellites in the study of genetic variation within and among livestock breeds has been documented by numerous studies.

Although the establishments of evolutionary significant units and management units for conservation have been basically defined in terms of phylogenetic distances and molecular markers, more emphasis has been recently given to a combination of phenotypic and genetic criteria. It is expected that a

comparison between molecular and quantitative estimates of genetic variation can shed light on the selective forces acting on populations (Toro and Caballero, 2005).

In view of this and also because of the slow disappearance of local animal genetic resources a study was undertaken with the following objectives:

1. To study the diversity of goat populations associated with different geographical areas of Kerala, based on qualitative and quantitative differences.

2. To evaluate the genetic basis for the phenotypic variability using microsatellite markers.

Review of Literature

2. REVIEW OF LITERATURE

Local goat populations have a very valuable genetic potential for sustainable agriculture, as they represent the consequence of local adaptation process. Unlike commercial breeds strongly selected for production purposes, local breeds evolved mostly as a result of natural selection for centuries, becoming a very interesting source of genetic variation. Most of the indigenous breeds are well adapted to harsh climates, long migration, poor nutrition and scarce water resources. Acharya (1982) described the important Indian breeds of goats primarily in terms of their home tract, population size, flock size, adult body size (body weight, length, height at withers and chest girth), physical conformation and their performance in terms of body weights (3 and 12 months), lactation performance and reproductive performance.

No published literature on local goat populations is available and the available literature on Indian breeds of goats and most relevant literature on other species are reviewed under the following heads.

1 Physical characters

2. Biometrical characters

3. Disease incidence and kid mortality

4. Microsatellite markers

2.1 PHYSICAL CHARACTERS

In India, a population of goats in a given locality, with characters distinct from the other populations in the vicinity and with a distinct local name has usually been considered as a breed. There is only one recognized breed of goat in Kerala: "Malabari", a dual-purpose goat of Northern Kerala. They are medium sized animals. Coat colour varies widely from completely white to completely

black. Thirty one percentage of the goats have long hair. Males and a small percentage of females (13%) are bearded. Both sexes have small slightly twisted horns directed outward and upward. Ears are medium sized, directed outward and downward. (Acharya, 1982). Only 12 percentage of animals have tassels (AICRP Research Report, 2005).

2.2. BIOMETRICAL CHARACTERS

2.2.1 Growth Characters

2.2.1.1 Body Measurements

Acharya (1982) reported that the height at withers, body length and heart girth of adult Malabari goats were 71.9 ± 2.1, 70.2 ± 1.6 and 73.8 ± 2.2 centimeters in males and 63.2 ± 0.3, 63.5 ± 0.5 and 67.4 ± 0.5 centimeters in females, respectively.

Menon (1994) observed that the mean body length (cm), chest girth (cm) and height at withers (cm) of female Malabari goats at 12 months were 58.36 ± 0.72, 59.07 ± 0.90 and 58.36 ± 0.72, respectively.

2.2.1.1.1 Prediction of Body Weight from Body Measurements

The assessment of powers of body measurements on the estimation of weight and the accuracies of body weights in the estimation of size among livestock species has been widely reported.

Mukherjee *et al.* (1982) compared the relationship between weight and body measurements in Grey and Brown Bengal goats of three agro climatic zones (Gangetic plain, Plateau and Sub- plateau). The result obtained indicated that in general, correlation of body weight with length and height was higher and body weight- heart girth associations lower in Brown Bengal goats than in Grey Bengals.

Menon (1994) reported that among phenotypic measurements the highest phenotypic correlations were observed between the body weight and heart girth in Malabari and Alpine x Malabari genetic groups.

Sharma and Das (1995) observed that during post weaning period, the coefficient of determination (R^2) was higher for prediction equation based on heart girth as compared to withers height or length at all ages in Jamunapari goats. However, heart girth in combination with withers height in the prediction equation gave R^2 value, which was quite comparable to that of equation based on all three measurements.

Mohammed and Amin (1996) suggested that the body weight of Sahel goats could be estimated in the field using morphometric measurements taken with a tape. They obtained a good correlation (r = 0.98) between body weight and heart girth in growing goats. For adult goats correlation between heart girth and weight was 0.96 for bucks, 0.83 for non-pregnant does and 0.71 for does in advanced pregnancy. Based on these observations an equation was derived for predicting body weight from measurements of heart girth for each category.

Varade *et al.* (1997) observed that the body weight is significantly and positively correlated with heart girth and body length and the R^2 value indicates that the body weight of doe could be predicted from heart girth with at least 73% reliability. A multiple regression equation including heart girth, abdominal girth and body length was obtained with low predictability. It was concluded that prediction of body weight from heart girth with the help of regression equation was of practical utility in field conditions in the instances of non-availability of weighing scales or weigh bridges in rural villages.

Awuah *et al.* (2000) proposed regression equations for predicting body weight of sheep by using linear body measurements. They obtained a coefficient

of determination (R^2) of about 0.76 for the model incorporating heart girth and body length as well as sex as separate independent variables.

Chitra (2002) derived linear regression equations for the does from known body weights with corresponding body measurements like body length, height and heart girth from 52 does. The prediction equation based on heart girth ($Y= -13.4480+ 0.5663X_3$) was found to be the most simple and effective with a coeffcient of correlation of 0.842.

Francis *et al.* (2002) developed a model that could be used to estimate body weight of cattle using linear body measurements. They found that the body weight was highly correlated ($r \geq 0.90$) with body length, heart girth and height at withers but particularly so with heart girth ($r = 0.96$). It was implied that heart girth alone could be used to develop a reliable prediction equation for body weight.

Nsoso (2003) opined that the prediction equations of live weight based on heart girth were good during the wet and dry seasons under semi- intensive management, but were very poor during the dry season under extensive management.

Thiruvenkadan (2005) reported that correlation coefficients between body weights and body measurements at different ages were positive and strongly correlated in Kanni Adu goats. The chest girth accounted maximum of 80.4 to 93.6 percentage of total variation in body weight. The coefficient of determination was highest (89.1% in males and 93.6% in females) in a regression model constructed using pooled data, within sexes from 0-12 months of age compared to equations constructed at different age groups.

Khan *et al.* (2006) obtained a high and significant correlation coefficient between height at withers and heart girth and body weight at 4- 18 months of age in Beetal goats. It was suggested that either of these variables or their

combination would provide a good estimate for predicting live body weight in Beetal goats at an early age.

2.2.1.2 Body Weight

Mukundan (1980) reported the body weight of Malabari goats at first kidding as 20.59 ± 1.32 Kg.

Raghavan (1980) observed a body weight of 15.24 ± 0.49 kg at one year of age in Malabari goats.

Acharya (1982) reported average adult body weight of Malabari goat as 38.96 ± 2.32 kg in males and 31.12 ± 0.9 kg in female animals. The body weight and measurements of various Indian goat breeds are given in Table 2.1

Mathew (1991) reported that the body weight of Malabari goats at 12 months of age as 16.8 ± 0.42 kg.

Menon (1994) observed no significant difference in the body weight at 12 months between female Malabari goats and its Alpine crosses.

Bindu (2006) reported that the body weight at first year of age in goat populations of Tanur, Thalassery and Badagara regions of Northern Kerala were 18.37 ± 2.07, 27.00 ± 1.75 and 25.58 ± 0.76 kg, respectively. The goats of Thalassery and Badagara were found to be heavier when compared to Tanur.

Table. 2.1 Body weight and measurements of adult female goats in India

Breed	Body weight (Kg)	Body length (cm)	Height at withers (cm)	Chest girth(cm)
Sirohi	22.54 ± 0.17	61.3 ± 0.2	68.4 ± 0.2	62.4 ± 0.2
Marwari	25.85 ± 0.29	63.51± 0.44	69.29 ± 0.22	68.60 ± 0.26
Beetal	34.97 ± 0.52	70.42 ± 0.88	77.13 ± 0.46	73.70 ± 0.70

Jhakrana	44.48 ± 0.52	77.74 ± 0.36	79.12 ± 0.29	79.13 ± 0.31
Barbari	22.56 ± 0.32	58.68 ± 0.38	56.18 ± 0.37	64.31 ± 0.40
Jamnapari	38.03 ± 0.63	75.15 ± 0.46	75.20 ± 0.38	76.11 ± 0.38
Mehsana	32.39 ± 0.38	68.0 ± 0.3	74.3 ± 0.2	73.0 ± 0.3
Gohilwadi	36.03 ± 0.38	72.4 ± 0.3	79.5 ± 0.3	75.2 ± 0.2
Zalawadi	32.99 ± 0.32	71.8 ± 0.3	78.5 ± 0.2	74.2 ± 2.3
Kutchi	39.29 ± 0.40	75.0 ± 0.3	82.4 ± 0.3	76.1± 0.2
Surti	32.03 ± 1.31	66.6 ± 0.85	70.1 ± 0.92	71.8 ± 1.02
Sangamneri	28.97 ± 0.49	62.5 ± 0.06	68.0 ± 0.12	71.0 ± 0.30
Malabari	31.12 ± 0.9	63.5 ± 0.5	63.2 ± 0.3	67.4 ± 0.5
Osmanabadi	32.36 ± 0.55	67.51 ± 0.38	74.79 ± 0.30	72.04 ± 0.40
Kaanaiadu	28.62 ± 0.37	67.30 ± 0.38	76.15 ± 0.46	70.83 ± 0.44
Ganjam	31.87 ± 0.37	67.6 ± 0.3	77.1± 0.3	74.6 ± 0.3
Black Bengal	20.38 ± 0.16	51.2 ± 0.16	55.4 ± 0.18	63.2 ± 0.16
Gaddi	24.72 ± 0.51	65.2 ± 1.18	58.1 ± 1.02	69.3 ± 0.48
Changthagi	19.75± 0.15	52.4 ± 0.23	51.6 ± 0.20	65.2 ± 0.29
Chigu	25.71 ± 0.33	69.3 ± 0.47	60.0 ± 0.96	73.7 ± 0.53

Source: Acharya (1982)

2.2.2 Production Characters

Mukundan (1980) reported mean milk yield and lactation length of Malabari to be 49.4 kg and 139.5 days, respectively.

According to Acharya (1982) the average lactation yield and lactation length of Malabari were 43.78 ± 2.55 Kg and 143.5 ± 8.11 days, respectively. Lactation milk yields of various goat breeds In India are given in Table 2.2

Stephen *et al.* (2005) observed that the average daily milk yield of Attapadi black goats was 170 ml in an average lactation period of 100days.

Table 2.2 Average daily milk yield recorded for various Indian goat breeds

Breed	Milk yield (in Kg)
Marwari	0.713 ± 0.047
Jhakrana	0.988 ± 0.06
Barbari	0.760 ± 0.158
Jamnapari	2.15 ± 0.30
Mehsana	1.323 ± 0.134
Gohilwadi	1.710 ± 0.145
Zalawadi	2.02 ± 0.18
Kutchi	1.84 ± 0.15
Sangamneri	0.819 ± 0.353
Osmanabadi	0.5- 1.5
Ganjam	0.319±0.02

Source: Acharya (1982)

2.2.3. Prolificacy

Shanmughasundaram (1957) recorded 58.5 percentage twinning and 42 percentage single births out of 103 kiddings in Malabari goats.

Mathew (1991) recorded a higher percentage of singles (68%) in Malabari goats followed by twins (29.5%) and triplets (2.5%).

Raghavan e*t al.* (2004) reported that under field conditions the percentage of singles, twins, triplets and quadruplets were 32.15, 53.14, 12.86 and 1.45 respectively in Malabari goats.

Stephen *et al.* (2005) reported that the overall average of litter sizes from first parity to sixth parity was 1.3 ± 0.02 in Attapadi Black goats. The incidence of singles was greater (73%) and the rate of twinning was 26 percentage. The incidence of triplets (0.8%) and quadruplets (0.1%) was low.

Bindu (2006) observed a higher percentage of twin births in Malabari goat populations, which was 58.95, 54.46 and 53.37, respectively in Tanur, Thalassery and Badagara regions of Northern Kerala.

Seena (2006) reported that the percentage of birth of singles, twins, triplets and quadruplets were 38.33, 51.67, 9.17 and 0.83, respectively in Malabari goats.

Prolificacy percentages of various goat breeds in India are presented in Table 2.3

Table 2.3 Prolificacy (in percentage) of various goat breeds in India

Breed	Singles	Twins	Triplets	Quadruplets
Sirohi	91.5	8.5	-	-
Beetal	40.66	52.6	6.52	0.22
Jhakrana	57.6	41.0	2.0	-
Barbari	49.64	49.32	1.04	-
Jamnapari	56.2	41.1	0.7	-
Mehsana	89.7	10.3	-	-
Gohilwadi	84.2	15.8	-	-
Zalawadi	82.1	17.9	-	-
Kutchi	84.1	15.2	0.7	-
Sangamneri	69.5	30.0	0.5	-
Malabari	50.5	42.4	6.6	0.5
Osmanabadi	70.5	29.0	0.5	-
Kaanaiadu	90	10	-	-
Ganjam	98.4	1.6	-	-
Black Bengal	22.4	54	20.9	2.7
Gaddi	85	15	-	-
Chigu	99.2	0.8	-	-

Source: Acharya (1982)

2.2.4. Discriminant Analysis Based on Biometrical Characters

Mathew (1991) compared economic traits of Malabari goats and its crosses with Alpine and Saanen breeds. Multivariate analysis of variance made by taking first lactation milk yield and length, age at first kidding and body weight at 12 months revealed that the genetic groups were significantly different. The maximum contribution to the total divergence was by first lactation length (33.79%), followed by first lactation milk yield (30.34%), weight at 12 months (29.84%) and the least was by age at first kidding (6.03%). The highest Mahalanobis D^2 value was estimated between Malabari and Saanen (50 %) X Alpine (25%) X Malabari (25%) crosses.

Capote *et al.* (1998) studied the variability of the Canary goat population by statistical analysis of 12 continuous variables (body length, breast width, breast height, height at end of the neck, height to the rump, head width, distance between the eyes, head length, hock length, rump width, thoracic circumference and shank circumference) and two qualitative variables (horn type and head profile). The analysis of variance, χ^2 independence test and canonical discriminant analysis revealed the existence of three types (Tinerfeno, Majorero and Palmero) and two subtypes within the Tinerfeno type.

Lanari *et al.* (2003) carried out phenotypic characterization of exterior traits in local Criollo goat population in Argentina. Correspodence analyses for qualitative and discriminant analyses for quantitative traits were performed using hair types as classification variable, and results were consistent in differentiating the four considered sub-areas.

Leotta (2004) performed linear discriminant analysis for 14 milk production traits to characterize individuals of three different cattle breeds in Italy (Italian Friesian, German Friesian and Jersey). The resulting classification of subjects was satisfactory: 79% of Italian Friesians, 73% of German Friesians and 100 % of the Jersey cows were classified correctly. The most important variables

were the percentage of fat and total solids for the first canonical variate and solids not fat, lactose and protein for the second.

Tabbaa *et al.* (2005) applied different discriminant analysis methods (simple, cluster, canonical, stepwise) on 20 metrical variables to discriminate different Jordanian goat breeds. Results identified four genetic groups: Damascus, Mountain, Dhaiwi and Desert in addition to a population of crossbred goats. The canonical discriminant analysis and the stepwise discriminant analysis revealed that nose shape was the most discriminating variable among different pair-wise breeds' comparisons, followed by withers height (WH) then body weight, ear type, color and teat placement. Chest width (CW), withers depth (WD), rump width showed small discriminatory power.

2.3. DISEASE INCIDENCE AND KID MORTALITY

Rajan *et al.* (1976) reported that percentages of mortality in Malabari during the first and second three months of their age were 44.3.

Acharya (1982) reported a mortality percentage of 32.8 in Malabari kids.

Pillai (1988) reported that 57.69 percentage of goat cases presented to field veterinary hospitals in Trichur district during the period 1982-1986 comprised of digestive disorders. It was shown that primary digestive disorders and acidosis were higher in summer followed by rainy and winter season.

Mathew (1991) analyzed the kid mortality rates in Malabari and its Alpine X Saanen crossbreds. The study revealed that highest mortality occurred within three months of age (26.5%), while the overall mortality rates were less than five percentage in other age groups (3-6 and 6-12 months). Period of birth and season influenced the mortality rate significantly.

Thoppil (1996) recorded the major health disorders encountered in adult goats of Malappuram and Palakkad districts. It was found that digestive disorders were more common (30%) followed by respiratory disorders (18%) under field conditions.

Maliekal (2000) reported that 1.12 % of the goat cases presented to the University hospital, Mannuthy, exhibited symptoms suggestive of polioencephalomalacia. The carbohydrate rich diet was found to be one of the predisposing causes for the development of polioencephalomalacia of goats in Kerala.

Sreeja (2005) reported that the occurrence of sub clinical mastitis based on California Mastitis Test in crossbred does from University Goat and Sheep farm was 30.2 percentage.

2.4. MICROSATELLITE MARKERS

Microsatellites are tandemly repeated sequences of 1-6 bp. The hyper variability of this simple sequence stretches were due to slippage events occurring during DNA replication and these are flanked by unique DNA sequences enabling the amplification using PCR. The degree of hyper variability is such that the length polymorphism within these regions may be exploited for relationship studies of individuals within and between populations and that they may serve as a general source of polymorphic DNA markers for genome mapping and linkage studies (Tautz, 1989).

The microsatellites evolve with three mutation models. Among the microsatellites the ones with 3-5 bp repeats evolve predominantly under the single step wise mutation model (SMM) (Shriver *et al.*, 1993) and therefore are less suitable than microsatellites with shorter repeats for which the mutation model usually includes multiple step mutation events (Di-Rienzo *et al.*, 1994) and hence closer to infinite allele model (IAM). The interrupted microsatellites

(Estoup *et al.*, 1995a) or compound microsatellites with motifs of different lengths (Estoup *et al.*, 1995b) are likely to fit the IAM better than pure repeat microsatellites.

Estoup *et al.* (1994) observed that microsatellites permit a highly precise dissection of the genetic structure of populations. They were easy to isolate and the PCR based typing of alleles could be readily automated.

There is high level of similarity between cattle, sheep and goat chromosomes and the bovine and ovine microsatellites are likely to detect polymorphisms in goats (Crawford *et al.*, 1995; Vaiman *et al.*, 1996).

Saitbekova *et al.* (1999) studied genetic diversity in eight Swiss goat breeds using 20 bovine microsatellites on 20 to 40 unrelated animals per breed and concluded that microsatellites were powerful tools to differentiate between goat breeds. From the comparison of microsatellite analysis with breeding histories, they opined that microsatellites gave the correct answer, regardless of whether the breeds were closely related or not.

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

2.4.1 Microsatellite Analysis

2.4.1.1 Isolation of Genomic DNA

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

Jeanpierre (1987) developed a simple method of DNA purification from blood involving the use of proteinase K which works efficiently in guanidium hydrochloride solutions followed by ethanol precipitation of DNA.

Mammalian DNA is usually isolated by the protocol given by Sambrook *et al.* (1989), which involved digestion of cells with proteinase K in the presence of EDTA and a detergent such as SDS, followed by extraction with phenol.

To overcome the obstacles involved in the use of hazardous organic solvents like phenol and chloroform, Miller *et al.* (1988) devised a salting out procedure for extraction of DNA from nucleated cells of the buffy coat obtained from anti- coagulated blood.

Laird *et al.* (1991) simplified the standard mammalian DNA isolation procedure in which the lysis buffer was adjusted to allow restriction digestion without organic solvent extractions.

Parzer and Mannhalter (1991) developed a DNA isolation method that allows the isolation of high quality DNA from frozen citrated blood, buffy coats and cell lines within 90 minutes avoiding the use of toxic agents.

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 yield good amount of high molecular weight DNA from cattle white blood cells whereas the guanidine hydrochloride method failed to yield pure DNA.

2.4.1.2 Polymerase Chain Reaction

The polymerase chain reaction (PCR) was developed by Mullis *et al.* (1986). This technique involves enzymatic amplification of a specific sequence of DNA using two oligonucleotide primers that flank DNA segment to be amplified resulting in many fold increase in amount of target sequence.

According to Saiki *et al.* (1988) a thermostable DNA polymerase isolated from *Thermus aquaticus* enable *in vitro* DNA amplification to be performed at

higher temperatures and significantly improves the specificity, yield, sensitivity and the length of the product amplified.

Weber and May (1989) found that application of polymerase chain reaction to type polymorphic DNA markers such as the (CA)n block markers consumed less DNA and was faster than standard blotting and hybridization. Ten nanograms of genomic DNA was sufficient for routine genotyping of the (CA)n block markers.

Lowe (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 hybridisation.

Holland (1991) utilized the 5'-3' exonuclease activity of the thermo stable enzyme *Thermus aquaticus* DNA polymerase in a polymerase chain reaction product detection system to generate a specific detectable signal concommitantly with amplification.

2.4.1.3 Sequencing

Maxam and Gilbert (1977) described reactions that cleave DNA preferentially at guanines, adenines, cytosines and thymines equally and at cytosines alone. When the products of these four reactions are resolved by size, by electrophoresis in a polyacrylamide gel, the DNA sequenced can be read from the pattern of radioactive bands. This technique will permit sequencing of at least 100 bases from the point of labeling.

Sanger *et al.* (1977) described a method for determining nucleotide sequences in DNA in which 2', 3' -dideoxy and arabinonucleoside analogues of the normal deoxynucleoside triphosphates, acted as specific chain terminating inhibitors of DNA polymerase. One of the nucleotides was radiolabelled so that

the mixture fractionated by electrophoresis could be subjected to autoradiography from which the required sequence could be read.

Biggin *et al.* (1983) showed that the use of deoxyadenosine 5'-α-(^{35}S)(thio) triophosphate as the label incorporated in dideoxynucleotide sequence reactions increased the sharpness of the bands on an autoradiograph and so increased the resolution achieved. But the basic disadvantage presented by ^{35}S is the longer exposure time required.

Innis *et al.* (1988) presented sequencing protocols that produce readable extension products of more than 1000 bases having uniform band intensities. A combination of high reaction temperatures and the base analog 7- deaza-2'-deoxyguanosine was used to sequence through G+C rich DNA and to resolve gel compressions. Polymerase chain reaction (PCR) conditions were modified for direct DNA sequencing of asymmetric PCR products, without intermediate purification by using *Taq* DNA polymerase.

Litt and Luty (1989) analysed primer preparations by electrophoresis in a standard sequencing gel after 5' end labeling with polynucleotide kinase and (γ ^{32} P) ATP.

Dideoxy sequencing ladders produced using M13, MP10 DNA as template was used as gel size standards by Weber and May (1989).

Bishop *et al.* (1994) used M13 and MP18 SSDNA sequencing ladders for comparison and computation of the approximate allele size of the amplified PCR products.

2.4.1.4. Microsatellite Markers

2.4.1.4.1. HUJ 1177

Van Hooft *et al.* (1999) detected five alleles with a size range of 190-212bp at the HUJ 1177 locus while testing the applicability of bovine microsatellite markers for population genetic studies in African buffalo.

Vallejo *et al.* (2003) used the bovine marker HUJ 1177 for assessing the genetic diversity and background linkage disequilibrium in North American Holstein cattle population and reported it to be polymorphic with six alleles.

Jacob (2005) detected 21 alleles at this locus with a size range of 189-229bp in four goat populations.

Thomas (2005) detected 13 alleles with a size range of 193-221 bp and 36 genotypes at this locus in crossbred cattle of Kerala.

Uthaman (2005) detected 14 alleles ranging in size from 193-225 bp at this locus in Vechur cattle. The allele 223 bp was found to be associated with a significantly higher milk fat percentage.

Bindu (2006) detected 19 alleles at this locus, with a size range of 188 to 230bp in Malabari goats. Heterozygosity values ranged from 0.660 to 0.909.

2.4.1.4.2 ILSTS 030

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

Mukesh *et al.* (2004) reported 4 alleles at this locus within a size range of 158 to 184 bp in Sahiwal cattle.

Jacob (2005) observed 17 alleles for the microsatellite locus ILSTS 030 with a size range of 146-178bp in four goat populations (Malabari, Attapadi black, Salem black and non-descript goats of Thrissur).

Tantia *et al.* (2006) used the microsatellite marker ILSTS 030 to study the population structure in three buffalo populations of India by multilocus genotyping. They reported an annealing temperature of 56°C for this marker and the allele size ranged from 155-167 bp. The number of alleles detected was five.

Bindu (2006) detected 13 alleles with a size range of 156 – 184 bp at this locus in Malabari goats.

2.4.1.4.3 INRA 063

Vaiman *et al.* (1994) assigned this dinucleotide microsatellite marker, INRA 063 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.

Saitbekova *et al.* (1999) observed seven alleles at INRA 063 locus with a size range of 154 to 168 bp in Swiss goat breeds.

Chenyambuga *et al.* (2004) reported ten alleles with a size range of 141 to 179 bp in goats of Sub-Saharan Africa. They reported an annealing temperature of 55 °C for this marker.

Marinez *et al.* (2004) detected five alleles at this locus with a PIC value of 0.48 in Andalusian goats.

Mukesh *et al.* (2004) observed three alleles with a size range of 170-188 bp at this locus, while microsatellite genotyping in Sahiwal cattle.

Jacob (2005) detected 11 alleles for the microsatellite locus INRA 063 with a size range of 152-172 bp in four goat populations(Salem Black, Attapadi Black, Malabari and Non-descript goats of Thrissur district).

Araujo *et al.* (2006) detected seven alleles at this locus with a size range of 162-206 bp, while assessing the genetic diversity among imported Alpine and Saanen dairy goats and naturalized Brazilian Moxoto breed. This locus showed a higher within population Fixation Index (F_{IS}) than other loci, indicating allele fixation.

Bindu (2006) recorded ten alleles with a size range of 155- 173 bp at this locus in Malabari goats.

2.4.1.4.4 TGLA 68

Chu *et al.* (2003) observed two alleles for the microsatellite marker TGLA 68 with a size range of 98 and 100 bp in Small Tail Han sheep. The heterozygosity observed was 0.497.

Seena (2006) observed a total of eight alleles with a size range of 98-114 bp for this marker in Malabari goats. The heterozygosity and PIC value obtained were 0.8024 and 0.7823, respectively. The allele 104 bp and the genotype 104/106bp were associated with significantly higher litter size in Malabari goats.

2.4.1.4.5 BM 1329

Bishop *et al.* (1994) used the microsatellite marker BM 1329 for constructing the genetic linkage map of cattle. They reported an annealing temperature of 58°C and allele size ranged from 145- 161bp. The number of alleles detected was eight.

Chu *et al.* (2003) observed a total of six alleles for the microsatellite marker BM 1329 with a size range of 160-180 bp in Small Tail Han sheep. The heterozygosity observed was 0.516.

Martinez *et al.* (2004) used the microsatellite marker BM 1329 for genetic characterization of Blanca Andaluza goat. Nine alleles were detected at this locus with a PIC value of 0.80.

Seena (2006) reported 15 alleles ranging in size from 167-195bp for this marker in Malabari goats. The heterozygosity observed for this marker was 0.8660 and the PIC value calculated was 0.8526.

2.5. STATISTICAL ANALYSIS

2.5.1 Heterozygosity

Heterozygosity is the most widely used parameter to measure diversity within populations, defined by Nei (1973) as the probability that two alleles chosen at random from the population are different.

Nei (1978) evaluated the magnitude of systemic 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;

$$H= 2n\ (1-\Sigma\ x_l^2)/2n-1$$

Takezaki and Nei (1994) opined that for the markers to be useful for measuring genetic variation, the average heterozygosity should be between 0.3 and 0.8 in the population.

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;

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

2.5.2 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;

$$PIC = 1 - [\sum_{i=1}^{k} P_I^2] - \sum_{i=1}^{k-1} \sum_{j=I+1}^{k} 2 P_I^2 P_j^2$$

2.5.3 Genetic Distance and Phylogenetic Reconstruction

Genetic distances estimated from polymorphic microsatellite markers have been the most popular method of choice to assess genetic diversity among populations. Genetic distance measures the evolutionary divergence of two populations.

According to Nei *et al.* (1983) the efficiency of constructing phylogenetic trees by means of genetic distances depends on the linear relationship with time and the sampling error of the distance measures used.

Takezaki and Nei (1996) observed that in both the infinite allele model and step wise mutation model, Cavalli- Sforza and Edward's chord distance (Dc) and D_A distance of Nei *et al.* generally show greater accuracy than other distance measures, whether the bottle neck effect exists or not. For estimating evolutionary time however, Nei's standard distance and $(\delta\mu)^2$ of Goldstein *et al.*'s are more appropriate than other distances.

Barker (1999) argued that phylogenetic diversity based on microsatellite loci provides the best objective criterion for making conservation decisions.

Notter (1999) recommended the importance of global programme to determine genetic distances among livestock breeds and to establish core collection of diversity within each species to ensure full range of genetic variation within species.

Yang *et al.* (1999) analyzed microsatellite variation in five Chinese indigenous goat breeds, which included four Cashmere breeds (Tibetan, Neimonggol, Liaoning, Taihang) and one Hubei local breed (Matou). In the neighbor-joining tree constructed using Nei's standard genetic distance (1978) Neimonggol and Liaoning were grouped together, then with Taihang; while Tibetan and Matou individually had their own branch. The genetic relationship of five breeds corresponded to their history and geographic origins.

The behaviour of different measures of genetic distances in the livestock context has been reviewed by Laval *et al.* (2002). They concluded that all distances strongly depend on the number of generations since the divergence and on the effective population size of the breeds and, therefore no phylogeny can be inferred from the tree in case of closely related breeds exhibiting different effective sizes. For this reason, it is generally assumed that in dealing with breeds of farm animals, the interpretation of trees in terms of phylogeny can be misleading.

Li *et al.* (2002) evaluated the genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. A phylogenetic tree based on the Nei's (1978) standard genetic distance displayed a remarkable degree of consistency with their different geographical origins and their presumed migration throughout China.

Chenyambuga *et al.* (2004) assessed genetic diversity of Sub- Saharan African goats using 19 microsatellite markers. Nei's D_A genetic distance values ranged from 0.087 to 0.382. It was concluded that the relationships were according to their geographical locations and within each sub- region goat populations could be differentiated according to morphological characteristics.

Kotze *et al.* (2004) measured the genetic relationship between the different populations within the Kalahari Red breed by determining the Nei's genetic distance between the populations. Genetic distance values were small and ranged from 0.072-0.171. A dendrogram based on these genetic distances revealed no specific link with geographic distance.

Tantia *et al.* (2006) genotyped three buffalo populations, viz. Bhadawari, Tarai and local buffaloes of Kerala using 24 heterologus polymorphic microsatellite loci. The three genetic distances, Nei's standard and D_A, and Goldstein, revealed closeness between Bhadawari and Tarai populations which was expected from geographical contiguity, while Kerala buffaloes were distant.

Maudet *et al.* (2002) investigated the genetic variability and relationships among six native French cattle breeds (Abondance, Tarentaise, Villard de Lans, Montbeliarde, Limousin and Charolais) and one foreign breed (Holstein) using 23 microsatellite markers. Neighbor- joining trees and principal component analysis showed that alpine breeds tend to cluster together. Abondance and Tarentaise breeds were closely related, whereas the Holstein was highly differentiated from all breeds analyzed.

Though no reports are available on Kerala's native goat population, the available reports on other native goat populations in India are reviewed above. Based on this it can be suggested that the quantitative assessment of the genetic diversity between and within populations can be mainly done on the basis of variation existing in physical and biometrical traits and genetic distance estimated based on molecular biological markers.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

A random sample of 400 adult female goats, 100 each from four geographically different areas in Kerala, viz. Kozhikode, Thrissur, Kottayam and Trivandrum formed the material for this study. Study sites were Muniyoor, Mantharathoor and Maiyanoor regions in Badagara of Kozhikode district, Thekkumkara and Madakathara panchayats in Thrissur district, Kadaplamatom and Kaduthuruthy panchayats in Kottayam district and Nedumangad, Tholikkode and Vithura panchayats in Trivandrum district. Data were collected by surveying methods using a structured questionnaire, onsite observation and physical measurements of goats. Pregnant animals were excluded in sampling.

3.2 PHYSICAL CHARACTERS

The morphological characters observed were coat colour, presence of horns/ tassels/ beard, ear size, and hair pattern.

3.3. BIOMETRICAL TRAITS

The peak yield and prolificacy of animals, disease incidence and kid mortality during the year 2006-2007 were recorded. The following body measurements were taken using a flexible measuring tape while the animal standing on a leveled surface. The reference points are also given below:

1. Head length: Distance from poll to nostril.

2. Head width: Distance between the outer canthus of right and left eye.

3. Height at withers: Distance from point of withers to toe region.

4. Chest depth: Distance from point of withers to chest floor/point of elbow.

5. Chest girth: Chest circumference / heart girth.

6. Shoulder point width: Distance between the right and left shoulder point.

7. Rump length: Distance from point of ischium to pin bone.

8. Rump width: Distance between the point of ischium.

9. Shin circumference: Canon bone perimeter.

10. Body length: Distance from point of shoulder to pin bone.

3.3.1 Prediction of Body Weight from Body Measurements

Data on chest girths, height at withers, body lengths and body weights of 100 adult female goats were collected from the records of All India Coordinated Research on Malabari goats. A multiple regression analysis was carried out to describe the relationship between the independent variables consisting of body length, chest girth and height at withers of adult females on the one hand and the live weight as the response variable on the other hand. In the present study, chest girth exhibited high and significant ($p<0.001$) phenotypic correlation to body weight (0.817) compared to body length (0.612) and height at withers (0.410) in adult female goats. Prediction equations were obtained using the backward elimination regression procedure of SPSS (Statistical Package for Social Sciences). Linear, logarithmic, inverse, quadratic, cubic, power, growth and exponential functions of chest girth were fitted against body weight by the least squares method. This allowed the possible inclusion of curvilinear functions in the prediction equations. Highest coefficient of determination ($R^2= 0.705$) was obtained for prediction equation based on power function of chest girth.

The prediction equation developed is presented below:

$$Y= A x^{b}$$

Where,

Y= Body weight in kilograms

A = Intercept (0.0416)

x= Independent chest girth measured in centimeters

b =Regression coefficient for the power function of chest girth (1.5301)

This equation was used for predicting the body weight of animals from different geographical areas.

3.4. MICROSATELLITE ANALYSIS

3.4.1 Source of DNA

Blood samples collected from 25 unrelated animals each from four geographically different areas in Kerala, viz. Thrissur, Kottayam, Kozhikode and Trivandrum were used as a source of DNA.

3.4.1.1 Collection of Blood

Blood samples (5ml each) were collected from the jugular vein aseptically using sterile disposable syringes and transferred immediately into sterile disposable centrifuge tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) as anticoagulant (1mg/ml of blood). The samples were brought on ice to the laboratory and stored at 4^0C till further processing.

3.4.1.2 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 followed was

1. To the 5ml blood, double the volume of ice cold RBC lysis buffer (150m*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 red blood cells

2. The leucocytes were pelleted by centrifuging at 3500rpm for 15 minutes, and the supernatant containing lysed RBCs was discarded.

3. The pellets were resuspended in ice-cold RBC lysis buffer and the above two steps were repeated till a clear pellet without any unlysed erythrocytes were obtained.

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

5. Resuspended the washed white cell pellet in 5 ml saline EDTA buffer (SE-75 m*M* NaCl, 35 m*M* EDTA) completely by vortexing so that no cell clumps remain. To this mixture 0.25 ml of 20 percent SDS and 25 µl of proteinase-K (20 mg/ml) were added, mixed well and incubated at 50°C in water bath with occasional mixing for a minimum of three hours.

6. Cooled the digested samples to room temperature, 300 µl of 5 *M* NaCl was added and given gentle mixing. An equal volume of phenol (pH 7.8) saturated with Tris- HCl, was added, mixed by gentle inversion of the tubes for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

7. 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.

8. Collected the aqueous phase in fresh tubes, equal volume of chloroform : isoamyl alcohol (24:1) was added, mixed for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

9. The supernatent was transferred to a sterile 50 ml beaker and one tenth volume of 3 *M* Sodium acetate (pH 5.5) was added and mixed.

10. To this mixture, 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.

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

3.4.2 Polymerase Chain Reaction (PCR)

3.4.2.1 Template DNA

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

3.4.2.2 Selection of Primers

A total of five microsatellite markers, viz. INRA 063, ILSTS 030, HUJ 1177, BM 1329 and TGLA 68 were chosen for the study which exhibited comparatively higher degree of polymorphism, based on earlier studies conducted at Center for Advanced studies in Animal Genetics and Breeding, Mannuthy. The primers for these markers were custom synthesized and typed for their polymorphism. The sequences of the forward and reverse primers for each locus were as follows.

INRA 063

F 5'- ATTTGCACAAGCTAAATCTAACC-3'

R 5'-AAACCACAGAAATGCTTGGAAG-3'

ILSTS 030

F 5'-CTGCAGTTCTGCATATGTGG-3'

R 5'-CTTAGACAACAGGGGTTTGG-3'

HUJ 1177

F 5'-TCCATCAAGTATTTGAGTGCAA-3'

R 5'-ATAGCCCTACCCACTGTTTCTG-3'

BM 1329

F 5'-TTGTTTAGGCAAGTCCAAAGTC-3'

R 5'-AACACCGCAGCTTCATCC-3'

TGLA 68

F 5'-ATCTTACTTACCTTCTCAGAGCT-3'

R 5'-GGGACAAAATTTTACATATACACTT-3'

The primers obtained in lyophilized form were reconstituted in sterile ultra pure 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 primers were prepared by diluting the stock solution.

3.4.2.3 Incorporation of Radioactivity: End-Labelling of Primers

For visualizing the PCR products by autoradiography, one of the primers was radio labeled. 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 Kit (Bangalore Genei).

The procedure for end labeling was as follows:

The following components were added to a 0.2ml-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
Y^{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 up to 40µl with sterile ultra pure water. One microlitre of this end-labeled primer was used for every 10µl PCR assay with sterile distilled water.

3.3.2.4 PCR Conditions

The PCR conditions for each microsatellite loci were standardized separately. Each reaction was carried out in 10µl volume. PCR reaction was set up with 1µl of 10 X PCR buffer (15m*M* $MgCl_2$, 100m*M* Tris-pH 8.3, 500m*M* KCl), 200µ*M* dNTP, 1µl of end-labeled and diluted forward primer, 5p*M* of reverse primer and 0.3U of Taq DNA polymerase. Concentration of $MgCl_2$ used was 1.25m*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 INRA 063, 62°C for ILSTS 030, 58°C for HUJ 1177 and BM 1329, 59°C for TGLA 68, for one minute and extension at 72°C for one minute. This was followed by a final extension for five minutes at 72°C. The samples were then cooled down to 4°C and stored at -20°C till further analysis.

3.4.2.5 Agarose Gel Electrophoresis

The presence of PCR products were checked in 1.5 per cent agarose in 1X TAE buffer in horizontal electrophoresis unit. 2µl of PCR products mixed with 6X gel loading buffer (Bromophenol blue 0.25 per cent, Xylene cyanol 0.25 per cent, sucrose 40 per cent) were loaded on the agarose gel. *HaeIII* digested pBR322 DNA was used as molecular size marker.

3.4.3 Sequencing M13 Bacteriophage DNA

Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (Amersham Biosciences Corporation,USA) for determining the allele size of microsatellite markers by comparison. The procedure followed was

1. Preparation of annealing mixture

The composition of the mixture was as follows:

M13 phage DNA (0.2μg/μl)	-	5μl
5X Sequenase reaction buffer	-	2μl
Primer(5p*M*/μl)	-	1μl
Sterile ultra pure water	-	2μl

The mixture was mixed well. Spun and incubated at 65°C for 2 minutes. Then cooled to room temperature over 15-30 minutes, and kept on ice.

2. Dilution of labeling mix

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

5X Labeling mix	-	0.5μl
Water	-	2.0μl

3. 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

4. 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^{32} PdATP (10µCi/µl) - 2µl

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

5. Four tubes labeled G, A,T and C were filled with 2.5µl of each dideoxy termination mixtures(ddGTP, ddATP, ddTTP and ddCTP).

6. To each termination tubes (G,A,T,C), 3.5µl of labeling reaction mixture was transferred, mixed well and incubated at 37°C for 5 minutes.

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

3.4.4 Denaturing Polyacrylamide Gel Electrophoresis

Denaturing polyacrylamide gel electrophoresis was performed on a Vertical Sequencer (Consort,Belgium) using 6 per cent denaturing polyacrylamide gel (6 per cent urea, 0.5 X TBE). The gels were set between two glass plates (41 x 33 cm) separated by 0.35 mm thick spacers.

3.4.4.1 Casting the Gel

The glass plates were cleaned thoroughly with soap and water and air dried. Traces of grease and oil were removed by repeated wiping with alcohol. The plates were assembled with spacers in between and the sides and bottom sealed with sealing tape. The gel was prepared by mixing 60 ml of 0.5 X TBE gel mix (6per cent acrylamide, 6*M* urea, 0.5 X TBE) and 125µl each of 10 per cent Ammonium persulphate solution and TEMED (N,N,N',N', Tetra Methyl

Ethylene Diamine) 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 30 minutes before electrophoresis. The tapes and clamps were removed, plates were cleaned and assembled in the sequencer. The upper and lower electrode tanks were filled with 1X TBE (pH 8.3) buffer (TBE- 0.045*M* Tris borate, 0.001 *M* EDTA) to the required level. The comb was removed, wells were cleaned with buffer solution and comb was then reinserted in opposite direction with the toothed surface now facing downwards to form sample-loading wells.

3.4.4.2 Loading of Samples

The PCR products were mixed with 3.5µl 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 minutes and cooled immediately on ice. About 3µl each of this mixture was loaded into each well. Sequenced products of M13 DNA, which were also denatured at 94°C for 5 minutes, were loaded simultaneously in the middle or side wells.

3.4.4.3 Electrophoresis

The gels were electrophoresed at 40 W for three hours maintaining a temperature of around 50°C. 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 Cyanol dye had a mobility equivalent to approximately 100 base fragment.

3.4.4.4. 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 gel was covered with klin film and dried in a gel drier connected with the motor pump at 80°C for one and a half hours.

3.4.5 Autoradiography

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

3.4.5.1 Development of X-Ray Film

The X-Ray film was developed in the dark room. Developing was done by transferring the film serially into IX developer solution (Kodak) for three to five minutes, one 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.

3.4.6 Microsatellite Typing

The genotypes of 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.

3.5. STATISTICAL ANALYSES

3.5.1. Biometrical Traits

For analysis of data SPSS statistical package was used. For the mean comparison of each biometrical characteristic for different populations one way analysis of variance was used and the pairwise comparison of means was done by using Duncan Multiple Range Test. Canonical discriminant analysis was

performed for identifying the set of biometrical characters that best discriminate the populations. Hotelling's T^2 value was found using the program MSTATC and the Mahalanobis distance (D^2) between the studied four goat populations was estimated using the formula,

$$D^2 = \frac{N_1 + N_2}{N1N2} \times T^2$$

Cluster analysis of the four goat populations based on biometrical characteristics was performed as per agglomerative hierarchical clustering. (Chatfield and Collins, 1980)

3.5.2. Microsatellite Analysis

3.5.2.1 Heterozygosity

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

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

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

3.5.2.2 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).

3.5.2.3 Genetic Distance

The genetic distance between the four populations were computed using Nei's method (1978). The distance measures were derived using the software POPGENE.

3.5.2.4 Dendrogram

A dendrogam representing the relationships of the animals belonging to the four goat populations was constructed using the POPGENE software.

Results

4. RESULTS

4.1. PHYSICAL CHARACTERS

The morphological characters of the four goat populations are given in Table 4.1. In Trivandrum, 96 percentage of the animals were horned, 94 percentage without tassels and 96 percentage not bearded. Ears were of medium type. Eighty nine percentage of the animals were short haired and 75 percentage white or a combination of white with either black or brown coat colour.

In goats of Kottayam population 60 percentage of animals were horned, 88 percentage without tassels and 80 percentage not bearded. Ears were of medium type. Of these animals 71 percentage was white or a combination of white with either black or brown coat colour. Short haired animals belonged to 77 percentage.

In goat population of Thrissur district, 74 percentage of animals possessed horns. Tassels were absent in 93 percentage and 92 percentage not bearded. Ears were medium sized. Eighty nine percentage of animals were white or a combination of white with either black or brown coat colour. The percentage occurrence of long haired variety (37) was slightly higher than short haired (31).

In the goat populations of Kozhikode district 89 percentage of animals were horned, 86 percentage without tassels, 95 percentage not bearded and 76 percentage short haired. They possessed medium size ears. Animals which were white or a combination of white with either black or brown coat colour belonged to 88 percentage.

4.2. BIOMETRICAL CHARACTERS

4.2.1 Growth Characters

4.2.1.1. Morphometric Measurements

For comparison of each morphometric variable among different populations, one way analysis of variance was done and the results obtained are presented in Table 4.2. In the case of variable head length, it was found that Trivandrum and Kottayam populations formed a homogeneous group, while Thrissur and Kozhikode another group. The populations Kottayam and Kozhikode were alike for the variables body length, rump length and head width, whereas Thrissur and Trivandrum were significantly different from others. The mean values of chest girth and height at withers were significantly different for all the populations. For shin circumference Trivandrum and Kottayam populations formed a homogenous group while Thrissur and Kozhikode were significantly different. For almost all traits, greatest average values corresponded to Trivandrum goat population while the smallest to Thrissur. Animals belonging to Kottayam and Kozhikode were in between.

4.2.1.1.1. Discriminant Analysis Based on Morphometric Traits

Multivariate analysis of variance performed taking ten morphometric variables (head length, head width, height at withers, chest depth, chest girth, shoulder point width, rump length, rump width, shin circumference and body length), revealed that all the four goat populations were significantly different. Hence canonical discriminant analysis was performed using SPSS statistical package. The most discriminant variables selected by stepwise procedure were, head width, height at withers, chest depth, rump length, rump width, shin circumference and body length. Analysis showed that the two first canonical variables represented a cumulative total of 89.9 % of total variation. Variables head width and body length were the most discriminative in canonical

correspondence with the ordinate axis (CAN1) and the shin circumference and rump length with coordinate axis (CAN2).

The Mahalanobis distance (D^2) estimated between the goat populations according to the morphometric variables studied is presented in Table 4.3. Greatest distance (D^2) value was obtained between Thrissur and Trivandrum populations (9.13866), while the Kottayam and Kozhikode populations had the least distance value (3.04962). On doing cluster analysis, it was found that Trivandrum, Kottayam and Kozhikode populations formed a group and Thrissur formed a separate group.

4.2.1.2. Body Weight

Body weights of adult female goats were predicted using the regression equation based on the power function of chest girth and are presented in Table 4.4. At 18 to 24 months, the mean body weights, in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 29.15 ± 0.66, 28.19 ± 0.45, 22.86 ± 0.41 and 26.92 ± 0.56 kg, respectively. At 24 to 36 months, the values for body weight were 29.92 ± 0.52, 29.55 ± 0.62, 25.73 ± 0.82 and 28.02 ± 0.87 kg, respectively. Weight recorded at 36 to 48 months in the respective populations were 32.29 ± 0.63, 30.32 ± 0.79, 26.64 ± 0.56 and 30.25 ± 0.75kg, respectively. The body weights above 48 months, were 32.79 ± 0.64, 30.95 ± 0.69, 29.37 ± 0.72 and 30.70 ± 0.86 kg, respectively.

4.2.2. Peak Yield

For comparison of mean values of peak yield in the four goat populations, one way analysis of variance was done, and it was found that there was significant variation between peak yields among different populations (Table.4.5). Highest mean value was recorded in Kottayam (832.5 ± 35.06ml), followed by Kozhikode (755 ± 38.49ml) and Trivandrum (699.5 ± 40.29ml), while the lowest value was recorded in Thrissur (444 ± 25.41ml). It was

observed that the Malabari goats of Kozhikode district were close to the goat populations in Kottayam and Trivandrum in peak yield.

4.2.3. Prolificacy

Prolificacy percentages of goat populations in Trivandrum, kottayam, Thrissur and Kozhikode are presented in Table 4.6.

Single births in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were found to be 32, 42, 45 and 39 percentage, respectively. The percentage occurrence of twins was higher in Trivandrum (58) when compared to Kottayam (46), Thrissur (48) and Kozhikode (52). The percentage of triplets born in Trivandrum, Kottayam, Thrissur and Kozhikode was found to be 10, 11, 7 and 8, respectively. Only one percentage birth of quadruplets was recorded in Kottayam and Kozhikode populations, while none was observed in Trivandrum and Thrissur populations. Highest percentage of multiple births was recorded in Trivandrum (68) followed by Kozhikode (61), Kottayam (58) and Thrissur (55).

4.2.4. Discriminant Analysis Based on Body Weight, Peak Yield and Prolificacy

Multivariate analysis of variance performed taking body weight, peak yield and prolificacy revealed that all the four goat populations were significantly different. Hence discriminant function analysis was carried out with these three variables using the statistical package SPSS. It was found that variables body weight and peak yield have significant discriminative capacity. Body weight accounted a maximum 84.6 percent of total variation with a canonical correlation value of 0.508, while peak yield contributed 15.3 per cent of total variation with a canonical correlation value of 0.243.

The Mahalanobis distance estimated between the four goat populations according to the variables studied is presented in Table 4.7. Goat populations of

Thrissur district were more distant from all other populations. Greatest distance value (D^2) was obtained between Thrissur and Kottayam goat populations (2.30842), while the least distance recorded between Kottayam and Kozhikode populations (0.19238). D^2 value estimated between Thrissur and Trivandrum population was 2.10488, between Thrissur and Kozhikode populations, 1.18574, between Kottayam and Trivandrum populations, 0.37338, and that between Trivandrum and Kozhikode was found to be 0.58476. Further on doing cluster analysis it was found that Trivandrum, Kottayam and Kozhikode formed a cluster and Thrissur a different cluster.

4.3. DISEASE INCIDENCE AND KID MORTALITY

The study revealed the occurrence of respiratory infections, deficiency diseases, mastitis, fungal infections and digestive disorders in the goat populations investigated. Percentage of adult female goats reported to have the occurrence of any of these disease conditions during the period 2006-2007 in Trivandrum, Kottayam, Thrissur and Kozhikode goat population was 11, 21, 14 and 8, respectively (Table 4.8). Vaccinations were done only against foot and mouth disease. Percentages of vaccinated animals in respective populations were 45, 34, 33 and 26, respectively and dewormed adults in the respective populations were 25, 78, 33 and 27 %, respectively during the same period.

Kid mortality percentages during pre weaning stage in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 4.49, 6.43, 15.43 and 2.92, respectively.

4.4. MICROSATELLITE ANALYSIS

4.4.1 Isolation of Genomic DNA

A total of 100 DNA samples were isolated from whole blood, 25 each from the four geographically different goat populations of Thrissur, Kottayam, Trivandrum and Kozhikode districts of Kerala.

4.4.2 PCR Analysis

Microsatellite primers were selected from published literature, custom synthesized and used for PCR amplification of the samples. Five markers, which were found to be highly polymorphic in goats, were chosen based on earlier studies conducted at Centre for Advanced studies in Animal Genetics and Breeding, Mannuthy. These were the bovine dinucleotide repeat microsatellites HUJ 1177, ILSTS 030, INRA 063, TGLA 68 and BM 1329. PCR amplification was performed on all the 100 samples using the primer pairs for the five selected loci.

4.4.2.1. Optimization of PCR

The conditions of PCR, temperature and time of cycles were optimized separately for each primer for the efficient amplification. The reaction conditions and cycle parameters for each primer pair is presented in Tables 4.9 and 4.10. The PCR products were checked for amplification on 1.5% agarose gels using *Hae*III digested pBR322 as the size standard. Amplified products of the expected size range were observed for all selected loci.

4.4.3. 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 visualized

by autoradiography. Direct counting method was employed for detecting the number of alleles at each locus. The allele sizes were checked by comparing with sequence of M13 mp18 single strand DNA, which was run along with the samples.

4.4.3.1 HUJ 1177

The highest polymorphism was observed at this locus (Fig.1). Twenty-four alleles ranging in size from 180-226bp were detected in the pooled population. The number of alleles observed for HUJ 1177 loci in Thrissur, Kottayam, Trivandrum and Kozhikode populations were 19, 14, 15 and 16, respectively. The allele size ranged between 180bp to 224bp in Thrissur, 188bp to 224bp in Kottayam, 186 to 226 bp in Trivandrum and 186 to 220 bp in Kozhikode. Four alleles (180, 182, 184 and 218 bp) were specific to Thrissur population while two alleles (192 and 196 bp) were specific to Kozhikode. The allele size and frequency at the same locus are summarized in the Table 4.11.

Twenty-one genotypes were recorded in goat populations of Kottayam, while 20 each were recorded in Thrissur and Kozhikode populations. In Trivandrum 18 genotypes were recorded. A total of 66 genotypes were recorded in the pooled population. The genotypes and their frequencies are presented in Table 4.12.

4.4.3.2 ILSTS 030

A total of 13, 12, 11 and ten alleles could be observed for ILSTS 030 locus in goats of Thrissur, Kottayam, Trivandrum and Kozhikode, respectively. The allele size and frequencies at ILSTS 030 locus are presented in Table 4.13. The allele size ranged from 148-172 bp in Thrissur, 152-178 bp in Kottayam, 148-174 bp in Trivandrum and 152-176 bp in Kozhikode populations. The maximum number of alleles (13) was observed in Thrissur population and the minimum (10) in Kozhikode. The allele 174 bp was specific to Trivandrum

population, while 178 bp was specific to Kottayam. The allele size of 158 bp occurred at the highest frequency (0.2500) in Thrissur, 162bp topped at a frequency of 0.2045 in Kottayam and 164 bp in Trivandrum and Kozhikode at frequencies of 0.1800 and 0.2609, respectively. The allele 164 bp occurred at higher frequencies in all goat populations except Kottayam, while the allele 162 bp was more frequent in all goat populations except Thrissur. The allele 168 bp was shared at a higher frequency by Kottayam and Trivandrum populations, and 156 bp occurred at a higher frequency in Kottayam and Kozhikode. 154 bp was observed at high frequencies in Trivandrum and Kozhikode and 158 bp in Trivandrum and Thrissur.

The genotypes and their frequencies at this locus are summarized in Table 4.14. In pooled population a total of 36 genotypes were observed. A total of 16, 11,14 and 10 genotypes were observed in Thrissur, Kottayam, Trivandrum and Kozhikode, respectively. In Thrissur, 148/158 genotype was observed with a maximum frequency of 0.1667, while in Kottayam 156/168 genotype recorded the highest frequency of 0.2727 and in Kozhikode 156/164 recorded highest with frequency of 0.2174. The genotypes 154/162, 154/164 and 162/174 were found at high frequencies (0.1200) in Trivandrum. Polymorphism at the locus ILSTS 030 is depicted in Figure 2.

4.4.3.3 INRA 063

The maximum number of alleles at this locus was observed in Kozhikode (8) whereas the minimum was recorded (5) in Thrissur. Seven alleles each were recorded in Trivandrum and Kottayam. The allele size ranged from 164-172 bp in Thrissur, 158-172 bp in Kozhikode and 160-172 bp both in Kottayam and Trivandrum. The allele size and frequency are summarized in Table 4.15 and the polymorphism is depicted in Figure 3. The allele 162 bp was highly frequent in all goat populations except Thrissur, while 164 bp occurred at higher frequencies in Trivandrum and Kozhikode. The alleles 166 and 170 bp were shared by all

goat populations at higher frequencies, while 168 and 172 bp occurred at higher frequencies in all populations except Trivandrum.

A maximum number of genotypes (13) were observed for INRA 063 locus in goats of Kozhikode, while the lowest number (7) was recorded in Thrissur. The genotype 164/170 recorded the highest frequency of 0.2272 in Trivandrum, whereas 166/168 recorded the highest in Kottayam (0.1764). In Thrissur 168/170 genotype was observed with maximum frequency of 0.2273, while in Kozhikode 164/168 and 166/170 genotypes occurred at highest frequencies (0.1739). The genotypes and their frequencies at this locus are summarized in Table 4.16.

4.4.3.4 TGLA 68

Nine alleles were detected at this locus with a size ranging from 98-114 bp (Fig.4). The maximum number of alleles at this locus was observed in Kottayam (9), whereas the minimum was recorded in Trivandrum(6). Thrissur and Kozhikode populations had 8 and 7 alleles within a size range of 100-114 and 98-110, respectively. The allele 106 bp occurred at highest frequencies in Thrissur (0.3200) and Kottayam (0.3043), while 104 bp occurred at highest frequency in kozhikode (0.3125) and Trivandrum (0.3182). The allele size and their frequencies are summarized in Table 4.17. The allele 102 bp was frequent in all populations except Kottayam, while the alleles 104, 106 and 108 bp occurred at high frequencies in all populations.

A maximum number of 13 genotypes were observed in Thrissur, while the lowest number of 9 was recorded in Trivandrum. 11 genotypes were detected in both Kottayam and Kozhikode. The genotype 104/106 occurred at highest frequencies in Trivandrum (0.2273) and Thrissur (0.4000), while 106/108 topped at a frequency of 0.2609 in Kottayam. The genotype 102/104 recorded the

highest frequency 0.2083 in Kozhikode. The genotypes and their frequencies are presented in Table 4.18.

4.4.3.5 BM 1329

At this locus twelve different alleles could be detected with a size range of 167-189 bp (Fig.5). The allele size and their frequencies are given in Table 4.19. The number of alleles recorded for BM 1329 locus in Kottayam and Kozhikode were 8 and 9, respectively, while 10 alleles each were detected in Trivandrum and Thrissur. The allele size of 173 bp occurred at highest frequency in Thrissur (0.4584) and Kozhikode (0.2619), while 171 bp topped at frequencies of 0.2333 and 0.2727 in Kottayam and Trivandrum, respectively. The allele 169 bp occurred at a higher frequency in Kottayam and Trivandrum and 177 bp in Kozhikode and Trivandrum. The alleles 171 and 173 bp were highly frequent in all populations investigated.

A total of 34 genotypes could be detected at this locus. The genotypes and their frequencies are presented in Table 4.20. In Trivandrum the genotypes 171/173 and 171/177 occurred in highest frequency (0.1818), while in Thrissur 173/175 recorded the highest frequency of 0.3333. The genotype 167/169 was specific to Kottayam, which recorded at highest frequency of 0.2667. In Kozhikode, the genotype 171/173 recorded the highest frequency (0.2857). Four genotypes were specific to Kottayam population (167/169,167/179,173/185 and 179/187), while five genotypes to Kozhikode (167/177, 169/173, 175/177, 177/179 and 179/189). Eight genotypes each were specific to the other two populations. (169/175, 169/177, 169/179, 169/185, 171/175, 171/183, 175/179 and 181/189 in Trivandrum and 169/181, 173/173, 173/175, 173/181, 173/183, 173/187, 177/177 and 185/185 in Thrissur).

4.4.4. Intra Population Variation

Genetic diversity within each population was calculated as the mean number of alleles per locus, average heterozygosity and average PIC values and is presented in Table 4.28.

4.4.4.1 Heterozygosity

The heterozygosity values for each locus were calculated by the method suggested by Ott (1992). The heterozygosity values for each locus in different goat populations are presented in Tables 4.22 to 4.26. The mean values obtained were 0.887 for HUJ 1177, 0.839 for ILSTS 030, 0.796 for INRA 063, 0.789 for TGLA 68 and 0.802 for BM 1329. The mean values are presented in Table 4.27.

4.4.4.2 Polymorphic Information Content

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 populations are presented in Tables 4.22 to 4.26. The mean PIC values obtained were 0.882 for HUJ 1177, 0.833 for ILSTS 030, 0.780 for INRA 063, 0.764 for TGLA 68 and 0.789 for BM 1329. The mean values are presented in Table 4.27.

4.4.5 Inter Population Variation

4.4.5.1 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. Nei's genetic distance between Thrissur and Kottayam populations was 0.7203, between Thrissur and Trivandrum population, 0.3547, between Thrissur and Kozhikode populations, 0.3776, between Kottayam and

Trivandrum populations, 0.6294, between Kottayam and Kozhikode 0.4389 and that between Trivandrum and Kozhikode was found to be 0.3564. The genetic distance matrix is presented in Table 4.29.

4.4.5.2 Dendrogram

Dendrogram of relationship between the four goat populations under study was plotted using the POPGENE program. In the dendrogram, Thrissur and Trivandrum goat populations were grouped together first and then with the Kozhikode goat population, while Kottayam population formed a unique branch. The dendrogram is presented in Fig.6.

Table.4.1. Percentages of each class of the studied qualitative variables in four goat populations

Variable		Trivandrum	Kottayam	Thrissur	Kozhikode	Total
Horns	Present	96	60	74	89	79.75
	Absent	4	40	26	11	20.25
Tassels	Present	6	12	7	14	9.75
	Absent	94	88	93	86	90.25
Beard	Present	4	20	8	5	9.25
	Absent	96	80	92	95	90.75
Ear size	Long	-	-	-	-	
	Medium	100	100	100	99	99.75
	Short	-	-	-	1	0.25
Coat Color	Black	7	8	4	1	5
	White	8	21	11	45	21.25
	Brown	7	4	2	4	4.25
	Black&White	29	32	36	30	31.75
	Black&Brown	11	7	5	7	7.5
	White&Brown	17	19	22	6	16
	Black,White& Brown	21	9	20	7	14.25
Hair pattern	Long haired	-	-	37	1	9.5
	Medium haired	1	6	11	8	6.5
	Short haired	89	77	31	76	68.25
	Short haired in forequarters&long haired in hind quarters	3	3	21	2	7.25
	Short haired in forequarters&medium haired in hindquarters	6	13	-	11	7.5
	Medium haired in forequarters&long haired in hindquarters	1	1	-	2	1

Table 4.2 Comparison of mean values of body measurements (cm) of adult females in the four goat populations.

Characteristics	Trivandrum	Kottayam	Thrissur	Kozhikode
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Head length	$19.16^{b} \pm 0.15$	$18.84^{b} \pm 0.16$	$17.79^{a} \pm 0.14$	$18.19^{a} \pm 0.15$
Head width	$16.08^{c} \pm 0.15$	$15.04^{b} \pm 0.11$	$13.37^{a} \pm 0.16$	$15.41^{b} \pm 0.12$
Height at withers	$70.78^{d} \pm 0.49$	$67.2^{c} \pm 0.43$	$62.34^{a} \pm 0.41$	$65.38^{b} \pm 0.46$
Chest depth	$34.17^{b} \pm 0.25$	$35.23^{c} \pm 0.28$	$31.5^{a} \pm 0.31$	$33.54^{b} \pm 0.27$
Chest girth	$75.35^{d} \pm 0.57$	$73.28^{c} \pm 0.56$	$66.68^{a} \pm 0.64$	$70.98^{b} \pm 0.64$
Shoulder point width	$17.99^{c} \pm 0.22$	$17.72^{bc} \pm 0.19$	$17.28^{ab} \pm 0.33$	$17.0^{a} \pm 0.21$
Rump length	$21.51^{c} \pm 0.19$	$20.08^{b} \pm 0.16$	$19.07^{a} \pm 0.19$	$19.61^{b} \pm 0.18$
Rump width	$13.75^{a} \pm 0.18$	$15.61^{b} \pm 0.14$	$14.07^{a} \pm 0.14$	$14.28^{a} \pm 0.52$
Shin circumference	$7.94^{b} \pm 0.04$	$8.09^{b} \pm 0.06$	$8.26^{c} \pm 0.07$	$7.39^{a} \pm 0.06$
Body length	$67.63^{c} \pm 0.56$	$63.94^{b} \pm 0.55$	$58.68^{a} \pm 0.48$	$65.04^{b} \pm 0.56$

* $p < 0.05$ significant at 5 % level.

** Means bearing same superscript in rows do not differ significantly.

Table 4.3. Mahalanobis distance between the goat populations based on morphometric measurements.

	Trivandrum	Kottayam	Thrissur	Kozhikode
Trivandrum	0	3.9794	9.13866	3.35988
Kottayam		0	6.94518	3.04962
Thrissur			0	7.8152
Kozhikode				0

Table 4.4 Average predicted body weights (kg) in different age groups in the four goat populations

Age group	Trivandrum	Kottayam	Thrissur	Kozhikode
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
18-24 months	29.15 ± 0.66	28.19 ± 0.45	22.86 ± 0.41	26.92 ± 0.56
24-36 months	29.92 ± 0.52	29.55 ± 0.62	25.73 ± 0.82	28.02 ± 0.87
36-48 months	32.29 ± 0.63	30.32 ± 0.79	26.64 ± 0.56	30.25 ± 0.75
> 48 months	32.79 ± 0.64	30.95 ± 0.69	29.37 ± 0.72	30.70 ± 0.86

Table 4.5. Comparison of mean values of peak yield in the four goat populations.

	Mean (in milliliters) ± SE
Trivandrum	$699.5^{b} \pm 40.2$
Kottayam	$832.5^{c} \pm 35.06$
Thrissur	$444^{a} \pm 25.4$
Kozhikode	$755^{bc} \pm 38.49$

*p< 0.05 significant at 5 % level.

** Means bearing same superscript do not differ significantly

Table 4.6. Prolificacy (in percentage) of goat populations in Trivandrum, Kottayam, Thrissur and Kozhikode districts.

	Singles	Twins	Triplets	Quadruplets	Total % of multiple births
Trivandrum	32	58	10	0	68
Kottayam	42	46	11	1	58
Thrissur	45	48	7	0	55
Kozhikode	39	52	8	1	61

Table 4.7. Mahalanobis distance between the goat populations based on body weight, peak yield and prolificacy

	Trivandrum	Kottayam	Thrissur	Kozhikode
Trivandrum	0	0.37338	2.10488	0.58476
Kottayam		0	2.30842	0.19238
Thrissur			0	1.18574
Kozhikode				0

Table 4.8 Disease incidence reported in adult female goats during the period 2006-2007 in the four goat populations

Sl.No	Disease condition	Trivandrum	Kottayam	Thrissur	Kozhikode	Total
1	Respiratory infections	-	3	2	1	6
2	Deficiency diseases	1	2	6	1	10
3	Digestive disorders	9	10	5	5	29
4	Fungal infections	-	1	1	-	2
5	Mastitis	1	5	-	1	7
A.	Total disease cases reported	11	21	14	8	54
B.	No diseases	89	79	86	92	346
C.	Total number of observations	100	100	100	100	400

Table 4.9 Standardized conditions for PCR assay

Sl.No	Parameter	Value
1	Template DNA(ng)	50
2	$MgCl_2$(m*M*)	1.25
3	10X Reaction Buffer(µl)	1
4	dNTPs (M*m*)	200
5	Forward primer(p*M*)	5
6	Reverse primer(p*M*)	5
7	Taq DNA polymerase(IU)	0.3
8	Reaction volume(µl)	10

Table 4.10 Standardized temperature and cycling conditions for the PCR at different microsatellite loci analyzed

Sl. No.	Parameter	Temperature/cycles				
		HUJ 1177	ILSTS 030	INRA 063	TGLA 68	BM 1329
1	Denaturation (^{0}C for 1min)	94	94	94	94	94
2	Annealing (^{0}C for 1min)	58	62	60	59	58
3	Extension (^{0}C for 1min)	72	72	72	72	72
4	Number of cycles	35	35	35	35	35
5	Final extension (^{0}C for 5min)	72	72	72	72	72

Table 4.11. Allele size and their frequencies at HUJ1177 locus in the four goat populations

Sl. No	Allele size (bp)	Trivandrum	Kottayam	Thrissur	Kozhikode	Pooled population
1	180	0.000000	0.000000	0.020000	0.000000	0.005102
2	182	0.000000	0.000000	0.100000	0.000000	0.025510
3	184	0.000000	0.000000	0.020000	0.000000	0.005102
4	186	0.020833	0.000000	0.040000	0.060000	0.030612
5	188	0.000000	0.020833	0.100000	0.100000	0.056122
6	190	0.062500	0.000000	0.040000	0.240000	0.086735
7	192	0.000000	0.000000	0.000000	0.040000	0.010204
8	194	0.020833	0.041667	0.040000	0.120000	0.056122
9	196	0.000000	0.000000	0.000000	0.040000	0.010204
10	198	0.020833	0.020833	0.100000	0.020000	0.040816
11	200	0.000000	0.020833	0.160000	0.000000	0.045918
12	202	0.020833	0.062500	0.040000	0.040000	0.040816
13	204	0.187500	0.166667	0.020000	0.080000	0.112245
14	206	0.166666	0.187500	0.060000	0.060000	0.117347
15	208	0.104166	0.125000	0.020000	0.040000	0.071429
16	210	0.020833	0.104166	0.040000	0.040000	0.051020
17	212	0.062500	0.104166	0.000000	0.040000	0.051020
18	214	0.062500	0.020833	0.000000	0.040000	0.030612
19	216	0.041666	0.000000	0.080000	0.020000	0.035142
20	218	0.000000	0.000000	0.020000	0.000000	0.005102
21	220	0.020833	0.062500	0.060000	0.020000	0.040816
22	222	0.000000	0.020833	0.020000	0.000000	0.010204
23	224	0.104166	0.041667	0.020000	0.000000	0.040816
24	226	0.083333	0.000000	0.000000	0.000000	0.020408

Table 4.12. Genotypes and frequencies at HUJ 1177 locus in the four goat populations investigated

Sl.No.	Genotype	Frequency			
		Trivandrum	Kottayam	Thrissur	Kozhikode
1	180/202	0.000000	0.000000	0.040000	0.000000
2	182/190	0.000000	0.000000	0.080000	0.000000
3	182/198	0.000000	0.000000	0.080000	0.000000
4	182/202	0.000000	0.000000	0.040000	0.000000
5	184/200	0.000000	0.000000	0.040000	0.000000
6	186/186	0.000000	0.000000	0.040000	0.040000
7	186/196	0.000000	0.000000	0.000000	0.040000
8	186/202	0.041666	0.000000	0.000000	0.000000
9	188/188	0.000000	0.000000	0.000000	0.040000
10	188/190	0.000000	0.000000	0.000000	0.080000
11	188/196	0.000000	0.000000	0.000000	0.040000
12	188/200	0.000000	0.000000	0.120000	0.000000
13	188/204	0.000000	0.041666	0.000000	0.000000
14	188/206	0.000000	0.000000	0.040000	0.000000
15	188/210	0.000000	0.000000	0.040000	0.000000
16	190/190	0.000000	0.000000	0.000000	0.120000
17	190/194	0.000000	0.000000	0.000000	0.120000
18	190/204	0.000000	0.000000	0.000000	0.040000
19	190/206	0.041666	0.000000	0.000000	0.000000
20	190/226	0.083333	0.000000	0.000000	0.000000
21	192/192	0.000000	0.000000	0.000000	0.040000
22	194/194	0.000000	0.000000	0.040000	0.040000

Table 4.12. (Continued) Genotypes and frequencies at HUJ 1177 locus in the four goat populations investigated.

23	194/198	0.000000	0.041666	0.000000	0.000000
24	194/204	0.041666	0.000000	0.000000	0.000000
25	194/206	0.000000	0.041666	0.000000	0.000000
26	194/208	0.000000	0.000000	0.000000	0.040000
27	198/200	0.000000	0.000000	0.040000	0.000000
28	198/206	0.041666	0.000000	0.000000	0.000000
29	198/212	0.000000	0.000000	0.000000	0.040000
30	198/216	0.000000	0.000000	0.080000	0.000000
31	200/200	0.000000	0.000000	0.040000	0.000000
32	200/208	0.000000	0.041666	0.000000	0.000000
33	200/216	0.000000	0.000000	0.040000	0.000000
34	202/208	0.000000	0.083333	0.000000	0.000000
35	202/210	0.000000	0.000000	0.000000	0.040000
36	202/212	0.000000	0.041666	0.000000	0.000000
37	202/214	0.000000	0.000000	0.000000	0.040000
38	204/204	0.000000	0.041666	0.000000	0.000000
39	204/206	0.000000	0.041666	0.000000	0.040000
40	204/208	0.083333	0.041666	0.000000	0.000000
41	204/210	0.000000	0.041666	0.000000	0.000000
42	204/212	0.041666	0.000000	0.000000	0.000000
43	204/214	0.083333	0.041666	0.000000	0.040000
44	204/216	0.000000	0.000000	0.000000	0.040000
45	204/220	0.000000	0.041666	0.000000	0.000000
46	204/224	0.125000	0.000000	0.040000	0.000000

Table 4.12. (Continued) Genotypes and frequencies at HUJ 1177 locus in the four goat populations investigated

47	206/206	0.041666	0.083333	0.000000	0.000000
48	206/208	0.041666	0.000000	0.040000	0.040000
49	206/210	0.041666	0.083333	0.000000	0.040000
50	206/212	0.083333	0.000000	0.000000	0.000000
51	206/216	0.000000	0.000000	0.040000	0.000000
52	206/222	0.000000	0.041666	0.000000	0.000000
53	208/210	0.000000	0.041666	0.000000	0.000000
54	208/212	0.000000	0.041666	0.000000	0.000000
55	208/220	0.041666	0.000000	0.000000	0.000000
56	208/224	0.041666	0.000000	0.000000	0.000000
57	210/212	0.000000	0.041666	0.000000	0.000000
58	210/218	0.000000	0.000000	0.040000	0.000000
59	212/220	0.000000	0.041666	0.000000	0.040000
60	212/224	0.000000	0.041666	0.000000	0.000000
61	214/226	0.041666	0.000000	0.000000	0.000000
62	216/224	0.041666	0.000000	0.000000	0.000000
63	216/226	0.041666	0.000000	0.000000	0.000000
64	220/220	0.000000	0.000000	0.040000	0.000000
65	220/222	0.000000	0.000000	0.040000	0.000000
66	220/224	0.000000	0.041666	0.000000	0.000000

Table. 4.13. Allele size and their frequencies at ILSTS 030 locus in the four goat populations

Sl. No	Allele size (bp)	Trivandrum	Kottayam	Thrissur	Kozhikode	Pooled population
1	148	0.040000	0.000000	0.083333	0.000000	0.031915
2	150	0.020000	0.000000	0.083333	0.000000	0.026596
3	152	0.000000	0.068181	0.083333	0.043478	0.047872
4	154	0.120000	0.045454	0.020833	0.195652	0.095745
5	156	0.040000	0.159090	0.020833	0.108695	0.079787
6	158	0.120000	0.068181	0.250000	0.021739	0.117021
7	160	0.000000	0.045454	0.125000	0.000000	0.042553
8	162	0.140000	0.204545	0.062500	0.173913	0.143617
9	164	0.180000	0.000000	0.125000	0.260869	0.143617
10	166	0.060000	0.045454	0.041666	0.043478	0.047872
11	168	0.100000	0.181818	0.020833	0.021739	0.079787
12	170	0.000000	0.090909	0.041666	0.000000	0.031914
13	172	0.040000	0.045454	0.041666	0.065217	0.047872
14	174	0.140000	0.000000	0.000000	0.000000	0.037234
15	176	0.000000	0.022727	0.000000	0.065217	0.021276
16	178	0.000000	0.022727	0.000000	0.000000	0.005319

Table 4.14. Genotypes and frequencies at ILSTS 030 locus in the four goat populations investigated

Sl.No.	Genotypes	Frequency			
		Trivandrum	Kottayam	Thrissur	Kozhikode
1	148/158	0.080000	0.000000	0.166666	0.000000
2	150/156	0.040000	0.000000	0.000000	0.000000
3	150/158	0.000000	0.000000	0.083333	0.000000
4	150/160	0.000000	0.000000	0.083333	0.000000
5	152/158	0.000000	0.045454	0.000000	0.000000
6	152/160	0.000000	0.090909	0.041666	0.000000
7	152/162	0.000000	0.000000	0.083333	0.086956
8	152/164	0.000000	0.000000	0.041666	0.000000
9	154/162	0.120000	0.045454	0.000000	0.217391
10	154/164	0.120000	0.000000	0.041666	0.173913
11	154/176	0.000000	0.045454	0.000000	0.000000
12	156/164	0.000000	0.000000	0.000000	0.217319
13	156/166	0.040000	0.045454	0.041666	0.000000
14	156/168	0.000000	0.272727	0.000000	0.000000
15	158/158	0.000000	0.000000	0.041666	0.000000
16	158/164	0.000000	0.000000	0.083333	0.000000
17	158/166	0.040000	0.000000	0.041666	0.000000
18	158/168	0.120000	0.090909	0.041666	0.043478
19	160/164	0.000000	0.000000	0.083333	0.000000
20	160/170	0.000000	0.000000	0.041666	0.000000
21	162/164	0.040000	0.000000	0.000000	0.000000
22	162/166	0.000000	0.045454	0.000000	0.000000

Table 4.14. (Continued) Genotypes and frequencies at ILSTS 030 locus in the four goat populations investigated

23	162/170	0.000000	0.181818	0.000000	0.000000
24	162/172	0.000000	0.090909	0.041666	0.043478
25	162/174	0.120000	0.000000	0.000000	0.000000
26	162/178	0.000000	0.045454	0.000000	0.000000
27	164/168	0.040000	0.000000	0.000000	0.000000
28	164/172	0.080000	0.000000	0.000000	0.043478
29	164/174	0.080000	0.000000	0.000000	0.000000
30	164/176	0.000000	0.000000	0.000000	0.086956
31	166/172	0.000000	0.000000	0.000000	0.043478
32	166/174	0.040000	0.000000	0.000000	0.000000
33	166/176	0.000000	0.000000	0.000000	0.043478
34	168/172	0.000000	0.000000	0.000000	0.000000
35	168/174	0.040000	0.000000	0.000000	0.000000
36	170/172	0.000000	0.000000	0.041666	0.000000

Table 4.15. Allele size and their frequencies at INRA 063 locus in the four goat populations

Sl.no	Allele size(bp)	Trivandrum	Kottayam	Thrissur	Kozhikode	Pooled population
1	158	0.000000	0.000000	0.000000	0.021739	0.005952
2	160	0.045454	0.029411	0.000000	0.043478	0.029762
3	162	0.159090	0.117647	0.000000	0.108695	0.095238
4	164	0.340909	0.029411	0.090909	0.195652	0.172619
5	166	0.159090	0.294117	0.159090	0.173913	0.190476
6	168	0.068181	0.235294	0.318181	0.173913	0.196429
7	170	0.204545	0.176470	0.295454	0.173913	0.214286
8	172	0.022727	0.117647	0.136363	0.108695	0.095238

Table 4.16. Genotypes and frequencies at INRA 063 locus in the four goat populations investigated

Sl.No.	Genotypes	Frequency			
		Trivandrum	Kottayam	Thrissur	Kozhikode
1	158/162	0.000000	0.000000	0.000000	0.043478
2	160/162	0.090909	0.058823	0.000000	0.000000
3	160/164	0.000000	0.000000	0.000000	0.086956
4	162/164	0.181818	0.058823	0.000000	0.000000
5	162/166	0.045454	0.000000	0.000000	0.043478
6	162/168	0.000000	0.000000	0.000000	0.086956
7	162/170	0.000000	0.058823	0.000000	0.043478
8	162/172	0.000000	0.058823	0.000000	0.000000
9	164/164	0.090909	0.000000	0.000000	0.000000
10	164/166	0.090909	0.000000	0.000000	0.043478
11	164/168	0.000000	0.000000	0.090909	0.173913
12	164/170	0.227272	0.000000	0.090909	0.086956
13	166/166	0.000000	0.117647	0.000000	0.000000
14	166/168	0.090909	0.176470	0.136363	0.043478
15	166/170	0.045454	0.058823	0.181818	0.173913
16	166/172	0.045454	0.117647	0.000000	0.043478
17	168/168	0.000000	0.058823	0.000000	0.000000
18	168/170	0.045454	0.117647	0.227272	0.043478
19	168/172	0.000000	0.058833	0.181818	0.000000
20	170/170	0.045454	0.058823	0.000000	0.000000
21	170/172	0.000000	0.000000	0.090909	0.000000
22	172/172	0.000000	0.000000	0.000000	0.086956

Table 4.17 Allele size and their frequencies at TGLA 68 locus in the four goat populations

Sl.no	Allele size(bp)	Trivandrum	Kottayam	Thrissur	Kozhikode	Pooled population
1	98	0.000000	0.021739	0.000000	0.020833	0.010869
2	100	0.068181	0.021739	0.020000	0.062500	0.043478
3	102	0.159090	0.086956	0.100000	0.208333	0.141304
4	104	0.318181	0.260869	0.300000	0.312500	0.298913
5	106	0.204545	0.304347	0.320000	0.166666	0.244565
6	108	0.181818	0.239130	0.120000	0.145833	0.173913
7	110	0.068181	0.021739	0.080000	0.083333	0.059782
8	112	0.000000	0.021739	0.040000	0.000000	0.016304
9	114	0.000000	0.021739	0.020000	0.000000	0.010869

Table 4.18. Genotypes and frequencies at TGLA 68 locus in the four goat populations investigated.

Sl.No	Genotypes	Frequency			
		Trivandrum	Kottayam	Thrissur	Kozhikode
1	98/100	0.000000	0.000000	0.000000	0.041666
2	98/102	0.000000	0.043478	0.000000	0.000000
3	100/102	0.000000	0.000000	0.000000	0.041666
4	100/104	0.136363	0.000000	0.000000	0.041666
5	100/106	0.000000	0.043478	0.040000	0.000000
6	102/102	0.000000	0.000000	0.040000	0.000000
7	102/104	0.181818	0.130434	0.080000	0.208333

Table 4.18. (Continued) Genotypes and frequencies at TGLA 68 locus in the four goat populations investigated

8	102/106	0.045454	0.000000	0.040000	0.041666
9	102/108	0.090909	0.000000	0.000000	0.125000
10	104/104	0.000000	0.043478	0.040000	0.000000
11	104/106	0.227272	0.217391	0.400000	0.166666
12	104/108	0.090909	0.000000	0.040000	0.083333
13	104/110	0.000000	0.043478	0.000000	0.125000
14	104/114	0.000000	0.043478	0.000000	0.000000
15	106/106	0.000000	0.043478	0.040000	0.000000
16	106/108	0.090909	0.260869	0.040000	0.083333
17	106/110	0.045454	0.000000	0.040000	0.041666
18	108/108	0.000000	0.086956	0.000000	0.000000
19	108/110	0.090909	0.000000	0.120000	0.000000
20	108/112	0.000000	0.043478	0.040000	0.000000
21	112/114	0.000000	0.000000	0.040000	0.000000

Table 4.19. Allele size and their frequencies at BM 1329 locus in the four goat populations

Sl. No	Allele size (bp)	Trivandrum	Kottayam	Thrissur	Kozhikode	Pooled population
1	167	0.000000	0.200000	0.000000	0.047619	0.048780
2	169	0.181818	0.133333	0.020833	0.071428	0.097561
3	171	0.272727	0.233333	0.125000	0.309523	0.231707
4	173	0.136363	0.166666	0.458333	0.261904	0.268293
5	175	0.068181	0.000000	0.166666	0.023809	0.073171
6	177	0.136363	0.033333	0.041666	0.166666	0.097561
7	179	0.068181	0.100000	0.020833	0.047619	0.054878
8	181	0.022727	0.000000	0.041666	0.000000	0.018293
9	183	0.022727	0.000000	0.020833	0.000000	0.012195
10	185	0.068181	0.100000	0.062500	0.047619	0.067073
11	187	0.000000	0.033333	0.041666	0.000000	0.018293
12	189	0.022727	0.000000	0.000000	0.023809	0.012195

Table 4.20. Genotypes and frequencies at BM 1329 locus in the four goat populations investigated

Sl.No	Genotypes	Frequency			
		Trivandrum	Kottayam	Thrissur	Kozhikode
1	167/169	0.000000	0.266666	0.000000	0.000000
2	167/173	0.000000	0.066666	0.000000	0.047619
3	167/177	0.000000	0.000000	0.000000	0.047619
4	167/179	0.000000	0.066666	0.000000	0.000000
5	169/171	0.090909	0.000000	0.000000	0.047619
6	169/173	0.000000	0.000000	0.000000	0.095238
7	169/175	0.045454	0.000000	0.000000	0.000000
8	169/177	0.045454	0.000000	0.000000	0.000000
9	169/179	0.045454	0.000000	0.000000	0.000000
10	169/181	0.000000	0.000000	0.041666	0.000000
11	169/185	0.136363	0.000000	0.000000	0.000000
12	171/171	0.000000	0.133333	0.000000	0.095238
13	171/173	0.181818	0.133333	0.208333	0.285714
14	171/175	0.045454	0.000000	0.000000	0.000000
15	171/177	0.181818	0.000000	0.000000	0.047619
16	171/183	0.045454	0.000000	0.000000	0.000000
17	171/185	0.000000	0.066666	0.041666	0.047619
18	173/173	0.000000	0.000000	0.083333	0.000000
19	173/175	0.000000	0.000000	0.333333	0.000000
20	173/177	0.045454	0.000000	0.000000	0.095238
21	173/179	0.045454	0.066666	0.041666	0.000000
22	173/181	0.000000	0.000000	0.041666	0.000000

Table 4.20. (Continued) Genotypes and frequencies at BM 1329 locus in the four goat populations investigated

23	173/183	0.000000	0.000000	0.041666	0.000000
24	173/185	0.000000	0.066666	0.000000	0.000000
25	173/187	0.000000	0.000000	0.083333	0.000000
26	175/177	0.000000	0.000000	0.000000	0.047619
27	175/179	0.045454	0.000000	0.000000	0.000000
28	177/177	0.000000	0.000000	0.041666	0.000000
29	177/179	0.000000	0.000000	0.000000	0.047619
30	177/185	0.000000	0.066666	0.000000	0.047619
31	179/187	0.000000	0.066666	0.000000	0.000000
32	179/189	0.000000	0.000000	0.000000	0.047619
33	181/189	0.045454	0.000000	0.000000	0.000000
34	185/185	0.000000	0.000000	0.041666	0.000000

Table 21. Number of genotypes observed in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations for different markers

Marker loci	Trivandrum	Kottayam	Thrissur	Kozhikode
HUJ 1177	18	21	20	20
ILSTS 030	14	11	16	10
INRA 063	11	12	7	13
TGLA 68	9	11	13	11
BM 1329	13	10	11	13

Table 4.22. Number of alleles, Allele size range, Heterozygosity and Polymorphic information content (PIC) at the HUJ 1177 locus in the four goat populations

Observation	Trivandrum	Kottayam	Thrissur	Kozhikode
No. of alleles	15	14	19	16
Size range	186-226	188-224	180-224	186-220
Heterozygosity	0.892	0.843	0.920	0.892
PIC	0.889	0.833	0.918	0.890

Table 4.23. Number of alleles, Allele size range, Heterozygosity and Polymorphic information content (PIC) at the ILSTS 030 locus in the four goat populations

Observation	Trivandrum	Kottayam	Thrissur	Kozhikode
No. of alleles	11	12	13	10
Size range	148-174	152-178	148-172	152-176
Heterozygosity	0.853	0.845	0.847	0.810
PIC	0.849	0.841	0.842	0.802

Table 4.24. Number of alleles, Allele size range, Heterozygosity and Polymorphic information content (PIC)at the INRA 063 locus in the four goat populations

Observation	Trivandrum	Kottayam	Thrissur	Kozhikode
No. of alleles	7	7	5	8
Size range	160-172	160-172	164-172	158-172
Heterozygosity	0.784	0.797	0.759	0.845
PIC	0.771	0.783	0.732	0.837

Table 4.25. Number of alleles, Allele size range, Heterozygosity and Polymorphic information content (PIC) at the TGLA 68 locus in the four goat populations

Observation	Trivandrum	Kottayam	Thrissur	Kozhikode
No. of alleles	6	9	8	7
Size range	100-110	98-114	100-114	98-110
Heterozygosity	0.789	0.772	0.774	0.798
PIC	0.772	0.748	0.751	0.783

Table 4.26. Number of alleles, Allele size range, Heterozygosity and Polymorphic information content (PIC) at the BM 1329 locus in the four goat populations

Observation	Trivandrum	Kottayam	Thrissur	Kozhikode
No. of alleles	10	8	10	9
Size range	169-189	167-187	169-187	167-189
Heterozygosity	0.839	0.837	0.736	0.794
PIC	0.831	0.831	0.717	0.780

Table 4.27. Mean Heterozygosity and PIC of the five microsatellite markers tested in the different goat populations

Parameter	Microsatellite loci				
	HUJ 1177	ILSTS 030	INRA 063	TGLA 68	BM 1329
Mean Heterozygosity	0.887	0.839	0.796	0.783	0.804
Mean PIC	0.882	0.833	0.780	0.764	0.789

Table 4.28. Total number of alleles, mean number of alleles per locus, mean heterozygosity and PIC value for 5 loci, within each goat population studied

Population	Total number of alleles	Mean number of alleles/locus	Mean heterozygosity	Mean PIC
Trivandrum	49	9.8	0.8264	0.8240
Kottayam	50	10	0.8188	0.8072
Thrissur	55	11	0.8072	0.7920
Kozhikode	50	10	0.8278	0.8184
Mean	51	10.2	0.8201	0.8104

Table 4.29. Nei' genetic distance matrix for the four goat populations analyzed.

	Thrissur	Kottayam	Trivandrum	Kozhikode
Thrissur	0.0000			
Kottayam	0.7203	0.0000		
Trivandrum	0.3547	0.6294	0.0000	
Kozhikode	0.3776	0.4389	0.3564	0.0000

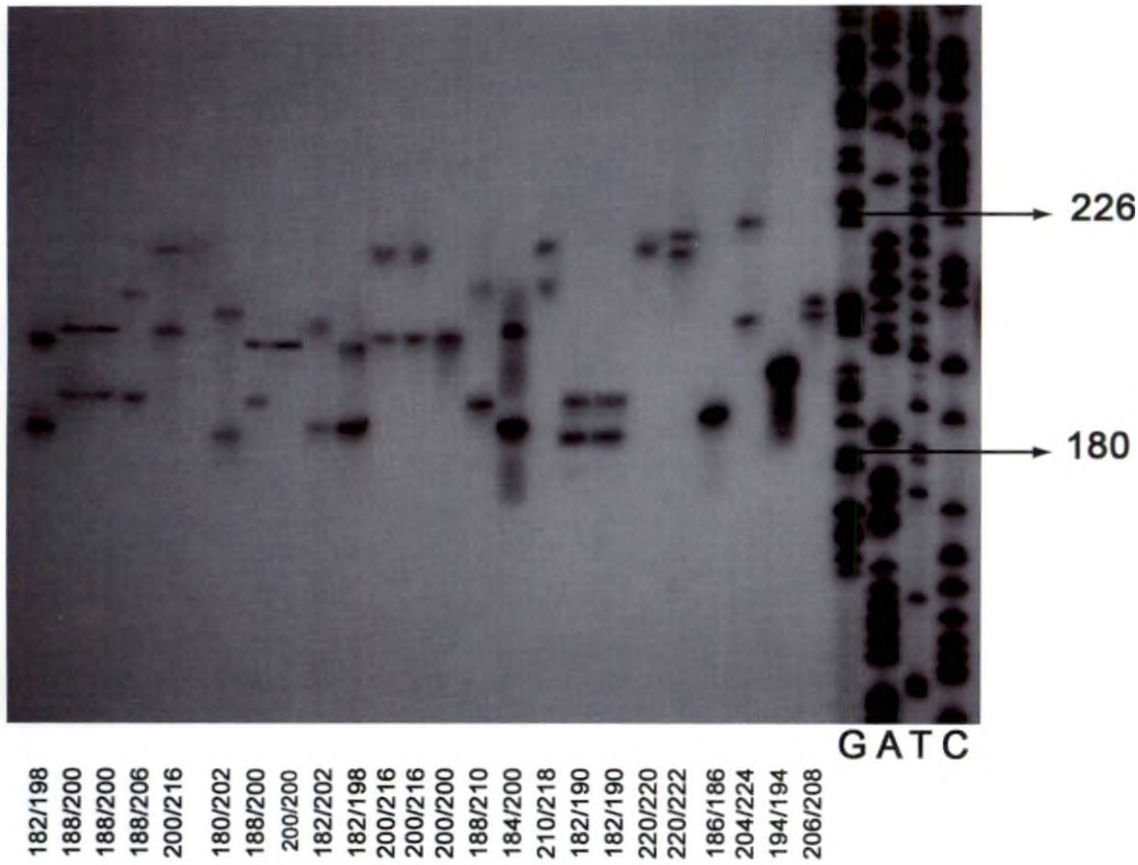

Fig. 1. Autoradiograph showing polymorphism at HUJ 1177 locus
GATC represents M13 sequence used as marker

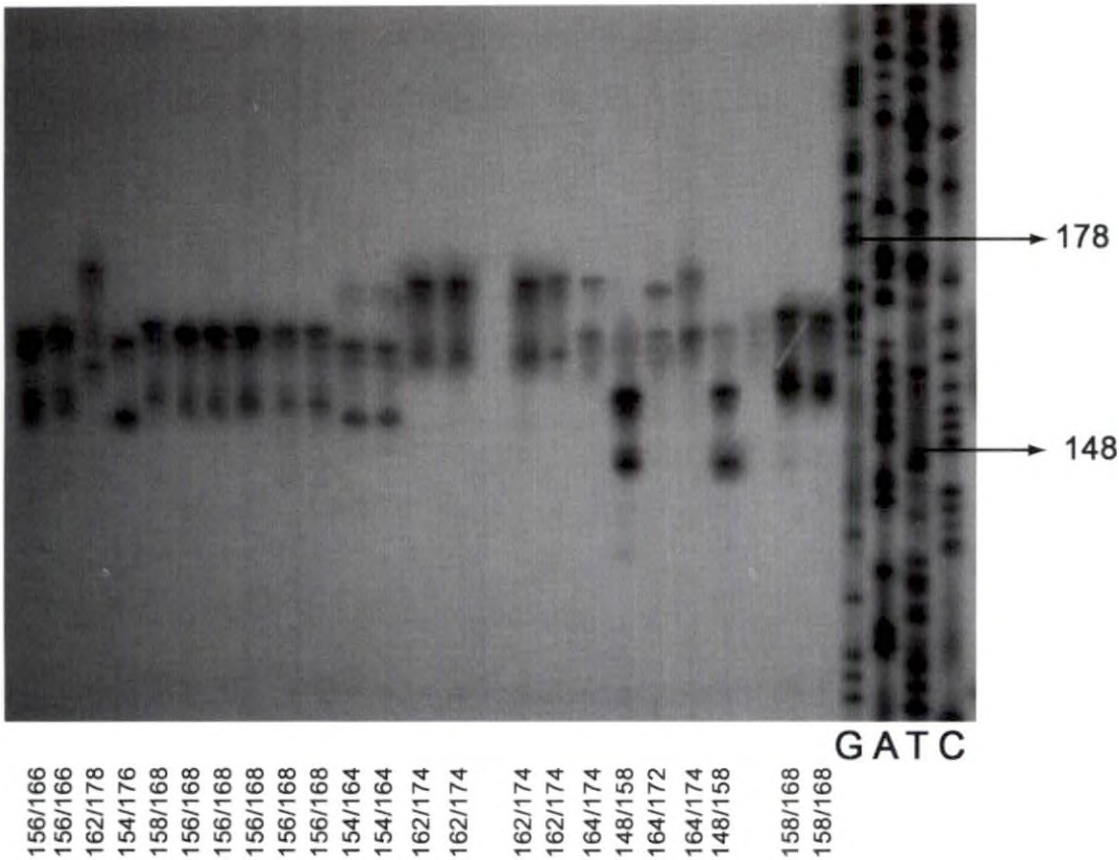

Fig. 2. Autoradiograph showing polymorphism at ILSTS 030 locus
GATC represents M13 sequence used as marker

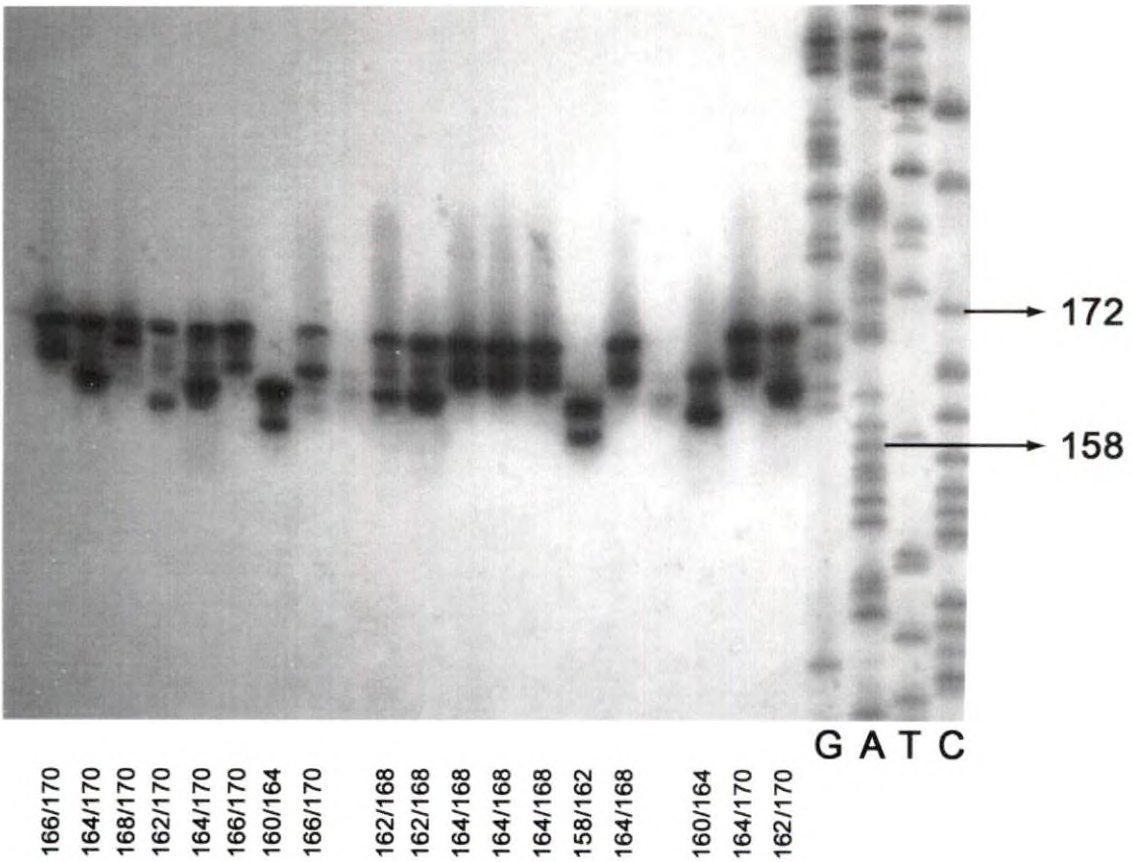

Fig. 3. Autoradiograph showing polymorphism at INRA 063 locus
GATC represents M13 sequence used as marker

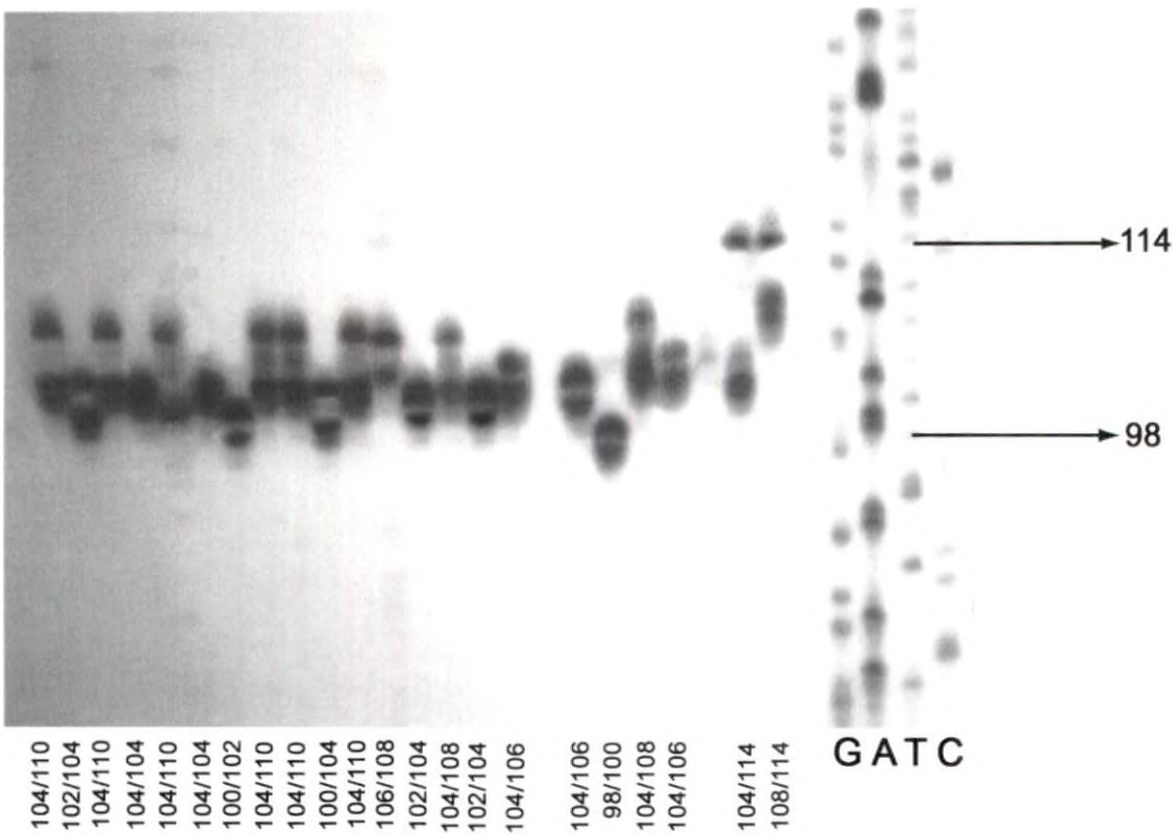

Fig. 4. Autoradiograph showing polymorphism at TGLA 68 locus
GATC represents M13 sequence used as marker

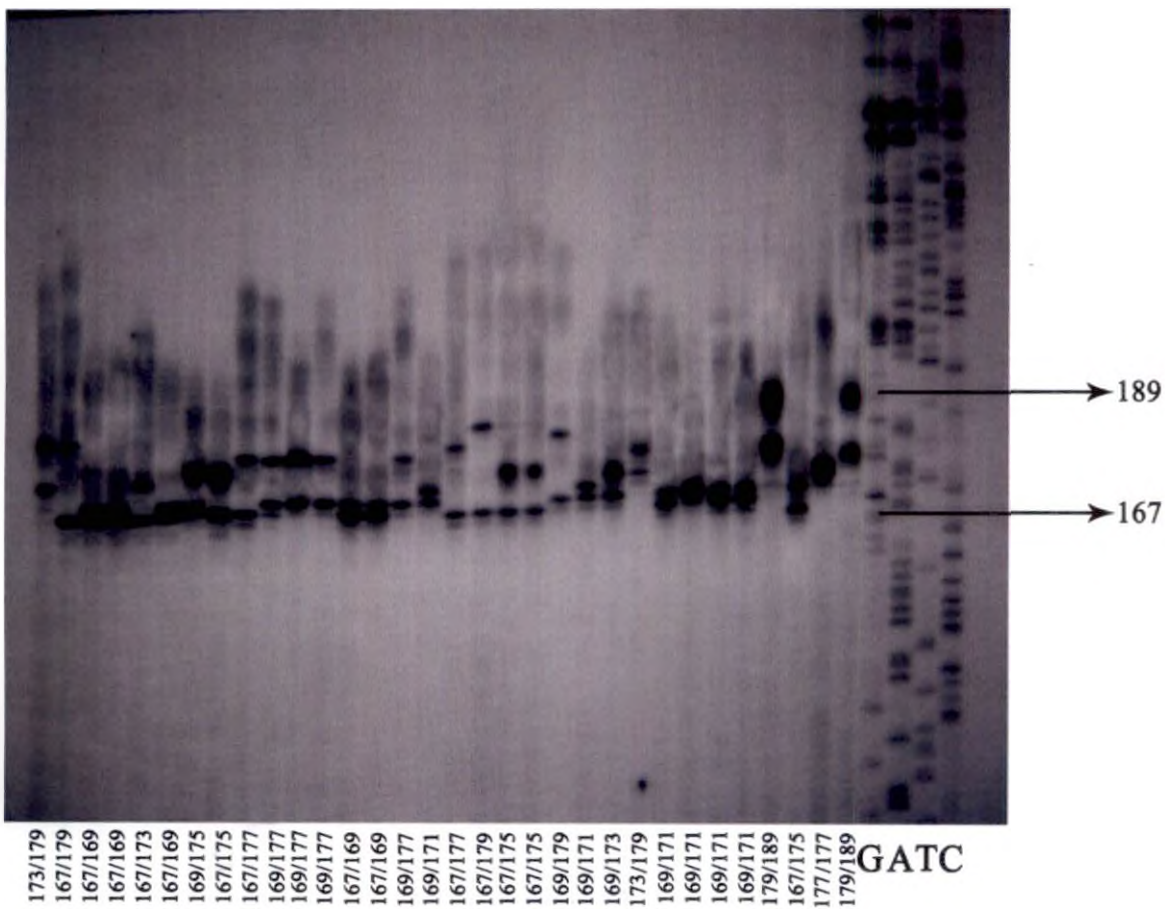

Fig. 5. Autoradiograph showing polymorphism at BM 1329 locus
GATC represents M13 sequence used as marker

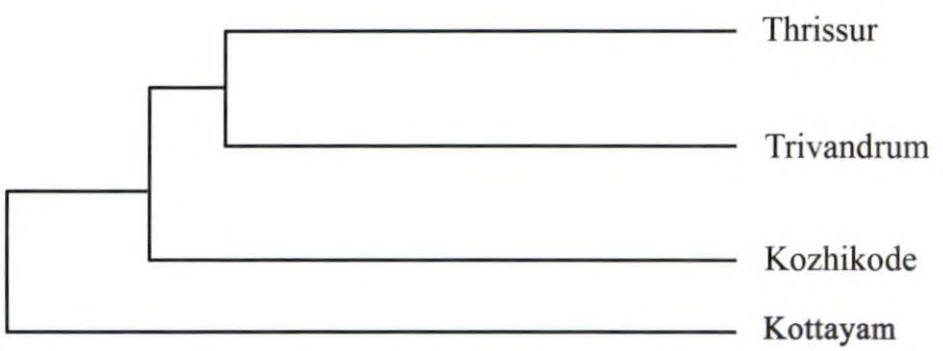

Fig. 6 Dendrogram Based Nei's (1978) Genetic distance: Method - UPGMA
-Modified from NEIGHBOR procedure of PHYLIP Version 3.5

Discussion

5. DISCUSSION

Goats, like other livestock species are recognized as important components of world biodiversity. The Indian subcontinent contains 20 well-characterized goat breeds, which vary in their genetic potential for the production of milk, meat and fibre, disease resistance, heat tolerance and fecundity (Joshi *et al.,* 2004). Worldwide improvement of caprine races has been fundamentally based on crossbreeding with improved races, which may lead to replacement of indigenous populations and the subsequent loss of their unique genetic traits. This trend is currently being reversed in favor of greater attention being focused on local breeds (Herrera *et al.*, 1996). The considerable variation observed among the goat populations, in terms of size and coat color has lead to some inconsistencies in the classification of various local populations into breeds or strains. Given the wide geographic area and the diversified climate and topography of the region, local populations in different places might have been isolated over a long period while also being subjected to varying selection pressures and genetic drift, thus have become genetically divergent (Chenyambuga *et al.,* 2004). However the extent to which various populations are different from each other is not clear. A range of innovations in molecular genetics has been developed for the study of genetic variation and evolution of populations using DNA marker genotype information. The analysis of microsatellites is currently regarded as the most useful technique in the study of genetic diversity of closely related populations (Yang *et al.*, 1999; Canon *et al.*, 2001; Kim *et al.*, 2002; Li *et al.*, 2002; Visser *et al.*, 2004; Hirbo *et al.*, 2006; Missohou *et al.*, 2006). This together with the conventional methods of studying the diversity using variation in physical and biometrical traits will be the best possible methods for studying the inter operation and intra population variations existing in different populations. So this study was planned with the objective of elucidating the diversity of four geographically different goat populations of Kerala. Four major criteria were considered for studying the diversity. They were.

1. Physical characters
2. Biometrical characters
3. Disease incidence and kid mortality
4. Microsatellite analysis

5.1 PHYSICAL CHARACTERS

Morphological variation is one of the tools for studying the genetic diversity of a population. Majority of goats studied in all the regions have a colour pattern of white and a combination of white with other colours. It was also observed that the colour pattern reported for goats of Trivandrum, Kottyam and Trichur is similar to that reported for Kozkikode goats (Malabari). Similar colour pattern for Malabari goats were reported by Raghavan *et al.* (2004). This may be because of two main reasons. Firstly the local goat population, which existed in the state, had a range of colours from white to black. Secondly, the predominantly white colored Malabri goats might have freely mixed with these goats resulting in the present colour pattern. This together with the fact that higher percentage of white coat colour offers a better resistance to heat stress in environments characterized by high solar radiation (Hensen, 1990) might have offered the white dominated colour pattern a selective advantage.

The study revealed high frequencies of horned condition (79.75%) among all the goat populations studied. Relatively small percentages of goats possessed tassels (9.25%) and beard (9.75%). The results were in conformity with other reports in Malabari goats. Malabari goats are generally horned and only a small percentage of females are bearded (Acharya, 1982).

Based on physical traits the populations were not very distinct and uniformity was seen with respect to coat colour, horn pattern, presence or absence of tassels and beard and hair pattern. This indicates that there was free

mixing of all these populations and no population can be classified as a distinct group based on physical characters.

5.2. BIOMETRICAL CHARACTERS

5.2.1. Growth Characters

5.2.1.1.Morphometric Measurements

The goats of Trivandrum district had significantly higher body weight and larger body dimensions than all other populations except for shin circumference and rump width. Animals of Thrissur district had the lowest body measurements and the populations of Kottayam and Kozhikode came in between. Based on the height at withers, goats are classified as large (>65cm), small (51-65cm) and dwarf (<50cm) (Devendra and Burns, 1983). Accordingly the goat populations of Trivandrum, Kottayam and Kozhikode can be grouped under large sized variety, while the goat populations of Thrissur belong to small sized category.

Though body weight was predicted using the regression equation based on power function of chest girth alone, the animals having higher predicted body weights had higher values for other measurements also. Similar trend for body measurements were also reported by Herrera *et al.* (1996).

5.2.1.1.1. Discriminant Analysis Based on Morphometric Traits

Discriminant analysis performed taking ten zoometric variables revealed that head width, height at withers, chest depth, rump length, rump width, shin circumference and body length were the most discriminant variables. Variables that contribute to differentiate populations were similar to those found by Herrera *et al.*(1996) in Andalusian goat breeds, Crepaldi *et al.*(2001) working on goat populations from Lombardy Alpines and Lanari *et al.(*2003) in local Criollo goats in Argentina. Variables head width and body length were the most discriminative

in canonical correspondence with the ordinate axis (CAN1) and the shin circumference and rump length with coordinate axis (CAN2).

The Mahalanobis distance estimated between the goat populations showed the greatest distance between Trivandrum and Thrissur goat populations (9.13866), while the Kottayam and Kozhikode populations bore a strong resemblance (3.04962) to each other with respect to morphometric traits.

5.2.1.2. Body Weight

Body measurements are used for the prediction of body weight in animals. In the present study a high and positive correlation (0.817) existed between chest girth and body weight and hence were used for prediction of body weight. Menon (1994) and Chitra (2002) reported a similar finding in Malabari goats. Mukherjee *et al.* (1986), Singh *et al.* (1981), Varade *et al.* (1997) and Thiruvenkadan (2005) reported high and significant correlation values of body weight with chest girth in various Indian goat breeds. Height at withers and body length were not found to be useful guides for estimating weight in adults since linear and vertical growth would have ceased in adults. Similar findings were reported by Mohammed and Amin (1996).

At 18 to 24 months, the average predicted body weights recorded in kilograms, in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 29.15 ± 0.66, 28.19 ± 0.45, 22.86 ± 0.41 and 26.92 ± 0.56, respectively. At 24 to 36 months, the values for body weight were 29.92 ± 0.52, 29.55 ± 0.62, 25.73 ± 0.82 and 28.02 ± 0.87 kg, respectively. Weight recorded at 36 to 48 months in the respective populations were 32.29 ± 0.63, 30.32 ± 0.79, 26.64 ± 0.56 and 30.25 ± 0.75kg, respectively. The body weights above 48 months were 32.79 ± 0.64, 30.95 ± 0.69, 29.37 ± 0.72 and 30.70 ± 0.86 kg, respectively. The present study revealed that goats of Trivandrum district were much heavier at all stages compared to other populations. Animals of Trivandrum and Kottayam had

higher body weight than Malabari goats from Kozhikode indicating that these animals have better gene pool for growth than Malabari goats.

5.2.2. Peak Yield

Highest mean value for peak yield was recorded in goat populations of Kottayam (832.5 ± 35.06ml), followed by Kozhikode (755 ± 38.49ml) and Trivandrum (699.5 ± 40.29ml), while the lowest value was recorded in Thrissur (444 ± 25.41). Kottayam animals had higher average than Malabari goat of Kozikode. A peak yield of 653.52 ± 46.30 was reported for Malabari goats (AICRP Research Report, 2005).

5.2.3 Prolificacy

The percentage of multiple births was higher in Trivandrum compared to other populations, indicating higher prolificacy for animals of this area. The percentage of twin births was higher compared to singles, triplets and quadruplets in all the populations studied. Similar observations were made by Shanmughasundaram (1957), Raghavan *et al.* (2004), Bindu (2006) and Seena (2006) in Malabari goats. High prolificacy is considered to be a character of local goat populations.

5.2.4. Discriminant Analysis Based on Body Weight, Peak Yield and Prolificacy

Multivariate analysis of variance made taking three variables, body weight, peak yield and prolificacy, revealed that the goat populations studied were significantly different. Body weight and peak yield contributed a cumulative 99.9 per cent of total variation. Low differentiation between these populations was supported by assignment test results. Only about 47.3 per cent of individuals were correctly classified to the population from which they were sampled. Low assignment rates may indicate either high gene flow or low power to assign because of too few variables used in analysis. (Hirbo *et al.*, 2006). The

distance value (D^2) calculated between populations revealed that the goat population of Thrissur district was more distant from all other populations. Cluster analysis using the above three variables indicated that the goat populations of Trivandrum, Kottayam and Kozhikode formed one cluster.

Based on the study on biometric observations it can be concluded that intrapopulation variation is high in all regions, and was found to be highest among Malabari goats (Kozhikode) where only 28 per cent of individuals could be correctly assigned and lowest in Trichur goats where 77 per cent of individuals could be assigned correctly. But the more uniform population of Trichur district had lower body size and prolificacy compared to other groups. Trivandrum, Kottayam and Kozikode populations formed a single group considering the traits body weight, peak yield and prolificacy. But when body weight alone is considered, Trivandrum animals topped the list. It is also note worthy that the role of prolificacy in discriminating the populations is very less.

5.3. DISEASE INCIDENCE AND KID MORTALITY

Respiratory infections, deficiency diseases, mastitis, fungal infections and digestive disorders were reported in the goat populations. Percentage of adult female goats reported to have the occurrence of any disease condition during the period 2006-2007 in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations was 11, 21, 14 and 8, respectively. Kid mortality percentages in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 4.49, 6.43, 15.43 and 2.92, respectively, which were lower than that of earlier reports in Malabari goats (Rajan *et al.*, 1976; Acharya, 1982; Mathew, 1991). In general, these local populations are considered as hardy with low overall mortality. This indicated its innate resistance to the harsh climatic conditions prevailing in its original habitat.

Comparatively higher kid mortality and incidence of disease are observed in Thrissur population though the highest disease incidence was seen in Kottayam population. However these aspects need further detailed study.

5.4. MICROSATELLITE ANALYSIS

5.4.1 Isolation of Genomic 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 (Tantia *et al.*, 2004; Araujo *et al.*, 2006; Hirbo *et al.*, 2006).

5.4.2 PCR Analysis

The polymerase chain reaction is an *in vitro* method of nucleic acid synthesis that enables the specific replication of a targeted segment of DNA, providing a rapid, highly sensitive and specific means of nucleic acid detection and isolation. A standard reaction mixture contains the sample DNA, two oligonucleotide primers, thermostable Taq DNA polymerase and four deoxynucleotide triphosphates (dNTPs) in a buffered solution. Through repeated cycles of heat denaturation of the DNA, annealing of the primers to their complimentary sequences, and extension of annealed primers, an exponential accumulation of the specific target fragment is achieved (Saiki *et al.*, 1988).

5.4.2.1. Optimization of PCR

Primers were selected from the database and available literature based on their allele frequency and heterozygosity. Five markers which were found to be highly polymorphic in earlier studies conducted at Center for Advanced Studies in Animal Genetics and Breeding, and were comparatively easy to analyse with

sharp bands were chosen for analysis (Jacob, 2005; Bindu, 2006; Seena, 2006). The markers selected for this study were bovine dinucleotide repeats HUJ 1177, ILSTS 030, INRA 063, TGLS68 and BM 1329. There is high level of similarity between cattle, sheep and goat chromosomes (Crawford *et al.*, 1995; Vaiman *et al.*, 1996), and bovine and ovine microsatellites are likely to detect polymorphism in goats. Bovine microsatellite primers were adapted for genetic diversity studies in goats by several workers (Saitabekova *et al.*, 1999; Yang *et al.,* 1999; Martinez *et al.*, 2004;Tantia *et al.*, 2004).

PCR conditions were optimized for the primers selected to obtain specific products. . Magnesium chloride concentration and annealing temperature of the primer were critical in obtaining specific amplifications. The annealing temperature for the primer pair was optimized using gradient thermal cycler in the range of 55°C to 65°C. An annealing temperature of 58°C was found optimal for the primers HUJ 1177 (Jacob, 2005; Bindu, 2006) and BM 1329 (Bishop *et al.*, 1994), 60°C for INRA 063, 59°C for TGLA 68 and 62°C for ILSTS 030. The annealing temperature used for the primer ILSTS 030 was higher than that of earlier reports. According to Don *et al.* (1991) the occurrence of spurious bands can be reduced by increasing the annealing temperature of PCR. All the primers selected yielded specific products at 1.25 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 (Cha and Thilly, 1993). These conditions were kept constant for all the primers used. A concentration of 0.3U/reaction of *Thermus aquaticus* DNA polymerase and 5p*M* of primer per reaction gave satisfactory results. Time for denaturation, annealing and extension was one minute with a final extension time of five minutes.

5.4.3. Number of Alleles, Size and Frequency

The number of alleles at different marker loci and their frequencies are indicators of genetic variability. It forms the basis for all the diversity indices for estimation of genetic distances and construction of phylogenetic trees (Nei and Roychoudhury, 1973).

Polymorphic loci which had at least five alleles were only selected to reduce the standard error of the distance estimates (Barker, 1994). The number of alleles ranged from five to nineteen in different populations. The mean number of alleles per locus varies from 9.8 in Trivandrum to 11 in Trichur with an overall average of 10.2. 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 tend to increase with increase in population size (Kotze *et al.*, 2004). Considering the fact that the sample size was constant for all the four populations, the genetic diversity of goat population based on molecular markers is considered to be more or less same for all populations studied. This is also evident from the more or less uniform value for the heterozygosity in all four populations studied.

At the HUJ 1177 locus, the alleles 204 bp and 206 bp were predominant in both Kottayam and Trivandrum. Allele 200 bp was more frequent in Thrissur, and allele 190 bp in Kozhikode. At the ILSTS 030 locus, the allele 158 bp was more frequent in Thrissur, 162 bp in Kottayam and 164 bp in both Trivandrum and Kozhikode. At the INRA 063 locus, alleles 168 bp and 170 bp were predominant in Thrissur, 166 bp and 168 bp in Kottayam and 164 bp and 170 bp in Trivandrum. Goat populations of Kozhikode had a predominance of four alleles, viz. 164 bp, 166 bp, 168 bp and 170 bp. Common alleles for INRA 063 occurred towards the upper extreme of size in all the four goat populations. At the TGLA 68 locus, alleles 104 bp and 106 bp were more frequent in both Thrissur and Kottayam. In Trivandrum and Kozhikode, allele 104 bp recorded the highest frequency. At the BM 1329 locus, allele 171 bp was more frequent in

Kottayam and Trivandrum and allele 173 bp in Thrissur. In Kozhikode alleles 171 bp and 173 bp were predominant. In most cases, the frequency distribution of alleles did not follow a consistent pattern. These results are in accordance with data previously reported for microsatellite markers in Malabari goats (Jacob,2005; Bindu,2006; Seena,2006).

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 these traits. At the TGLA 68 locus, allele 104 bp was predominant in all the four goat populations investigated. The frequency of genotypes 102/104 and 104/106 were more frequent in all populations studied. Seena (2006) reported that the allele 104 bp is associated with a significantly higher litter size in Malabari goats. Another probable reason for the predominance of certain alleles in a population is the higher rate of inbreeding within the populations, due to low numbers of males in most domestic animal populations.

5.4.3.1 HUJ 1177

This marker was found to be the most polymorphic of the five markers used, detecting a total of 24 alleles in the goat populations analysed. The size range of alleles varied between 180 and 226 bp. The highest number of 19 alleles was observed in Thrissur followed by 16 in Kozhikode, 15 in Trivandrum and 10 in Kottayam. These observations were in concordance with that reported by other workers who used the same marker in goats. Jacob (2005) detected 21 alleles with a size range of 189-229 bp in four goat populations (Salem Black, Attapadi Black, Malabari and nondescript goats of Thrissur). Bindu (2006) recorded 19 alleles ranging in size from 188 to 230 bp and 62 genotypes in Malabari goats.

5.4.3.2 ILSTS 030

A total of sixteen alleles were detected at this locus with a size range of 148-178 bp. The numbers of alleles recorded were 13, 12, 11 and 10 in Thrissur, Kottayam, Trivandrum and Kozhikode, respectively. A total of 36 genotypes were reported in the pooled population. This finding is in accordance with the observations of Jacob (2005), who reported that this bovine primer was very well amplified in goats with a total number of 17 alleles and within a size range of 146-178 bp. Nineteen alleles were detected with an average size of 175 bp at this locus in Swiss goats by Saitbekova *et al.* (1999). Allele size range 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.

5.4.3.3 INRA 063

In the present study the bovine microsatellite primer INRA 063 was well amplified with caprine DNA. Eight alleles were observed at this locus considering all the four goat populations. The highest number of eight alleles was recorded in Kozhikode, followed by seven in Trivandrum and Kottayam. Lowest number of alleles (5) at this locus was observed in Thrissur. The size range of alleles varied between 158 and 172 bp. Pepin *et al.* (1995) reported that this bovine primer could produce strong amplification with only one band (in case of homozygous individuals) or two bands (in case of heterozygous individuals) of 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. Chenyambuga *et al.* (2004)

reported ten alleles with a size range of 141-179 bp in the goats of sub Saharan Africa. In Malabari goats a total of nine (Jacob,2005) and ten (Bindu,2006) alleles were recorded with a size range of 154-170 bp and 155-173 bp, respectively.

5.4.3.4 TGLA 68

A total of nine alleles with a size range of 98-114 bp were observed for this locus. The highest number of nine alleles were recorded in Kottayam, followed by eight in Thrissur and seven in Kozhikode, whereas the minimum was recorded in Trivandrum (6). A total of 21 genotypes were observed in the pooled population. Seena (2006) reported eight alleles with a size range of 98-114 bp and 12 genotypes for the microsatellite marker TGLA 68 in Malabari goats. Chu *et al.* (2003) reported two alleles with a size range of 98 and 100 bp for this locus in Small Tail Han sheep. The allele 104 bp was predominant in all the four goat populations investigated. A possible linkage with prolificacy may be the probable reason (Seena , 2006) .

5.4.3.5 BM 1329

This bovine marker 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. This non-specificity may be attributed to a lower degree of homology between this particular bovine microsatellite and the corresponding caprine sequence (Jacob, 2005). A total of twelve alleles were detected at this locus, ten each in Thrissur and Trivandrum followed by nine in Kozhikode and eight in Kottayam. The size of alleles ranged from 167 to 189 bp. A total of 34 genotypes could be observed at this locus. Chu *et al.* (2003) reported six alleles with a size range of 160 to180 bp in Small Tail Han sheep. Seena (2006) observed 15 alleles with a size range of 167 to195 bp and 34 genotypes in Malabari goats.

5.4.4 Intra Population Variation

Genetic diversity within each population was calculated as the mean number of alleles per locus, average heterozygosity and average PIC values. The highest mean number of alleles was found in Thrissur with a mean number of 11 per locus. The mean number of alleles observed in the present study was higher than that reported by Ganai and Yadav (2001) in three Indian goat breeds using heterologus microsatellite markers- Sirohi (4.12), Jamnapari (4.00) and Barbari (3.37). It is reported that there was a positive relationship between the number of dinucleotide repeats and number of alleles at a given locus and that the number of alleles per locus might range from one to 18 (Bishop *et al.*, 1994). Based on the heterozygosity and PIC values all populations were found to be more or less similar with values ranging from 0.8072 to 0.8278 for heterozygosity and 0.792-0.824 for PIC. In indigenous goat breeds of South Africa, the estimated heterozygosity values were 0.63 to 0.69 and in Boer goats, it was 0.49 (Visser *et al.* 2004). PIC values obtained in the present study were higher than that reported by Kim *et al.* (2002) for Korean (0.350), Chinese (0.620) and Saanen (0.570) goats. This indicates that high variability exist within each population with respect to these alleles.

5.4.4.1. Heterozygosity

Heterozygosity is the most widely used parameter to measure diversity within populations, defined by Nei (1973) as the probability that two alleles chosen at random from the population are different. The heterozygosity values were found to be high for all the populations at all loci and ranged from 0.736 to 0.92 with an overall average of 0.887,0.839,0.796, 0.783 and 0.804 for HUJ 1177, ILSTS 030, INRA 063, TGLA 68 and BM 1329, respectively. The mean heterozygosity values were more or less same for all populations studied. The higher values of heterozygosity indicated that the population has retained the presence of several alleles. This implies a substantial amount of genetic variability in goat populations that might be used in planning breeding strategies

particularly in population of small sizes. Furthermore, such variability could also be employed to detect genetic markers linked to quantitative trait loci (QTL) (Mukesh *et al.*, 2004). Similar values for heterozygosity were also reported in Malabari goat populations by other workers (Jacob,2005; Bindu,2006; Seena,2006). Since the marker was highly polymorphic and mean heterozygosity was high in Kozhikode, the population is considered to be highly diverse.

5.4.4.2. Polymorphic Information Content

Polymorphic Information Content (PIC) values which ranged from 0.717 to 0.918 revealed the polymorphic nature and suitability of the used set of microsatellite markers for the measurement of within breed diversity. The average values were 0.882, 0.833, 0.780, 0.764 and 0.789 for the markers HUJ 1177, ILSTS 030, INRA 063, TGLA 68 and BM 1329, respectively. The average PIC values were similar for all populations studied. Similar PIC values were also reported in Malabari goats (Jacob, 2005; Bindu,2006; Seena,2006). The high degree of PIC values indicated that these primers can be used for linkage mapping program and other applications like parentage control (Arora and Bhatia, 2006).

5.4.5 Inter Population Variation

5.4.5.1 Genetic Distance

Genetic distances estimated from polymorphic microsatellite markers have been the most popular method of choice to assess genetic diversity among populations (Toro and Caballero, 2005). Genetic distance measures the evolutionary divergence of two populations. In the present study, Nei's genetic distance between populations ranged from 0.3547 to 0.7203. The highest value of genetic distance was found to be between Thrissur and Kottayam goat populations, while the lowest value was recorded between Thrissur and Trivandrum. The results indicate no differentiation between the different

populations that can be linked to geographical separation. Goat populations of Thrissur, Trivandrum and Kozhikode showed a closer relationship. Jacob (2005) reported a low distance value between Malabari goats and nondescript goats of Thrissur. This is substantiated by the fact that cross breeding of nondescript goats and Malabari is practiced by goat farmers.

5.4.5.2 Dendrogram

A dendrogram was constructed from the genetic distance data using the POPGENE version 3.2. According to Takezaki and Nei (1996) unweighted pair group method with arithmetic mean (UPGMA) seems to be useful for allele frequency data when the evolutionary rate is nearly the same for all populations. Dendrogram based on UPGMA showed that Thrissur and Trivandrum goat populations were grouped together in one cluster and Kozhikode goat population was closer to it, while Kottayam population formed a unique branch. Dendrogram based on these genetic distances revealed no specific link with geographic distance. Similar pattern was observed by Kotze *et al.* (2004) in Kalahari Red goat populations of southern Africa.

The behaviour of different measures of genetic distances in the livestock context has been reviewed by Laval *et al.* (2002). They concluded that all distances strongly depend on the number of generations since the divergence and on the effective population size of the breeds and, therefore no phylogeny can be inferred from the tree in case of closely related breeds exhibiting different effective sizes. For this reason, it is generally assumed that in dealing with breeds of farm animals, the interpretation of trees in terms of phylogeny can be misleading. However, some authors (Barker, 1999) have argued that phylogenetic diversity provides the best objective criterion for making conservation decisions.

It was found that the set of microsatellite markers tested in this study could be used satisfactorily for molecular characterization and genetic diversity

studies in goat populations of Kerala. Quantitative and molecular measures of diversity produced unrelated distance values. Similar findings were reported by Crepaldi *et al.* (2001) in goat populations of Lombardy Alps. It may be suggested that the morphological distinctness of Thrissur goat population is mostly determined by environmental factors and selection pressure rather than by different origin. Conversely goat populations of Kottayam district seem to have diverged from the other populations at the genome level. However, use of more number of markers and increasing the sample size would allow a better differentiation and characterization of populations.

The genetic diversity studies based on molecular markers can be summarized as follows:

Some alleles were found to be more frequent in all populations studied, which is suggestive of possible linkage with traits of economic importance for which selection pressure has been applied. At the TGLA 68 locus, allele 104 bp was predominant in all the four goat populations investigated and a possible linkage of this with higher litter size has been pointed out earlier in Malabari goats. High rate of inbreeding may also be a possible reason. Based on the heterozygosity and PIC values, all populations were found to be more or less similar with high variability measures. A high amount of genetic variability in the populations can be employed to detect markers associated with quantitative trait loci. The high degree of PIC values obtained for all primers indicated that these primers can be used for linkage mapping program and other applications like parentage control. The results on genetic distance values and dendogram indicate no differentiation between the different populations that can be linked to geographical separation.

In the present study the diversity of goat populations in Kerala was studied using physical and biometrical traits and molecular biological markers. The intrapopulation and inter-population variations were studied in four different goat populations of the state. The study indicated that the variations found in

different populations based on physical traits were not in line with the variations found based on biometrical and molecular biological traits.

In physical traits, the diversity found was more or less similar in all populations studied. This was not reflected in biometrical traits like body measurements, body weight, peak yield and prolificacy. Thrissur population was found to be more uniform compared to other populations. All populations except Thrissur could be grouped together by cluster analysis based on biometrical traits. Based on molecular biological markers, Trivandrum and Thrissur populations could be grouped together and Kozhikode population was closer to it. Kottayam goat population was found to be distant. Qualitative, quantitative and molecular measures of diversity produced unrelated distance values. Since biometrical traits are more important from stand point of improving production, Trivandrum goats can be rated best followed by Kottayam and Kozhikode. This difference might have arisen because of local selection. A special feature to be noted is that on average, goats of north Kerala (Malabari) were not found to be distinct from goats of other parts of the state. Rigorous selection of the native population to explore the heterozygosity existing in different goat populations is the best way to improve the performance of goats of our state.

Summary

6. SUMMARY

Goat population in Kerala is highly variable. Local selections were reported to have favorable characters like high growth rate, increased prolificacy, higher milk yield and low disease incidence. The intra population and inter population variability of goats of Kerala have not been scientifically studied so far. Hence, a study was conducted to assess the diversity of goat populations associated with different geographical areas in Kerala, based on physical and biometrical traits and molecular markers. Four hundred adult female goats, one hundred each from Trivandrum, Kottayam, Thrissur and Kozhikode districts of Kerala, were used in the study.

The physical characters observed were the presence of horns/tassels/ beard, ear size, coat colour and hair pattern. More than 70 per cent of animals studied in all these goat populations had white coat colour or a combination of white with either black or brown, and were predominantly horned. Percentage of animals with tassels was high in Kozhikode goat population (14). Beard was absent in majority of animals studied. Ears were of medium type. Short haired animals were predominantly seen in all goat population except in Thrissur, where a higher percentage (48 per cent) of medium and long haired varieties was observed. Based on physical traits, no distinction could be made between different populations except for hair pattern and Malabari goats of Kozhikode were not distinct from other populations.

Biometrical characters observed in the present study were body measurements (head length, head width, height at withers, chest depth, chest girth, shoulder point width, rump length, rump width, shin circumference and body length), peak yield and prolificacy. The mean body length, chest girth and height at withers of adult female goats were 67.63 ± 0.56, 75.35 ± 0.57 and 70.78 ± 0.49cm in Trivandrum, 63.94 ± 0.55, 73.28 ± 0.56 and 67.2 ±0.43cm in Kottayam, 58.68 ± 0.48, 66.68 ± 0.64 and 62.34 ± 0.41cm in Thrissur and 65.04 ± 0.56, 70.98 ± 0.64 and 65.38 ± 0.46cm in Kozhikode populations, respectively.

Body weights of adult female goats were predicted using the regression equation based on the power function of chest girth. At 18 to 24 months, the mean body weights, in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 29.15 ± 0.66, 28.19 ± 0.45, 22.86 ± 0.41 and 26.92 ± 0.56 kg, respectively. At 24 to 36 months, the values for body weight were 29.92 ± 0.52, 29.55 ± 0.62, 25.73 ± 0.82 and 28.02 ± 0.87 kg, respectively. Weight recorded at 36 to 48 months in the respective populations were 32.29 ± 0.63, 30.32 ± 0.79, 26.64 ± 0.56 and 30.25 ± 0.75kg, respectively. The body weights above 48 months, were 32.79 ± 0.64, 30.95 ± 0.69, 29.37 ± 0.72 and 30.70 ± 0.86 kg, respectively. Animals belonging to Trivandrum had higher average for all traits, which was also reflected in the body weight predicted. Thrissur animals had lowest average for all body measurements and had low predicted body weight. Animals belonging to Kottayam and Kozhikode came in between.

For peak yield, highest value was recorded in Kottayam (832.5 ± 35.06ml), followed by Kozhikode (755 ± 38.49ml) and Trivandrum (699.5 ± 40.29ml), while the lowest value was recorded in Thrissur (444 ± 25.41ml).

The percentage of multiple births was higher in Trivandrum (68) compared to other populations, indicating high prolificacy. Percentage of multiple births recorded in Kozhikode, Kottayam and Thrissur populations were 61, 58 and 55, respectively.

Canonical discriminant analysis was performed using ten morphometric variables. The variables having high discriminative capacity were head width, height at withers, chest depth, rump length, rump width, shin circumference and body length. Variables head width and body length were the most discriminative in canonical correspondence with the ordinate axis (CAN1) and the shin circumference and rump length with coordinate axis (CAN2). The Mahalanobis distance (D^2) estimated between the goat populations based on morphometric measurements revealed the greatest distance value between Thrissur and Trivandrum populations (9.13866), while the Kottayam and Kozhikode

populations had the least distance value (3.04962). The Mahalanobis distance between Trivandrum and Kottayam population was 3.9794, between Trivandrum and Kozhikode populations, 3.35988, between Kottayam and Thrissur populations 6.94518, and that between Thrissur and Kozhikode populations was found to be 7.8152.

Discriminant analysis based on body weight, peak yield and prolificacy revealed that only body weight and peak yield variables have significant discriminative capacity. The Mahalanobis distance estimated between the four goat populations indicated that goat populations of Thrissur district were more distant from all other populations. Greatest distance value (D^2) was obtained between Thrissur and Kottayam goat populations (2.30842), while the least distance was recorded between Kottayam and Kozhikode populations (0.19238). D^2 value estimated between Thrissur and Trivandrum population was 2.10488, between Thrissur and Kozhikode populations, 1.18574, between Kottayam and Trivandrum populations, 0.37338, and that between Trivandrum and Kozhikode was found to be 0.58476. Goat populations of Trivandrum, Kottayam and Kozhikode formed one cluster.

Trivandrum population had higher averages for all morphmetric traits and the predicted body weight and Trichur animals recorded lowest. But when body weight, peak yield and prolificacy were considered populations of Trivandrum, Kozikode and Kottayam formed one cluster. Body weight and peak yield were the traits by which the populations were mainly discriminated.

Disease conditions reported in adult females of the four goat populations were respiratory infections, deficiency diseases, mastitis, fungal infections and digestive disorders. Percentage of adult female goats reported to have the occurrence of any of these disease conditions during the period 2006-2007 in Trivandrum, Kottayam, Thrissur and Kozhikode goat population was 11, 21, 14 and 8, respectively. Kid mortality percentages during pre-weaning stage in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 4.49,

6.43, 15.43 and 2.92, respectively. The mortality and disease incidence reported were high in Kottayam population.

Microsatellite analysis was performed by PAGE, using radio labeled isotope. Five microsatellite markers, viz. HUJ 1177, ILSTS 030, INRA 063, TGLA 68 and BM 1329, which were reported to be highly polymorphic in Malabari goats, were selected for the study.

The number of alleles observed for HUJ 1177 loci in Thrissur, Kottayam, Trivandrum and Kozhikode populations were 19, 14, 15 and 16, respectively. Twenty-four alleles ranging in size from 180-226bp and 66 genotypes were detected in the pooled population. Heterozygosity values calculated at this locus were 0.892, 0.843, 0.920 and 0.892 in Trivandrum, Kottayam, Thrissur and Kozhikode, respectively and the PIC values recorded were 0.889, 0.833, 0.918 and 0.890 in the respective populations.

A total of 13, 12, 11 and ten alleles could be observed for ILSTS 030 locus in goats of Thrissur, Kottayam, Trivandrum and Kozhikode, respectively. The numbers of genotypes observed in the respective populations were 16, 11, 14 and 10, respectively. A total of 36 genotypes were observed in pooled population. Heterozygosity values obtained at this locus were 0.853, 0.845, 0.847 and 0.810 in Trivandrum, Kottayam, Thrissur and Kozhikode, respectively and the PIC values reported were 0.889, 0.833, 0.918 and 0.890 in the respective populations.

A maximum number of 13 genotypes were observed for INRA 063 locus in goats of Kozhikode, while the lowest number of seven was recorded in Thrissur. The maximum number of alleles at this locus was observed in Kozhikode (8) whereas the minimum was recorded (5) in Thrissur. Seven alleles each were recorded in Trivandrum and Kottayam. Heterozygosity values observed at this locus were 0.784, 0.797, 0.759 and 0.845 in Trivandrum,

Kottayam, Thrissur and Kozhikode, respectively and the PIC values recorded were 0.889, 0.833, 0.918 and 0.890 in the respective populations.

Nine alleles were detected at the locus TGLA 68 with a size ranging from 98-114 bp. The maximum number of alleles at this locus was observed in Kottayam (9), whereas the minimum was recorded in Trivandrum (6). A maximum number of 13 genotypes were observed in Thrissur, while the lowest number of 9 was recorded in Trivandrum. Heterozygosity values calculated at this locus were 0.789, 0.772, 0.774 and 0.798 in Trivandrum, Kottayam, Thrissur and Kozhikode, respectively and the PIC values obtained were 0.772, 0.748, 0.751 and 0.783 in the respective populations. The alleles 104 and 106 bp were predominant in all goat populations investigated, suggesting a probable linkage with traits of economic importance.

At BM 1329 locus twelve different alleles could be detected with a size range of 167-189 bp. A total of 34 genotypes could be detected at this locus. Heterozygosity values observed at this locus were 0.839, 0.837, 0.736 and 0.794 in Trivandrum, Kottayam, Thrissur and Kozhikode, respectively and the PIC values recorded were 0.831, 0.831, 0.717 and 0.780 in the respective populations.

The total number of alleles for 5 loci reported in Trivandrum, Kottayam, Thrissur and Kozhikode goat populations were 49, 50, 55 and 50, respectively with an average value of 9.8, 10, 11 and 10 per locus in the respective populations. The mean heterozgosity values were 0.8264, 0.8188, 0.8072 and 0.8278 and the mean PIC values were 0.8240, 0.8072, 0.7920 and 0.8184 in the respective populations. All populations remained highly heterozygous with respect to the loci screened.

Nei's genetic distance between populations ranged from 0.3547 to 0.7203. The highest value of genetic distance was found to be between Thrissur and Kottayam goat populations, while the lowest value was recorded between Thrissur and Trivandrum. Dendrogram based on UPGMA showed that Thrissur

and Trivandrum goat populations were grouped together in one cluster and Kozhikode goat population was closer to it, while Kottayam population formed a unique branch.

The results of microsatellite analysis are not in line with that reported for physical and biometric characters. The mean heterozygosity and PIC values are higher in all populations studied indicating the possibility of using these markers to unravel the variability at molecular level in all these populations. 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 these traits. At the TGLA 68 locus, allele 104 bp which was reported to have associated with high litter size was predominant in all the four goat populations investigated. Another probable reason for the predominance of certain alleles in a population is the higher rate of inbreeding within the populations, due to low numbers of males in most domestic animal population.

The results indicated that these local goat populations were not very distinct and more uniform in physical characteristics. However, biometrical and molecular measures of variation revealed unrelated distance values having no specific link with geographical distance. The results obtained for Malabari (Kozhikode population) indicated that this population is not distinct from other populations of the state and there is a connecting link between all these populations. Though use of more number of molecular markers possibly may explain the variation encountered in these populations, the present investigation reveals that selection within each population for traits of economic importance like body weight and milk production is the best method to improve the performance of goats of Kerala.

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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 was 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 Gelmix	60 ml
TEMED	0.125 ml
Ammonium persulphate (10 %)	0.125 ml

Mixed well without air bubbles.

EDTA (0.5*M*, pH 8.3)

Dissolved 18.61 g 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 in 10 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	10m*M*

Gel loading buffer

Bromophenol blue	0.25 %	50 mg
Xylene cyanol	0.25 %	50 mg
Sucrose	40 %	8 g

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

Phenol (Saturated, pH 7.8)

Commercially available crystalline phenol melted at 65°C in a water bath. Hydroxyquinolone 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 minutes in 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 minutes. 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

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, stored at 4 °C.

Sodium dodecyl sulphate (SDS) 20 %

SDS	20 g
Distilled water 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 (pH 8.0)	20 ml
Distilled water up to	1000 ml

Autoclaved and stored at room temperature.

Tris-Borate (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 (0.5 X)

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

Mixed well in 700 ml distilled water, volume made up to 1000 ml 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 pH adjusted to 7.4. Made up the volume to 1000 ml, filtered, autoclaved and stored at 4°C.

Tris EDTA (TE) buffer (pH 8.0)

Tris base	10 mM	1.2114 g
EDTA	0.1 mM	0.3722 g

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

Tris 1M (pH 8.0)

Tris base	121.14 g

Added distilled water up to 1000 ml, pH adjusted to 8.0, filtered and *stored at room temperature.*

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.
PNK	-	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
RAPD	Random Amplified Polymorphic DNA
DNA	Deoxy Nucleic Acid
PAGE	Polyacrylamide Gel Electrophoresis
PIC	Polymorphic Information Content
EDTA	Ethylene Diamine Tetraacetic Acid
DTT	Dithiothreitol
TEMED	N, N, N′, N′ Tetramethylethylenediamine
APS	Ammonium Persulphate
PNK	Polynucleotide Kinase
He	Heterozygosity
µl	microlitres
µg	microgram
mg	milligram
mM.	millimolar
cm	centimeter
nm	nanometer
mCi	millicurie
Kb	Kilo basepair
Rpm	Revolutions per minute
SDS	Sodium Dodecyl Sulphate
SPSS	Statistical Package for Social Sciences

dNTP.	Deoxy Nucleotide Triphosphate
ddATP.	Dideoxy Adenosine Triphosphate
ddCTP	Dideoxy Cytosine Triphosphate.
ddGTP.	Dideoxy Guanosine Triphosphate
ddTTP.	Dideoxy Thymidine Triphosphate

GENETIC AND PHENOTYPIC VARIATIONS OF GEOGRAPHICALLY DIFFERENT GOAT POPULATIONS OF KERALA

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ABSTRACT

Genetic diversity of four geographically different goat populations in Trivandrum, Kottayam, Thrissur and Kozhikode districts in Kerala was analysed based on physical, biometrical traits and microsatellite markers.

Based on physical traits, the populations were not very distinct and uniformity was seen with respect to coat colour, horn pattern, presence or absence of tassels or beard and hair pattern. The animals were predominantly horned and short haired with coat colour of white or a combination of white with either black or brown. Majority of animals did not possess tassels or beard.

The biometrical traits observed in the present study were body measurements, peak yield and prolificacy. Body weight of adult female goats was predicted using the regression equation based on the power function of chest girth. It was shown that the animals of Trivandrum district had higher values for almost all body measurements and predicted body weight, while the Thrissur population had lowest values for these growth traits. Animals of Kottayam and Kozhikode came in between. Percentage of multiple births was also higher in Trivandrum goat population (68) compared to other populations indicating high prolificacy. Highest peak yield was recorded for Kottayam goat population.

Discriminant analysis based on morphometric measurements revealed that the most discriminative variables were head width and body length, followed by shin circumference and rump length. Mahalanobis distance calculated based on morphometric traits indicated that Thrissur and Trivandrum populations were more distant, while least distance was observed between Kottayam and Kozhikode. Discriminant analysis based on body weight, peak yield and prolificacy revealed that only body weight and peak yield variables have significant discriminative capacity. Trivandrum, Kottayam and Kozhikode

populations were grouped together in one cluster based on the distance value. Thrissur population was distant from all other populations.

Microsatellite analysis revealed that all the five loci were highly polymorphic with five to nineteen alleles in different populations. The total number of alleles, the mean number of alleles per locus, mean heterozygosity and mean polymorphic information content were 51, 10.2, 0.8201 and 0.8104, respectively. The values indicate that the markers can be successfully used to study the variations existing in the populations. Based on Nei's genetic distance Thrissur and Trivandrum population were grouped together first and then with Kozhikode population, while the Kottayam population formed a unique branch in dendrogram.

Unrelated distance values produced by quantitative and molecular measures of variation may be attributed in part to the environmental influences and local selection pressures. Though use of more number of markers may possibly explain the variation encountered in these traits, the present investigation reveals that selection within each population for traits of economic importance like body weight and milk production is the best method to improve the performance of goats of Kerala.