MARKER ASSISTED SELECTION FOR MILK PRODUCTION TRAITS IN VECHUR CATTLE

SHYMAJA UTHAMAN

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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

DECLARATION

I hereby declare that this thesis entitled **"MARKER ASSISTED SELECTION FOR MILK PRODUCTION TRAITS IN VECHUR CATTLE"** is a bonafide record of research work done by me during the course of research and that this 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.

Shymaja U

Place : Mannuthy,
Date : 22-8-2005

SHYMAJA UTHAMAN

CERTIFICATE

Certified that the thesis entitled **"Marker Assisted Selection for milk production traits in Vechur cattle"** is a record of research work done independently by Dr. Shymaja Uthaman, under my guidance and supervision and that it had not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr. K.V. Raghunandanan
(Chairman, Advisory Committee)
DIRECTOR
Centre for Advanced Studies in
Animal Genetics and Breeding
College of Veterinary and Animal
Sciences,Mannuthy

Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Shymaja Uthaman,** a candidate for the degree of Master of Veterinary Science in Animal Breeding and Genetics, agree that this thesis entitled **"MARKER ASSISTED SELECTION FOR MILK PRODUCTION TRAITS IN VECHUR CATTLE"** may be submitted by Shymaja Uthaman, in partial fulfillment of the requirement of the degree.

Dr. K.V. Raghunandanan
(Chairperson, Advisory Committee)
Director
Centre for Advanced Studies in Animal Genetics and Breeding
College of Veterinary and Animal Sciences, Mannuthy, Thrissur

Dr. K. Anilkumar
(Member, Advisory Committee)
Assistant Professor
Department of Animal Breeding
and Genetics
College of Veterinary
and Animal Sciences
Mannuthy

Dr. A.P.Usha
(Member Advisory Committee)
Assistant Professor
Department of Animal Breeding
and Genetics
College of Veterinary and
Animal Sciences
Mannuthy

Dr. G. Girish Varma
(Member, Advisory Committee)
Assistant Professor
Department of Physiology
College of Veterinary and Animal Sciences
Mannuthy

External Examiner
(C. S. Nagaraja)
Associate Professor
Dept. of ABGB
Veterinary College
Hebbal, Bangalore-24

ACKNOWLEDGEMENT

My unreserved gratitude and indebtedness goes to Dr. K.V.Raghunandanan, DIRECTOR, CASAGB and the chairperson of the advisory committee. His able guidance, creative suggestion, sustained encouragements and critical comments throughout the course of my study was the major factor that helped me in my accomplishment.

I honestly acknowledge and express my profound gratitude to Dr. Anilkumar, K., Assistant Professor, Dept. of Animal Breeding and Genetics and member of the Advisory Committee for his personal attention, moral support, expert advise and meticulous guidance extended to me liberally. His incessant support and keen interest right from the beginning of the research work till shaping up of the thesis in its present form have been a source of great inspiration and motivation.

I am greatly indebted to Dr. A.P. Usha, Assistant Professor, Dept. of Animal Breeding and Genetics, and member of the Advisory Committee for her valuable guidance and immense generosity extended to me in the supply of all materials required for the conduct of the research work.

I extend my heartfelt thanks to Dr. G. Girish Varma, Assistant Professor, Dept. of Physiology for his valuable advice and help proffered to me in his respective position as the member of the Advisory Committee.

I am thankful to Dr. K.C. Raghavan, Associate Professor, Dr. T.V. Aravindakshan, Assistant Professor and Dr. K.A. Bindu, Assistant Professor of Dept. of Animal Breeding and Genetics for their expert advice and support offered throughout the course of the study.

The facilities provided by Dr. T.V. Viswanathan, Head, Department of Nutrition and Dr. M. Munkundan, Head, Department of Dairy Science for the analysis of milk samples are greatly acknowledged. I also thank Dr. Dr. K. Ally, Assistant Professor, Dept. of Nutrition for her expert advice and help rendered.

I am grateful to Mr. K.K. Gopalan and all the labourers of the Vechur Conservation Centre for their whole hearted help and kindness rendered to me in the collection of data and samples from the farm.

The constant inspirations given to me by Dr. Naicy Thomas, my colleague and companion had helped solve many problems thanks to her logical thinking and loving concern.

The sincere help and co-operataion rendered to me by my colleague and friend Dr. Amrita Susan Jacob is hereby gratefully remembered. I also thank my colleague Dr. Jeeba. K. George for her concern and well wishes.

The invaluable help, assistance and advice offered to me by my seniors Dr. Preethy, M.S., Dr. Bindu Mathew and Dr. Kowsigaraj are hereby duly acknowledged.

A special thanks to my other colleagues, Dr. Seena, T.X., Dr. Sharad Jose and Dr. Ariprasath for their encouragement and support.

I express my thanks to my friends, Dr. Smitha, N.F. and Dr. Lekshmy, M.A. for their concern, advice and encouragement rendered to me during the entire course of the study.

I am short of words to express my deep sense of gratitude and indebtedness to my uncle Mr. C.H. Raveendran, whose personal concern, moral and unconditional support, constant encouragement and timely financial help had enabled me the successful completion of my research.

I express my heartfelt gratitude and thanks to my relatives and friends, Mr. C.H. Surendran, Mr. C.H. Rajendran, Mrs. Chrisilda Devdas, Mr. T.C. Prakashan, Mrs. Ajitha Asokan and their family for their loving concern and help rendered to me at times of distress.

It is beyond words to express my love and gratitude to my mother for her loving concern, kind hearted co-operation and understanding. I also thank my grandfather for his personal concern and constant support.

Finally the blessings of my late father Mr. C.H .Uthaman had given me courage and determination throughout the course of my study.

I also thank the Almighty for enabling me to complete this work in time.

Shymaja Uthaman

CONTENTS

LIST OF TABLES

LIST OF PLATES

Introduction

1. INTRODUCTION

The legendry Vechur cattle is considered as one of the dwarf cattle breeds of the world. It originated from Vechur village of Kottayam district in Kerala. The dwarfness of this breed is emphasized by the fact that its height ranges from 83-105 cm for bulls and 81-91 cm for cows. The coat colour of the animals of this breed can be light red, black, fawn or white. Farmers favour Vechur cattle because of their small body size, good adaptation and high disease resistance traits. The Vechur cattle are usually maintained by the farmers of Kerala as house cows on the fodder available in and around the house and the kitchen wastes. A Vechur cow produces on an average 2-3kgs of milk per day which is mainly used for household consumption. These animals are also highly adapted to hot humid climate of the state and hence do not require sophisticated managemental practices.

One of the unique characteristics of Vechur breed is the smaller size of the milk fat globules when compared to that of other cattle breeds. The mean size of the fat globules in Vechur cattle was found to be 3.21μ and they range from 2.54-4.07μ. The size of milk fat globule of Vechur cattle is higher than that of goat, but smaller than that of crossbred cattle and buffalo. Estimation of iodine value for milk fat indicates that Vechur milk has less unsaturated fatty acids when compared to the milk of other cattle breeds. The smaller size of fat globules associated with larger surface area indicates high levels of phospholipids. Phospholipids play a major role in the development of the nervous system. Higher levels of saturated fatty acids enhance the digestibility of fat, facilitating the use of Vechur milk in malabsorption syndromes. Milk of Vechur cows is believed to be more suitable for feeding infants and the sick.

The Government policy of grading up local cattle with Red Sindhi was started in 1950's. The massive crossbreeding policy was applied to all the local cattle including Vechur cattle thereby increasing the population of crossbreds in the state. The crossbred cows produce double the amount of milk than indigenous cows

making them more preferable. This has led to an almost near extinction of the Vechur cattle.

The Vechur cows produce relatively high quantity of milk when compared to other local genotypes of cattle in the state. In order to exploit this ability, there arose a necessity to identify genetically superior animals and use them for breeding purposes. Earlier, selection was performed based on the individuality or on the pedigree records. With the introduction of artificial insemination, it was possible to assess the superiority of the sires based on their large number of progenies. Even though this method of selection is widely used, the rate at which genetic progress made is rather slow. To increase the rate of genetic progress, selection has to be performed at a very early age itself and with higher degree of accuracy when compared to conventional methods.

Recent advances made in quantitative genetics suggest that genetic gain can be achieved using genetic markers that are closely linked to genes that affect quantitative traits. It is possible to detect linkage of QTL to marker loci by scoring the individuals genotype at the marker locus and their phenotype for the quantitative trait. From the difference in the mean phenotype among marker genotype classes, the presence of QTL linked to marker can be inferred. This makes possible to select animals based on their genotype at the QTL in a procedure called Marker Assisted Selection (MAS).

The use of microsatellite markers in the whole genome scans led to the discovery of several QTL's affecting the various economic traits located on several different chromosomes of the bovine genome. Microsatellite markers were found to be linked to QTL and this formed the basis for MAS. A high density genetic linkage map gives all the information on the different polymorphic microsatellite markers associated with the QTL of interest. It is therefore possible to identify the individuals inheriting the alleles for marker loci linked to a favourable allele at the QTL. Marker assisted selection also increases the scope for selection of QTL and making selection decisions even before the trait is manifested.

The Vechur cattle because of its unique characteristics are of great demand by the farmers and animal lovers in different parts of the state and country. Presently random mating is being carried out for the cattle maintained at the Vechur Conservation Centre, care taken to avoid inbreeding. This cattle have not been subjected to any form of selection be it conventional or advanced. Marker Assisted Selection is the most advanced method of selection currently practiced. MAS can be applied on the Vechur cattle in order to select genetically superior animals pertaining to economic traits.

A successful attempt had been made for molecular characterization of Vechur cattle, maintained at the Vechur Conservation Centre, Mannuthy. No attempt has so far been made to perform MAS in the existing Vechur cattle population. The purpose of this study was to study the association of different microsatellite markers with the economic traits for milk composition phenotypes like milk fat percentage, protein percentage, solids not fat (SNF) and production parameters like age at first calving (AFC) and intercalving period so as to apply this information for future selection.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ISOLATION OF GENOMIC DNA

DNA sample for use in microsatellite typing can be obtained from the blood, semen, milk and tissue samples. Different methods are adopted for extraction of DNA from various tissues. The method for extraction of DNA from blood and semen are reviewed.

2.1.1 Blood

Andersson *et al.* (1986) isolated DNA from 10 ml of whole blood by using proteinase K followed by phenol, chloroform – isoamyl alcohol extractions to remove the protein. DNA was recovered by ethanol precipitation.

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.

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 the nucleated cells of the buffy coat obtained from anti-coagulated blood.

Oliver *et al.* (1989) prepared DNA from 25 ml of blood collected in heparinised vacutainers using phenol chloroform method of isolation. The DNA was spooled out after precipitation with ethanol, dried and resuspended in TE buffer.

Trommelen *et al.* (1993) extracted DNA from blood using phenol chloroform method. Ethanol precipitated DNA was dried and dissolved in 10m*M* Tris HCl (pH 8.0) and 3µg/ml of 1m*M* Sodium EDTA.

Apparao *et al.* (1994) developed a rapid and modified method to extract genomic DNA from the venous blood of cattle, buffaloes, goat, sheep and pigs.

Aravindakshan *et al.* (1998) compared three different methods of DNA extraction (Guanidine hydrochloride method, phenol – chloroform extraction method and high salt method) and concluded that extraction of DNA from cattle white blood cells by both phenol chloroform and high salt methods produced good yield of high molecular weight DNA.

2.1.2 Semen

Andersson *et al.* (1986) used a modified protocol for isolation of DNA from semen samples. The semen sample was treated with sperm lysis buffer and proteinase K to digest the proteins followed by standard phenol and chloroform – isoamyl alcohol extraction.

Bahnak *et al.* (1988) modified the method used for isolation of RNA to extract DNA and found that sperm cells readily lysed in guanidinum thiocyanate without homogenization and resulted in good yields of DNA.

Trommelen *et al.* (1989) used a modified method to extract the DNA from semen where the pellet consisting of sperm cells was resuspended in 10m*M* Tris HCl (pH-8.0), 10m*M* Sodium EDTA, 100m*M* NaCl, 5% SDS, 500µg/ml of proteinase K and 6mg/ml of dithiothretiol. After incubation at 65°C for 4 hours, DNA was extracted by phenol chloroform method.

Ng-Kwai-Hang *et al.* (1991) extracted DNA from semen of one to two insemination straws after washing the sperms with PBS to remove the diluent. The pellet was then treated with sperm lysis buffer followed by phenol and chloroform-isoamyl alcohol extraction of DNA.

Nadesalingam *et al.* (2001) extracted DNA from semen straws (200-500 µl). Semen was washed thrice in I X SSC and 2m*M* EDTA to remove the cryoprotectant and then treated with proteinase K to remove the contaminating epithelial cells before the extraction process.

2.2 MICROSATELLITE ANALYSIS

2.2.1 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 10 fold increase in amount of target sequence. Analysis of allelic variation with as little as 1 ng of DNA was possible with PCR.

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 products amplified.

White *et al.* (1989) stated that the product of each cycle of polymerase chain reaction can again serve as a template for attachment of primer thereby doubling the amount of DNA fragment produced in the previous cycle. Each cycle results in exponential accumulation of a specific target to several millions in few hours.

Weber and May (1989) used polymerase chain reaction to type polymorphic DNA markers and amplification was possible using a simple template molecule.

Eckert and Kunkel (1990) described Taq polymerase as the enzyme of choice in amplification of genetic information. This enzyme is capable of DNA synthesis using a wide range of in vitro reaction conditions though it lacks 3' – 5' proofreading exonuclease activity. Fidelity of DNA synthesis *in vitro* by Taq DNA polymerase responds strongly to change in relative $MgCl_2$ concentrations and pH.

The concentration of enzyme and primers as well as the annealing and extension time and number of cycles affect the specificity of the PCR (Erlich *et al.*, 1991).

Don *et al.* (1991) interpreted the appearance of spurious small bands in the PCR product spectrum to be due to mispriming of one or both of the oligonucleotide

primers internal or external to the target template. The occurance of spurious bands can be reduced by increasing the annealing temperature in PCR.

Arezi *et al.* (2003) stated that PCR being an exponential process, very small changes in the amplification efficiency can result in dramatic differences in the amount of final product even if the initial number of target molecules is the same.

2.2.2 Evaluation of allelic size

Dideoxy sequencing ladders consists of four chain terminating reactions G, A, T, C which are run along with amplified microsatellite products to assign band size in order to facilitate genotyping in microsatellite analysis.

Sanger *et al.* (1977) devised a new method for determining the nucleotide sequences in the DNA by making use of the 2'-3'- dideoxy and arabinonucleoside analogues of the normal deoxynucleoside triphosphates, which act as specific chain-terminating inhibitors of DNA polymerase.

According to Biggin *et al.* (1983), incorporation of (dATP [α^{35}S]), instead of [α^{32}P] dATP as the radioactive label in dideoxy sequencing reactions lead to an improved resolution of bands in a sequencing gel autoradiograph. The decay characteristics of ^{35}S are well suited for use in the dideoxy sequencing method.

A chemically modified phage T7 DNA polymerase was used for DNA sequencing by dideoxy method to increase the electrophoretic resolution and to ensure uniform radioactive intensity of the bands in the gels (Tabor and Richardson, 1987).

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

Tautz (1989) resolved PCR reaction products directly on 6% sequencing gel using sequencing reaction as marker.

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

Allelic size of microsatellite PCR product can be evaluated by running the amplified products along with DNA sequencing reaction sample on a polyacrylamide gel. The size of G, A, T, C dideoxy termination band can be calculated by its distance from the sequencing primer and the size of the PCR product can be determined by comparison with the bands of M13 ladder (Oh and Mao, 1999).

2.2.3 Endlabeling

Incorporation of radioactivity in the PCR enables the visualization of the amplified microsatellite products run on polyacrylamide gels. This is possible in two ways- (i) the radioisotope can be directly incorporated in the polymerase chain reaction or (ii) the forward primer can be endlabeled with the radioisotope.

γ^{32}P ATP, γ^{32}P CTP and α^{35}S dATP are the isotopes commonly used for this purpose. Endlabeling of forward primer with radioisotope is reviewed.

Gyllensten and Erlich (1988) labeled oligonucleotides with (γ^{32}P) dATP by using polynucleotide kinase.

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

Bacteriophage T4 polynucleotide kinase catalyses the transfer of the γ-PO_4 of ATP to a 5' terminus of DNA or RNA (Sambrook *et al.*, 1989).

The 5' end of the primers were endlabeled using γ^{32}P-ATP and polynucleotide kinase and 1 p*M* of this labeled primer was used in each PCR reaction (Tautz, 1989).

The insulin – like growth factor I (IGF1)CA strand oligonucleotide primer was endlabeled for 1 hour at 37°C in a 50 µl reaction consisting of 72 p*M* (480 ng) primer, 33 p*M* γ^{32}P-ATP at 3000Ci/m*M*, 10m*M* $MgCl_2$, 5m*M* DTT, 150m*M* Tris (pH – 7.6) and 50 units T4 polynucleotide kinase (Weber and May, 1989).

Bishop *et al.* (1994) stated that ^{32}P endlabeled primers are preferred over direct incorporation of ^{32}P into amplified products. Direct incorporation resulted in increased sub banding and hindered scoring.

According to Kappes *et al.* (1997) primers of amplified products can be endlabeled to reduce sub banding.

Segev *et al.* (1998) endlabeled one of each pair of primer with T4 kinase and $\gamma(^{32}P)$ ATP. 4ng of this labeled primer and 20 ng of the unlabeled primer were used in a 10 µl PCR reaction containing 5-20 ng genomic DNA.

A 50 µl endlabeling reaction consists of 40 p*M* of one primer, 1 µl bovine serum albumin 15 µl of 10 X T4 kinase buffer, 40 µCi $(\gamma^{32}P)$ATP and 10U T4 DNA polynucleotide kinase. Mixture was allowed to incubate for 10 min. at 37°C followed by heat inactivation of the enzyme at 95°C for 5 minutes (Oh and Mao, 1999).

Nadesalingam *et al.* (2001) used 0.5 p*M* of kinase endlabeled (γ^{32}P dCTP) forward primer in 12.5 µl volume PCR reaction.

2.2.4 Electrophoresis

The amplified PCR products are run on denaturing polyacrylamide gels to facilitate the separation of the microsatellite products according to their size.

Biggin *et al.* (1983) used gel containing 6% acrylamide and 460 gms of urea/liter, polymerized by addition of 1.5 µl of 25% ammonium persulfate stock solution and 1.5 µl of N, N, N', N' – tetramethyl ethylene diamine per ml of gel mix. The gels were dried and saran wrap removed for subsequent autoradiography of ^{35}S – labeled reaction products.

Tabor and Richardson (1987) electrophoresed sequencing reactions incorporated with (α-[^{35}S] thio)dATP on polyacrylamide gels containing 7M Urea and buffer of 100 m*M* Tris borate (pH 8.3) and 1 m*M* EDTA. Kodak OM-1 films were used for autoradiography to provide higher contrast and resolution.

3-5 µCi of α- [^{32}P]dCTP was included in each PCR reaction tube and the amplified samples were run in alternate lanes of a DNA sequencing gel. Gels were autoradiographed for 1-3 days usually without intensifying screens (Litt and Luty, 1989).

Weber and May (1989) used γ^{32}PATP endlabeled primer in 50µl PCR reaction. Aliquots of amplified DNA along with two volume of formamide sample buffer were electrophoresed on standard polyacrylamide sequencing gels after which the gels were fixed, dried and processed for autoradiography.

Tautz (1989) used γ^{32}PATP endlabeled primer in the PCR reaction. The amplified PCR products were resolved on 6% sequencing gels, gel dried and autoradiographed.

Bishop *et al.* (1994) radioisotopically labeled PCR products with 0.1 µCi of [α-^{32}P] dATP. The amplified products diluted with equal volume of loading buffer was electrophoresed on 7% denaturing polyacrylamide gels between 3 and 6 hrs at 40 V/cm. Gels were vacuum dried for 15 min onto 3MM chromatography paper and exposed overnight on X-ray film.

Primers endlabeled with γ[^{32}P] ATP was used in 10 µl PCR reaction and the products were separated on a 40% formamide and 8.3 M/L urea – 6% polyacrylamide gels. The gels were dried and autoradiographed for 4-48 hrs at room temperature or at 70°C (Segev *et al.*, 1998).

Microsatellite PCR products are analysed either on polyacrylamide or high resolution agarose gels. Polyacrylamide gels offer better band resolution and higher sensitivity when used with labeled PCR products (Oh and Mao, 1999).

Curi and Lopes (2002) used 6% polyacrylamide gel to permit good separation of amplified DNA fragments of different sizes. A constant potency of 40W was applied for 2-4 hrs (depending on the average size of the alleles) for fragment migration.

2.2.5 Microsatellite Markers

Microsatellites are short (1-6 bp) simple tandemly repeated nucleotide sequences present as multiple copies in the genome.

Litt and Luty (1989) stated that $(TG)_n$ repeats (microsatellites) might be polymorphic when 12 different allelic fragments was detected in 37 unrelated individual by using PCR to amplify the microsatellite.

Simple sequence stretches are generally considered to be hypervariable in length, this variability mostly resulting from slippage occurring during replication or DNA repair. Simple sequence loci hypervariability thus opens a new way for determining individual identity (Tautz, 1989).

Weber and May (1989) stated that variation in the number of repeats within a block of tandem repeats appear to be a universal feature of eukaryotic DNA, regardless of length of the repeat. The DNA sequences flanking microsatellite sequences can be used as primers to PCR amplify tandem repeat sequences in specific genomic loci.

Weber (1990) stated that the repeat sequences may be perfect repeat sequences without interruptions in the run of CA or GT dinucleotides, imperfect repeat sequences with one or more interruptions in the run of repeats or compound repeat sequences with adjacent tandem simple repeats of a different sequence. Informativeness of $(dC\text{-}dA)_n$ $(dG\text{-}dT)_n$ polymorphisms were found best predicted by longest run of uninterrupted CA or GT repeats.

Bishop *et al.* (1994) reported an additional 172 microsatellites to the 118 previously reported microsatellites of the bovine genome map. Highly polymorphic

markers made available on high density linkage maps helps to identify polygenic loci affecting quantitative traits.

Enrichment of single stranded DNA library using CA or GT primers provided a sufficient source of microsatellites resulting in high percentage of informative markers (Stone *et al.*, 1995).

Rubinsztein *et al.* (1995) stated that microsatellites can evolve directionally and at different rates in closely related species.

Progressive discovery of new microsatellites had enabled the development of a comprehensive linkage map of bovine genome. A high resolution linkage map with 1250 polymorphic loci covering 2990 cM was published by Kappes *et al.* (1997).

Microsatellites are useful for comparative genetics and genomic mapping because of their high degree length polymorphism and their abundant and even distribution in the genome (Oh and Mao, 1999).

Noor *et al.* (2001) stated that extreme caution has to be exercised in use of microsatellite as their flanking region size shows continuous variation and in some species an altogether different variable region is present between primer sequences.

According to Sonstegard *et al.* (2001) web sites containing reference linkage maps like that available from the USDA meat animal research centre contains additional information related to ease of scoring genotypes and PCR amplification characteristic which helps in selection of most useful marker map for any given location in the genome.

2.3 QUANTITATIVE TRAIT LOCI

A large number of genes affect the traits of economic interest and are collectively known as Quantitative Trait Loci (QTL). QTL is a segment of chromosome affecting a trait and does not imply a single locus.

According to Soller (1990), the first experiment designed to map loci affecting quantitative traits through linkage to Mendelian marker loci was carried out in1923 and the advent of DNA level markers had made possible the attempts to map most of quantitative trait loci affecting traits of economic importance in dairy cattle.

Weller *et al.* (1990) stated that the progeny of a sire heterozygous for both a marker locus and a linked QTL which inherit different alleles for the marker will have different trait means thus making it possible to map QTL of economic importance in cattle using daughter and granddaughter designs.

A bovine genetic linkage map can be used for mapping of QTL with high probability of success as most of the microsatellite loci are linked to QTL controlling various economic traits facilitating its detection (Barendse *et al.*, 1994).

Bovenhuis *et al.* (1997) reported that several studies have indicated that knowledge about genetic markers linked to genes affecting quantitative traits can increase the selection response of animal breeding programs especially for traits that are difficult to improve when using traditional selection methods.

Arranz *et al.* (1998) stated that whole genome scans are undertaken in the elite dairy cattle populations to identify segregating QTL's.

2.3.1 QTL affecting milk production traits

Ron *et al.* (1994) verified the presence of segregating QTL having significant effect on milk production linked to locus D21S4.

Bovenhuis and Weller (1994) identified different QTL's affecting milk, fat and protein production linked to milk protein genes like β-lactoglobulin, κ-casein and β-casein.

Georges *et al.* (1995) identified the presence of highly segregating QTL affecting milk production on chromosomes 1, 6, 9, 10 and 20.

Two QTL alleles were identified, one of which causes an increase in milk production accompanying a dilution in fat and protein constituent (Arranz *et al.*, 1998).

Ashwell *et al.* (1998) found strong associations between two microsatellite markers viz., BM415 and BM6425 and QTL affecting protein percentage on chromosome 6 and 14 respectively.

Lipkin *et al.* (1998) conducted a quantitative trait loci mapping in dairy cattle by means of selective milk DNA pooling which showed evidence of a linkage existing between the marker ILSTS006 and a QTL affecting protein percentage.

Ron *et al.* (1998) found that the locus CSSM66 has a significant effect on fat percentage at $P<10^{-7}$ and a highly significant effect on fat yield which has a correlation of 0.54 with fat per cent.

Velmala *et al.* (1999) scanned the cattle chromosome six with ten microsatellites and a casein haplotype and identified the existence of two QTLs with an effect on milk production traits.

Kuhn *et al.* (1999) reported the presence of a QTL with effects on milk fat and milk protein yield on BTA6.

Heyen *et al.* (1999) confirmed the presence of a QTL for fat percentage on chromosome 14 by using daughter design in a half sib family of Israeli Holstein dairy herds.

van Tassell *et al.* (2000) used 105 markers located throughout the bovine genome to identify associations between microsatellite markers and QTL affecting milk production. The results of this study concluded that a QTL affecting milk yield may be present on BTA10 and a QTL affecting protein per cent may be present on BTA3, 6, 14 and 28.

Literature based survey of significant marker-associated effects on milk production traits given by Mosig *et al.* (2001) shows that marker ILSTS096 was associated with milk yield, protein yield, protein per cent, fat yield and fat per cent

and marker BL41 was associated with fat per cent and protein per cent on chromosome 3, marker HUJII77 was associated with protein per cent on chromosome 4 and marker BM4305 was associated with protein per cent and milk yield on chromosome 4.

Ashwell *et al.* (2001) conducted a whole genome scan to identify QTL and concluded that QTL affecting fat yield and fat percentage may be present on BTA10.

Plante *et al.* (2001) confirmed the presence of QTL on 10 chromosomes of Holstein cattle affecting milk production trait, previously identified in other studies.

Nadesalingam *et al.* (2001) used least square interval mapping approach based on daughter yield deviations of sons for 305 day milk, fat yield, protein yield, fat percentage and protein percentage. A significant QTL affecting fat and protein yield and fat per cent were identified on chromosome 1 and QTL for milk yield and protein per cent on chromosome 6.

Ron *et al.* (2001) reported significant effects on milk production traits associated with markers BM143 and BM415 by ANOVA analysis across all families with heterozygous sires.

Suggestive QTL for one or several of the five milk traits (milk yield, protein per cent, protein yield, fat percentage and fat yield) were detected on chromosomes 3, 5, 6, 11, 13, 18 and 20 (Olsen *et al.*, 2002).

Rodriguez-Zas *et al.* (2002a) reported that marker BL41 was associated with decrease in milk yield during mid lactation and the marker HUJII77 was associated with changes in milk yield and protein per cent suggesting a QTL with pleiotropic effects or multiple QTL in the region.

Rodriguez-Zas *et al.* (2002b) used single marker, interval mapping and composite interval mapping to identify a QTL on BTA3 affecting protein yield and another QTL affecting fat yield.

Freyer *et al.* (2003) confirmed the presence of a pleiotropic QTL for fat and protein yield on BTA6.

Viitala *et al.* (2003) identified a new QTL affecting all yield components on BTA12 in Finnish Ayrshire cattle by granddaughter design.

Weller *et al.* (2003) found out the association between the 225 allele of the ILSTS039 and K allele of DGAT1 with decreased milk production and increased fat production and fat and protein percentage.

Ashwell *et al.* (2004) confirmed that a mutation in the bovine acyl CoA diacyl glycerol acyl transferase (DGAT1) gene on BTA14 is associated with a major effect on milk fat content. Another mutation in the bovine growth hormone receptor gene is associated with effect on milk yield and composition.

The central region of BTA6 and the centromeric region of BTA14 were found to have genome wide significance on protein and fat percentage respectively in a complete genome scan conducted on Israeli Holstein by Ron *et al.* (2004).

2.4 MARKER ASSISTED SELECTION

Stuber *et al.* (1980) stated that marker loci with no direct effect on the characters of interest can be utilized in selection because of statistical associations (linkage disequilibrium) between alleles at the marker loci and QTL's.

Lande and Thompson (1990) suggested that further increase in relative efficiency of Marker Assisted Selection (MAS) was possible when individuals that do not express the phenotypic traits of interest can be selected on the basis of their molecular markers. MAS can be applied to sex limited traits or when additional selection has to be exerted on juveniles before the development of the adult phenotype.

Several studies have indicated that knowledge about genetic markers linked to genes affecting quantitative traits can increase the selection response of animal breeding programs especially for traits that are difficult to improve when traditional selection methods are used (Meuwissen and van Arendonk, 1992).

Brascamp *et al.* (1993) studied dairy cattle QTL to identify those that can be utilized in marker assisted selection. MAS has been evaluated for dairy cattle and have shown that rate of genetic gain can be increased by implementation of MAS.

Bishop *et al.* (1995) opinioned that it was possible to increase the selection intensity and shorten the generation interval by integrating several reproductive technologies (embryo transfer, IVM, IVF and culture) with MAS for QTL's and quantitative information.

Ruane and Colleau (1996) showed that the use of markers to estimate QTL effects more accurately led to an increased QTL response but a reduced polygenic response beginning from first generation of selection.

Spelman and Garrick (1997) stated that utilizing genetic markers in conjunction with phenotypic observation will provide more information on the genetic merit of the animal than phenotypic information alone.

According to Davis and deNise (1998), MAS program relies on 3 phases for commercialization of technology (i) the detection phase in which QTL are located and their effects on phenotype measured (ii) the evaluation phase in which markers are evaluated in commercial populations and (iii) the implementation phase in which the markers are combined with phenotypic and pedigree information in genetic evaluation for predicting the genetic merit of the individuals within population.

The top down marker assisted selection scheme identifies sires that are heterozygous for the locus based on grand daughter design and uses quantitative tract loci information in pre-selection of grandsons entering progeny testing. The bottom up MAS scheme identifies sires heterozygous for QTL based on daughter design and uses information in the pre-selection of sons entering progeny testing (Spelman and Garrick, 1998).

Sonstegard *et al.* (2001) said that information from genetic markers that identify desirable alleles of economically important traits could be used along with breeding values to guide mating decisions resulting in genetic gains over a broader range of traits. In addition marker assisted selection (MAS) could be used to select

the most desirable phenotypes affected by non additive gene action or epistatic interactions between loci.

Georges (2001) said that MAS could be distinguished into three distinct phases depending on accuracy of information available about Economic Trait Loci (ETL). In MAS I, an ETL is located with respect to relatively distinct flanking markers. Associations between specific marker and ETL alleles hold within specific families only and needs to be re-established for each family to allow MAS. In MAS II, an ETL has been fine mapped with respect to closely linked markers which are in linkage disequilibrium with ETL. Associations between specific marker haplotypes and ETL alleles hold across the populations and need not be re-established for individual family.

According to Stella *et al.* (2002) maximum progress was obtained, when selection was applied for each generation for several sets of markers neighboring multiple potential QTL, rather than only on markers near the single most likely QTL.

Schulman and Dentine (2005) used two stages of selection using marker information and progeny testing and reported that response in total genetic gain was faster with MAS than with traditional selection.

2.5 STATISTICAL ANALYSIS

2.5.1 Heterozygosity

The average heterozygosity per locus (H) is defined as the mean of population heterozygosity at a locus over all structural loci in the genome. Estimation of average heterozygosity and genetic distance requires a large number of loci rather than a large number of individuals per locus when total number of genes to be used is fixed. Population heterozygosity at a locus is given by $H = 1-\Sigma p_i^2$ where p_i is the frequency of the i^{th} allele at a locus in the population (Nei, 1978)

According to Ott (1992), newly generated markers should be screened for high polymorphism on the basis of a small panel of individuals in order to classify a marker as having heterozygosity of 70% or higher.

Van Hooft *et al.* (1999) reported a mean observed heterozygosity of 0.61 per marker for all the markers and a mean observed heterozygosity of 0.67 for polymorphic markers in an analysis conducted on 101 markers for polymorphisms.

2.5.2 Polymorphic Information Content (PIC)

Informativeness gives the probability that a given offspring of a parent carrying the rare allele at the index locus will allow deduction of parental genotype at the marker locus. PIC of each marker locus is therefore given by the summation of the product of the mating frequencies and the probability that an offspring will be informative (Botstein *et al.,* 1980). Formula for calculating the polymorphic information content is given by

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-i} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

(Hai-Guo et al., 2003, Li *et al.,* 2004 and Jang *et al.,* 2005)

Velmala *et al.* (1995) reported a PIC value of casein haplotypes as 0.70 in a study conducted on Finnish Ayrshire cattle.

The PIC values varied from 0.03 (ETH3) in Sahiwal cattle to 0.89 (HEL9) in Hariana cattle with an average of 0.55, 0.62 and 0.65 in Sahiwal, Hariana and Deoni cattle respectively in the genetic diversity study of Indian native cattle by Mukesh *et al.* (2004)

Materials and Methods

3. MATERIALS AND METHDOS

Fifty pure bred Vechur cattle maintained at Vechur Conservation Centre, Mannuthy were used for the polymorphism study of the markers. Thirty four animals belonging to seven different families were used in this microsatellite study.

3.1 SOURCE OF DNA

The blood and semen samples were used as source of DNA. Five ml of blood was collected aseptically using EDTA as anticoagulant.

In case of bulls used in the study, the semen samples were used to isolate the DNA. Both fresh and frozen semen straws containing around 300-500 million sperms were used for the study.

3.1.1 Isolation of genomic DNA from blood

DNA was extracted from whole blood using phenol chloroform method. The procedure used was as follows.

- Five ml of blood was collected in a 15 ml centrifuge tube.
- Two third volume of ice-cold RBC lysis buffer (150m*M* NH_4Cl, 10m*M* KCl, 0.1m*M* EDTA) was added mixed well and kept in ice with occasional mixing for 10 minutes for complete lysis of RBC.
- The leukocytes were pelleted by centrifuging at 3500 rpm for 15 minutes and the supernatant containing lysed RBC's was discarded.
- The pellet was resuspended in ice-cold RBC lysis buffer and the above two steps were repeated till a clear pellet without any unlysed erythrocytes were obtained.
- The pellet was then washed twice with 10 ml of Tris Buffered saline (TBS-140m*M* NaCl, 0.5m*M* KCl, 0.25m*M* Tris) by vigorous vortexing followed by centrifugation at 3000 rpm for 10 min.

- The washed white blood cell pellet was resuspended completely by vortexing in 5 ml of saline EDTA buffer (SE – 75m*M* NaCl, 35m*M* EDTA) and to this mixture 0.25 ml of 20% SDS and 25 µl of proteinase K (20mg/ml) were added, mixed well and incubated at 50°C for a minimum of 3 hours.

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

- The aqueous phase containing the DNA was collected in fresh tubes to which an equal volume of saturated phenol : chloroform : isoamylalcohol (25:24:1) was added. The contents were mixed thoroughly and centrifuged at 3500 rpm for 15 minutes.

- The aqueous phase containing the DNA was collected in a fresh tube to which an equal volume of chloroform : isoamylalcohol (24:1) was added, mixed and centrifuged at 3500 rpm for 15 minutes.

- The supernatant was transferred to a sterile 50 ml beaker and 1/10 the volume of 3M sodium acetate (pH 5.5) was added.

- To this mixture an equal volume of chilled isopropyl alcohol was added and the precipitated DNA was spooled out using a fresh micropipette tip, washed in 70% ethanol and air-dried.

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

3.1.2 Isolaton of genomic DNA from semen

- Semen sample containing approximately 300-500 million sperms (1-2 ml fresh semen) was used for isolation of DNA. The sample was washed twice with 10 ml of PBS and centrifuged at 3500 rpm for 10 minutes. The pellet was resuspended in 2 ml of PBS by vortexing.

- 6 ml of warm sperm lysis buffer (100m*M* Tris, 500m*M* NaCl, 10m*M* EDTA, 1% SDS, 2% Mercaptoethanol) was added mixed well and incubated at 55°C in water bath for 30 minutes with occasional mixing.

- 100µl of Proteinase K (20mg/ml) was added and incubation was continued for 3 hours at 55°C.

- To the completely digested cellular protein, equal volume of saturated phenol (pH 7.8) was added, mixed thoroughly for 10 minutes and centrifuged at 3500 rpm for 15 minutes.

- To the upper aqueous phase collected in a fresh centrifuge tube, equal volume of saturated phenol : chloroform : isoamylalcohol (25:24:1) was added and mixed for 10 minutes. The tubes were centrifuged at 3500 rpm for 15 minutes. The aqueous supernatant was collected in fresh centrifuge tubes.

- To the aqueous phase collected, an equal volume of chloroform : isoamylalcohol (24:1) was added, mixed well for 10 minutes and then centrifuged at 3500 rpm for 15 minutes.

- The above step was repeated once again and aqueous phase was transferred to a sterile 50 ml beaker. To this one-tenth volume of 3M sodium acetate (pH 5.5) was added and mixed well.

- Equal volume of chilled isopropyl alcohol was added and the precipitated DNA was spooled using a fresh micropipette tip. The spooled out DNA was immediately washed with 70% ethanol and air-dried. The air dried DNA was resuspended in 500 µl TE buffer and stored at –20°C.

3.2 DETERMINATION OF YIELD AND PURITY OF DNA

20 µl of the DNA stock solution was diluted to 2 ml with sterile distilled water. Optical densities (OD) were measured at 260 nm and 280 nm wavelengths in a UV spectrophotometer. Yield and Purity of DNA samples were estimated as follows.

3.2.1 Yield

An OD of 1 at 260 nm wavelength corresponds to approximately 50 µg/ml of double stranded DNA. Concentration of DNA stock solution was calculated as follows.

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

The total yield of DNA obtained from blood was calculated by multiplying the concentration with the volume of DNA stock solution.

3.2.2 Purity

Purity of DNA sample was assessed by estimating the ratio between the readings at 260 nm and 280 nm wavelengths. A ratio of 1.6-1.8 indicated the presence of DNA. The ratio below 1.6 and above 1.8 indicated the presence of protein and RNA respectively. The quality of the DNA sample was assessed electrophoretically on 0.7% agarose gel in 1XTris acetate EDTA (TAE) buffer in horizontal submarine gel electrophoresis.

3.3 POLYMERASE CHAIN REACTION

3.3.1 Template DNA

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

3.3.2 Selection of primers

A panel of 10 microsatellite markers was selected and primers were custom synthesized. The polymorphisms of each of these markers were determined and five markers viz. HUJII77, ILSTS096, BM4305, BL41 and BM1508 with higher degree of polymorphism were chosen for the study. The sequences of the forward and reverse primers for each of the microsatellites are as follows.

Marker		Primer sequence (5' – 3')
HUJII77	F	CTCCATCAAGTATTTGAGTGCA
	R	GTGTTCATAGCCCTACCCAC
ILSTS096	F	GTGACCTGGAGAAGTTTTCC
	R	ACCACGCTCTGACTTGTAGC
BM4305	F	CCAAGACATGAAAGCAATCTG
	R	CTCTAGGTACATCCATGTTGCA
BL41	F	CCTCGCCATCTTTATTCCT
	R	AAGATCAACTTATTATTCCTCACAGTGG
BM1508	F	CAGGTGTACAGCAAACTGAATC
	R	CGTCAAAACATTCGTTCAGG

The primers were reconstituted using sterile ultra pure water to make a stock solution of 200p*M*/µl concentration. The reconstituted primers were incubated at room temperature for one hour and then stored at –20°C. Working solutions were prepared from dilutions of stock solutions.

3.3.3 Endlabeling of primers

The forward primer of each marker was radio endlabelled at the 3' end with γ^{32}P ATP. Endlabelling was carried out using DNA endlabelling kit.

For a 10 µl endlabelling reaction, the following components were added in order.

10X Polynucleotide Kinase (PNK) buffer	- 1µl
Forward Primer (200 pM/µl)	- 1µl
T4 Polynucleotide Kinase (5U/µl)	- 0.5 µl
γ^{32}P ATP (10µCi/ml)	- 1 µl
Nuclease free water	- 6.5 µl

This mixture was incubated at 37°C for 30 minutes and final volume was made up to 40 µl with double distilled water.

3.3.4 PCR conditions

PCR reactions were carried out in an automated thermal cycler. Each 10 µl PCR reaction consisted of 1µl of 10X PCR buffer (*10*m*M* Tris (pH 8.3), 500m*M* KCl), 200µ*M* dNTP, $MgCl^2$ whose concentration varies from 1.25m*M* per reaction for markers ILSTS096, BM4305 and BM1508 to 1.5m*M* per reaction for the markers BL41 and HUJII77, 1µl of endlabeled primer with a concentration of 5p*M*/µl, 5p*M* of reverse primer and 0.3U of Taq DNA polymerase. Thermal cycling was carried out for 35 cycles and the samples were finally cooled to 4°C and stored at –20°C.

3.4 AGAROSE GEL ELECTROPHORESIS

The amplification of the products after PCR was checked on 1% agarose gel in 1X Tris acetate EDTA (TAE) buffer in horizontal electrophoresis unit. One µl of PCR product with $1/6^{th}$ volume of 6X gel loading buffer (0.25% Bromophenol blue, 0.25% Xylene cyanol, 40% sucrose) was loaded on the agarose gel. *Hae III* digested pBR322 DNA was used as molecular size marker.

3.5 DENATURING POLYACRYLAMIDE GEL ELECTROPHORESIS

Denaturing polyacrylamide gel electrophoresis was performed on a vertical DNA sequencer. 6% denaturing polyacrylamide gel (6% Urea, 0.5X TBE) set between two glass plates separated by 0.35 mm thick spacers was used for this purpose. A sharp toothed comb was used which enabled the loading of 40 samples on a single gel.

The PCR products mixed with 2.5 µl of formamide loading buffer (0.02% Xylene cyanol, 0.02% Bromophenol blue, 10mM EDTA, 98% deionised formamide) was denatured at 95°C for 5 min and kept in ice. 3.5 µl of the denatured product was loaded in each well. Sequenced products of M13 DNA phage were run in the centre of the gel, which is required for genotyping of the products.

The gels were run at 40W for 2-3 hrs depending on the expected size range of the product at 45°C. Bromophenol blue and xylene cyanol dye has a mobility

equivalent of approximately 25 base pairs and 100 base pairs respectively and hence used as an indicator of the mobility of the amplified PCR product.

3.6 SEQUENCING OF M13 BACTERIOPHAGE DNA

Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (M/s Amersham Biosciences Corporation 1 USA).

Annealing mixture

The following components are added together in order.

M13 phage DNA (0.2µg/µl) - 5 µl
5X sequenase reaction buffer - 2 µl
Forward primer (0.5pM/µl) - 1 µl
Double distilled water - 2 µl

This mixture was mixed well, incubated at 65°C for 2 minutes, cooled to room temperature over 15-30 min and chilled on ice.

Dilution of Labeling Mix

The labeling mix available in the kit was diluted in the ratio of 1:4 as follows

5X Labeling mix - 0.5 µl
Water - 2.0 µl

Dilution of enzyme

The enzyme (sequenase version 2.0) was diluted with sequenase dilution buffer in the ratio of 1:8 as follows.

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

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

Labeling reaction

To the ice cold annealed DNA mixtures, the following were added.

Dithiothretiol (DTT 0.1M)	- 1 µl
Diluted labeling mix	- 2 µl
Diluted enzyme	- 2 µl
α^{35}S dATP (10µCi/ml)	- 1 µl

The contents were mixed well and incubated at 37°C for five minutes.

The reaction was stopped by addition of 4 µl of stop solution and stored at – 20°C.

3.7 DRYING OF GELS

The glass plates were separated carefully and the gel transferred to a filter paper. The gel was covered with kling film and dried in a gel drier.

3.8 AUTORADIOGRAPHY

The dried gels were placed in a cassette (Kiran Hypercassette) fitted with intensifying screen and a X-ray film (Kodak, 35.5 x 43.2 cm) was loaded in the cassette. The X-ray film was developed after 24-48 hrs depending on the intensity of the radioactive signal.

3.9 GENOTYPING OF MARKERS

The number of different alleles for each marker was counted. The size of each allele was determined by comparison with M13 sequencing ladder. The frequency of each allele was worked out.

3.10 MILK ANALYSIS

Milk samples (100 ml) collected from Vechur cows were analysed for fat %, protein % and solids not fat (SNF). Fat percentage was estimated by Gerber's method (IS: 1224 – Part I 1977). Total solids were estimated by gravimetric method (IS: 1479 – Part II 1961). Protein percentage was determined by Micro Kjeldhal

method (AOAC, 1990). Solids not fat (SNF) content of milk was determined by finding the difference between total solids and fat content of milk.

3.11 DATA COLLECTION

Data for milk yield, age at first calving, intercalving period and pedigree were collected from the records maintained at the Vechur Conservation Centre, Mannuthy, Thrissur.

3.12 STATISTICAL ANALYSIS

3.12.1 Heterozygosity

3.12.1.1 Direct count heterozygosity

Heterozygosity of microsatellite markers was calculated by the following statistical formula (Nei, 1978).

$$H = 1 - \Sigma P_i^2$$

Where P_i was the frequency of i^{th} allele at a locus in the population.

3.12.1.2 Unbiased Heterozygosity

The unbiased heterozygosity was calculated using the formula of Pandey *et al.* (2002).

$$H = [2n/(2n-1)][1- (\sum_{i=1}^{k} P_i^2)]$$

Where Pi is the frequency of i^{th} allele and n is the number of observations.

3.12.2 Polymorphic Information Content (PIC)

Polymorphic Information Content (PIC) was calculated using the formula.

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - \left[\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2 \right]$$

Where P_i and P_j stand for frequency of band i and band j in one population and n is the number of alleles from a certain locus.

3.12.3 Analysis of economic traits in population

One way analysis of variance was performed to find the effect of the families for different production parameters like milk fat percentage, protein percentage, SNF, total solids, peak yield, days to attain peak yield, age at first calving (AFC) and intercalving period. This was done using the method of Snedecor and Cochran (1985).

3.12.4 Analysis of economic traits in the families

A simple T-test was performed to identify the families which differ significantly from each other for the production parameters viz. milk fat percentage, protein percentage, SNF, total solids, peak yield, and days to attain peak yield, AFC and intercalving period. The method used was that suggested by Snedecor and Cochran (1985).

3.12.5 Analysis of allelic effect

The production traits showing significant difference were analysed for their allelic effects. The allelic effect was calculated for the alleles of all the ancestors. Large sample Z-test was used to compare the mean of the population having the allele to those of the population not having this allele for the different production parameters. Method followed was that suggested by Snedecor and Cochran (1985).

Results

4. RESULTS

4.1 ISOLATION OF GENOMIC DNA

DNA was isolated from whole blood, fresh and frozen semen samples using phenol: chloroform extraction procedure.

4.1.1 Yield and Quality of DNA

Good quality DNA was obtained from blood, fresh and frozen semen. The purity of DNA was checked by agarose gel electrophoresis. Pure DNA appeared as a single band.

4.2 PCR ANALYSIS

Five markers were selected based on their polymorphisity and the established association with QTL in cattle. The primers were custom synthesized for use in PCR for amplification of DNA samples. Five markers viz., HUJII77, ILSTS096, BL41, BM1508 and BM4305 were typed on 50 unrelated DNA samples collected from the Vechur cattle.

4.3 NUMBER, SIZE AND FREQUENCIES OF ALLELES

The PCR products were first checked in agarose gel and then resolved on denaturing polyacrylamide gels. The amplified products were visualized as bands by autoradiography of the dried gels. The number of alleles was counted and their size were obtained by comparison with the sequence of M13 DNA run alongside the samples in the gel. The alleles for each marker appeared as double/single bands and the frequencies of the alleles for each locus were worked out separately. The number, size range and frequencies of alleles for each locus are presented in Table 4.1.

4.3.1 HUJII77

The HUJII77 locus was found to be highly polymorphic with 14 alleles ranging in size from 193-225 bp. The polymorphism at the HUJII77 locus is presented in Plate. 1. The allele size and frequencies obtained are presented in Table 4.2. The allele of size 221 bp had the highest frequency at the HUJII77 locus. The lowest frequency was obtained for the alleles of size 193, 195 and 219 bp.

4.3.2 ILSTS096

The products obtained at the ILSTS096 locus ranged in size from 188-212 bp. The polymorpshism at the ILSTS096 locus is presented in Plate. 2. Twelve alleles were identified at the ILSTS096 locus. The allele size and frequencies obtained are presented in Table 4.3. The highest frequency obtained at the ILSTS096 locus was for 208 bp size allele. The allele of size 194 bp had the lowest frequency.

4.3.3 BL41

The allele size and frequencies obtained are given in Table 4.4. Eleven alleles were prevalent at the BL41 locus sizes from 238-258 bp. Plate.3 shows the polymorphism at the BL41 locus. The allele of size 250 bp had the highest frequency at the BL41 locus. The allele having the lowest frequency was 254 bp in size.

4.3.4 BM1508

The least polymorphic of the five markers studied was BM1508, with 9 alleles ranging in size from 103-119bp. The polymorphism at the BM1508 locus is shown in Plate. 4. The allele size and frequencies are presented in Table 4.5. Highest frequency was present for the allele of size 107 bp and the lowest frequency obtained at this locus was for 119 bp size allele.

4.3.5 BM4305

Plate.5 shows the polymorphism at the BM4305 locus. The 11 alleles were identified at the BM4305 locus and their size ranged from 146-168 bp. The allele size and frequencies obtained are tabulated in Table 4.6. The allele of size 158 bp had the highest frequency of 0.21 and the lowest obtained was for allele of size 154 bp (0.03).

4.4 STATISTICAL ANALYSIS

4.4.1 Heterozygosity

The heterozygosity value (H), indicates the usefulness of the markers in the study of genetic polymorpshism and was calculated by the method of Ott (1992). Heterozygosity values obtained were 0.899 for HUJII77, 0.862 for ILSTS096, 0.868 for BL41, 0.840 for BM1508 and 0.875 for BM4305 respectively. Unbiased heterozygosity was calculated using the method of Pandey *et al.* (2002). Unbiased heterozygosity values obtained for HUJII77, ILSTS096, BL41, BM1508 and BM4305 were 0.932, 0.899, 0.909, 0.889 and 0.917 respectively

4.4.2 Polymorphic Information Content

Polymorphic Information Content (PIC) gives the level of informativeness of the markers and was calculated using the method of Botstein *et al.* (1980). Highest PIC value was obtained for HUJII77 (0.890) followed by BM4305 (0.863). The PIC values for BL41, ILSTS096 and BM1508 were 0.854, 0.847 and 0.820 respectively.

Table 4.1 Number of alleles, Heterozygosity and PIC

Observation	HUJII77	ILSTS096	BL41	BM4305	BM1508
No. of alleles	14	12	11	11	9
Size range of alleles	193-225	188-212	236-258	146-168	103-119
Direct Count heterozygosity	0.899	0.862	0.868	0.875	0.840
Unbiased heterozygosity	0.932	0.899	0.909	0.917	0.889
PIC	0.890	0.847	0.854	0.863	0.820

Table 4.2 Allele sizes and frequencies at HUJII77 locus in Vechur cattle of Kerala

S. No.	Size in base pairs	Allele frequencies
1	193	0.02
2	195	0.02
3	199	0.12
4	201	0.05
5	203	0.10
6	205	0.03
7	209	0.13
8	211	0.09
9	213	0.10
10	217	0.04
11	219	0.02
12	221	0.17
13	223	0.04
14	225	0.07

Table 4.3 Allele sizes and frequencies at ILSTS096 locus in Vechur cattle of Kerala

S. No.	Size in base pairs	Allele frequencies
1	188	0.02
2	190	0.04
3	192	0.03
4	194	0.01
5	196	0.03
6	198	0.11
7	200	0.14
8	202	0.13
9	204	0.11
10	206	0.10
11	208	0.25
12	212	0.03

Table 4.4 Allele size and frequencies at BL41 locus in Vechur cattle of Kerala

S. No.	Size in base pairs	Allele frequencies
1	236	0.03
2	238	0.06
3	240	0.19
4	242	0.10
5	244	0.05
6	246	0.14
7	250	0.21
8	252	0.09
9	254	0.02
10	256	0.07
11	258	0.04

Table 4.5 Allele sizes and frequencies at BM1508 locus in Vechur cattle of Kerala

S. No.	Size in base pairs	Allele frequencies
1	103	0.02
2	105	0.12
3	107	0.24
4	109	0.17
5	111	0.16
6	113	0.16
7	115	0.07
8	117	0.05
9	119	0.01

Table 4.6 Allele size and frequencies at BM4305 locus in Vechur cattle of Kerala

S. No.	Size in base pairs	Allele frequencies
1	146	0.05
2	148	0.10
3	152	0.06
4	154	0.03
5	156	0.12
6	158	0.21
7	160	0.18
8	162	0.09
9	164	0.05
10	166	0.05
11	168	0.06

4.4.3 Production parameters of different families

The mean values of fat% of milk of seven families are presented in Table 4.7 Analysis of Variance for fat% of seven families is given in Table 4.10. Fat% in milk of animals of seven families studied was found to be significantly different. Students't' test was performed to compare the mean fat percentage of each of the seven families with the population mean. The mean fat percentage of V43 and V88 families were found to be significantly different.

The mean values of the protein%, total solids and Solids Not Fat (SNF) of the milk of five families are presented in Table 4.8. Analysis of Variance for these milk constituents are presented in the Tables 4.11, 4.12 and 4.13 respectively. No significant difference was found for these constituents in the five families studied.

The mean peak yield, days to attain peak yield, age at first calving (AFC) and intercalving period of seven families are presented in Table 4.9. There were no significant differences for peak yield, days to attain peak yield and intercalving period. The Analysis of Variance for these traits are presented in the Tables 4.14, 4.15 and 4.16 respectively. The effect of families on AFC was found to be significant and the Analysis of Variance is given in the Table 4.17. Students't' test was performed to compare the family means with the population mean of AFC and V43, V44, V88 and V117 families were found to differ significantly from the other families studied.

4.4.4 Allelic Means of Production parameters for different ancestral families

4.4.4.1 HUJII77 locus

Allelic means for fat percentage of seven families are given in Table 4.18. Statistical analysis using Students't' test revealed significant difference in fat percentage for alleles of size 205, 209, 221 and 223 bp. The means for AFC did not

show any significant difference for the alleles at the HUJII77 locus and are presented in the Table 4.19.

4.4.4.2 ILSTS096 locus

Allelic means of fat percentage and AFC at the ILSTS096 locus are presented in Table 4.20. Significant difference was obtained for fat percentage for allele of size 208 bp. But there were no significant difference in AFC between the alleles of the locus.

4.4.4.3 BL41 locus

Mean fat percentage for animals with 252 bp size allele was found to be significantly different from that of animals without this allele. The allelic means of fat percentage at BL41 locus is presented in Table 4.21. The allelic means for AFC showed a significantly higher value for animals with 238 bp size allele and is presented in the Table 4.22.

4.4.4.4 BM4305 locus

Allelic means at BM4305 locus for fat percentage differed significantly for the allele of size 158 bp. There was no significant difference for AFC with regard to the alleles of the locus. Allelic means for fat percentage and AFC at BM4305 locus is given in Table 4.23.

4.4.4.5 BM1508 locus

Allelic means of fat percentage and AFC at the BM1508 locus are given in the Table 4.24. The allele of size 113 bp at this locus showed significant difference for mean fat percentage. There was no significant difference for AFC of the different alleles at this locus.

Table 4.7 Families and mean fat percentage in the milk of progenies

S.No.	Family	Fat %
1	V87 (4)	4.475 ± 0.17^{b}
2	V88 (9)	5.42 ± 0.27^{a}
3	V43 (2)	5.58 ± 0.24^{a}
4	V117 (4)	4.47 ± 0.15^{b}
5	V009 (5)	4.40 ± 0.10^{b}
6	V44 (2)	4.5 ± 0.08^{b}
7	V83 (2)	4.17 ± 0.08^{b}

Means bearing the same superscript do not differ significantly
Figures in parenthesis are number of observations

Table 4.8 Families and mean values of protein percentage, total solids and SNF in the milk of progenies

S.No.	Family	Protein %	Total solids %	SNF %
1	V87 (2)	3.20±0.07	11.25±0.88	8.70±0.14
2	V88 (5)	3.05±0.19	11.80±0.81	9.13±0.22
3	V43 (2)	2.95±0.03	12.75±0.17	9.20±0.14
4	V117 (2)	3.10±0.07	11.90±0.42	8.65±0.17
5	V009 (4)	3.22±0.11	12.15±0.59	9.10±0.18

Figures in parenthesis are number of observations

Table 4.9 Families and mean values of peak yield, peak days, AFC and intercalving period in the milk of progenies

S. No.	Family	Peak Yield (kgs)	Days to attain peak yield	AFC (days)	Intercalving period (days)
1	V87	1.86±0.25 (3)	27.66±4.83 (3)	942.60±38.34 (5)	423.87±49.58 (2)
2	V88	1.87±0.23 (9)	30.33±5.26 (9)	980.00±30.23[b] (9)	418.17±24.93 (6)
3	V43	3.30±0.77 (2)	37.00±2.82 (2)	1294.50±67.52[c] (2)	544.79±56.95 (2)
4	V117	2.10±0.36 (6)	20.66±3.19 (6)	961.83±57.73[b] (6)	420.37±21.10 (4)
5	V009	1.58±0.42 (6)	18.16±3.57 (6)	1065.00±41.01 (6)	404.65±12.55 (5)
6	V44	1.56±0.35 (3)	21.33±5.96 (3)	878.66±17.86[a] (3)	406.87±37.56 (2)
7	V83	0.66±0.13 (3)	18.66±8.82 (3)	1077.00±75.97 (3)	413.70±7.99 (2)
8	Mean	1.87±0.14 (39)	27.10±1.96 (39)	1084.65±50.86 (41)	425.75±13.34 (23)

Means bearing the same superscript do not differ significantly (P<0.05)
Figures in parenthesis are number of observations

Table 4.10 Analysis of variance for effect of families on milk fat percentage of progenies

Source	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Families	6	8.304657	1.38411	3.856028*
Error	21	7.537886	0.358947	
Total	27	15.84254		

*(P<0.05)

Table 4.11 Analysis of variance for effect of families on protein percentage of progenies

Source	Degrees of freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Families	4	0.11788	0.2947	0.336523^{NS}
Error	10	0.87572	0.087572	
Total	14	0.9936		

NS-Non Significant

Table 4.12 Analysis of variance for effect of families on total solids of progenies

Source	Degrees of freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Families	4	4.213833	1.053458	2.620869^{NS}
Error	10	4.0195	0.40195	
Total	14	8.233333		

NS-Non Significant

Table 4.13 Analysis of variance for effect of families on the Solids Not Fat (SNF) of progenies

Source	Degrees of freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Families	4	0.699333	0.174833	0.719479^{NS}
Error	10	2.43	0.243	
Total	14	3.129333		

NS-Non Significant

Table 4.14 Analysis of variance for effect of families on peak yield of progenies

Source	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Sires	6	9.663194	1.610532	1.960663^{NS}
Error	25	20.53556	0.821422	
Total	31	30.19875		

NS-Non Significant

Table 4.15 Analysis of variance for effect of families on the days to attain peak yield in progenies

Source	Degrees of Freedom	Sum of Squares	Mean of Sum Squares	F Value
Between Sires	6	1476.996	246.1659	1.376723^{NS}
Error	25	4470.142	178.8057	
Total	31	5947.137		

NS-Non Significant

Table 4.16 Analysis of variance for effect of families on intercalving period in progenies

Source	Degrees of Freedom	Sum of Squares	Mean of Sum Squares	F Value
Between ancestor	6	32035.91	5339.318	1.374424^{NS}
Error	16	62156.3	3884.769	
Total	22	94192.21		

NS-Non Significant

Table 4.17 Analysis of variance for effect of families on AFC of progenies

Source	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	F Value
Between Sires	6	288915	48152.51	3.567291*
Errors	27	364455.2	13498.34	
Total	33	653370.2		

* ($P<0.05$)

Table 4.18 Allelic Means of milk fat percentage at HUJII77 locus in Vechur cattle.

S.No.	Alleles	Fat%
1	203 (2)	5.6±0.42
2	205 (4)	4.52 ± 015^{b}
3	209 (2)	4.22 ± 0.12^{b}
4	213 (8)	5.3±0.27
5	219 (3)	4.63±0.14
6	221(5)	4.5 ± 0.13^{b}
7	223 (4)	6.3 ± 0.11^{a}

Means bearing same superscript do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

Table 4.19 Allelic means of AFC at HUJII77 locus in Vechur cattle

S.No.	Alleles	Age at first calving (days)
1	199 (3)	1069.33±131.39
2	203 (2)	1071.00±90.50
3	205 (6)	944.15±31.99
4	209 (5)	997.60±52.39
5	213 (14)	1200.92±130.96
6	219 (3)	1033.66±57.93
7	221 (10)	1020.70±65.33
8	223 (6)	1040.66±52.17

Figures in parenthesis are number of observations

Table 4.20 Allelic Means of fat percentage and AFC at ILSTS096 locus in Vechur cattle

S.No.	Alleles	Mean Values	
		Fat%	Age at first calving (days)
1	198	5.63 ± 0.47^{b} (3)	996.83±50.18 (6)
2	200	5.40 ± 0.32^{b} (3)	1087.5±89.89 (4)
3	202	4.62 ± 0.10^{b} (5)	1082.88±116.90 (9)
4	208	4.96 ± 0.17^{a} (21)	1020.68±25.05 (32)

Means bearing same superscript do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

Table 4.21 Allelic Means of fat percentage at BL41 locus in Vechur cattle

S.No.	Alleles	Fat%
1	244 (6)	5.32 ± 0.34^{b}
2	246 (15)	4.98 ± 0.19^{b}
3	252 (8)	4.50 ± 0.12^{a}

Means bearing same superscripts do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

Table 4.22 Allelic Means of AFC at BL41 locus in Vechur cattle

S.No.	Alleles	Age at first calving (days)
1	238 (3)	1541.00 ± 229.49^{a}
2	244 (7)	1095.85 ± 58.86^{b}
3	246 (24)	1017.66 ± 31.06^{b}
4	252 (9)	972.11 ± 42.99^{b}

Means bearing same superscript do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

Table 4.23 Allelic Means of fat percentage and AFC at BM4305 locus in Vechur cattle

S.No.	Alleles	Mean Values	
		Fat%	Age at first calving (days)
1	148	5.20±0.49[b] (3)	1024.83±52.60 (6)
2	158	4.27±0.15[a] (2)	1064.12±74.85 (8)
3	160	5.02±0.18[b] (15)	1098.24±78.28 (25)
4	166	4.91±0.22[b] (10)	1034.09±48.81 (11)
5	168	4.90±0.22[b] (13)	1077.00±69.32 (16)

Means bearing same superscript do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

Table 4.24 Allelic Means of fat percentage and AFC at BM1508 locus in Vechur cattle

S.No.	Alleles	Mean values	
		Fat%	Age at first calving (days)
1	105	4.97±0.39[b] (5)	1015.71±47.77 (7)
2	107	5.12±0.21[b] (14)	1014.82±25.02 (23)
3	109	4.92±0.27[b] (8)	1164.14±131.50 (14)
4	111	4.89±0.22[b] (10)	1027.30±45.54 (13)
5	113	4.53±0.02[a] (3)	1032±90.24 (7)

Means bearing same superscript do not differ significantly ($P<0.05$)
Figures in parenthesis are number of observations

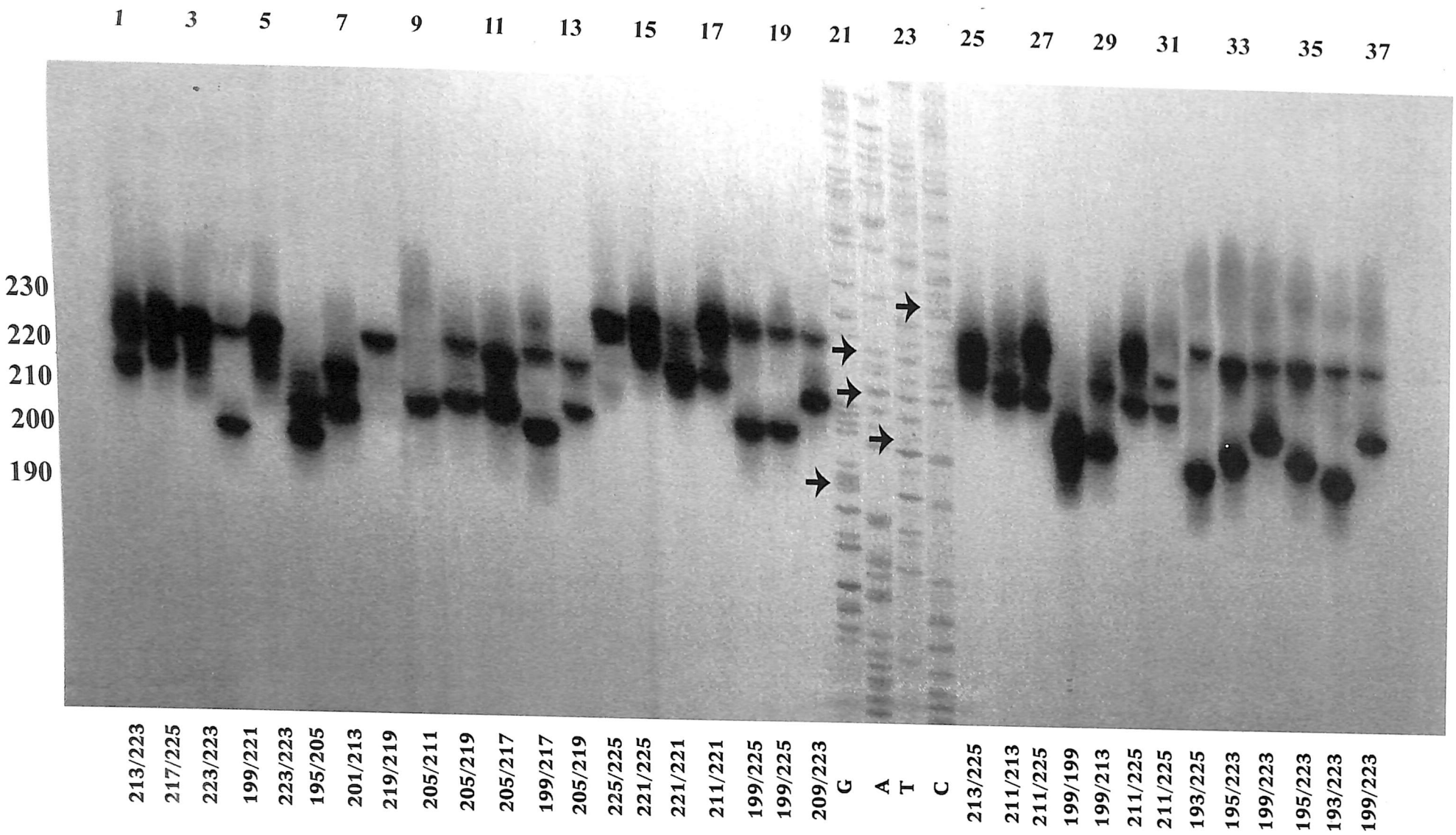

Plate.1. Autoradiograph of polymorphism at HUJII77 locus in Vechur cattle of Kerala
Lanes 1- 20; samples

21-24; G,A,T,C sequences of M13 DNA

25-37; samples

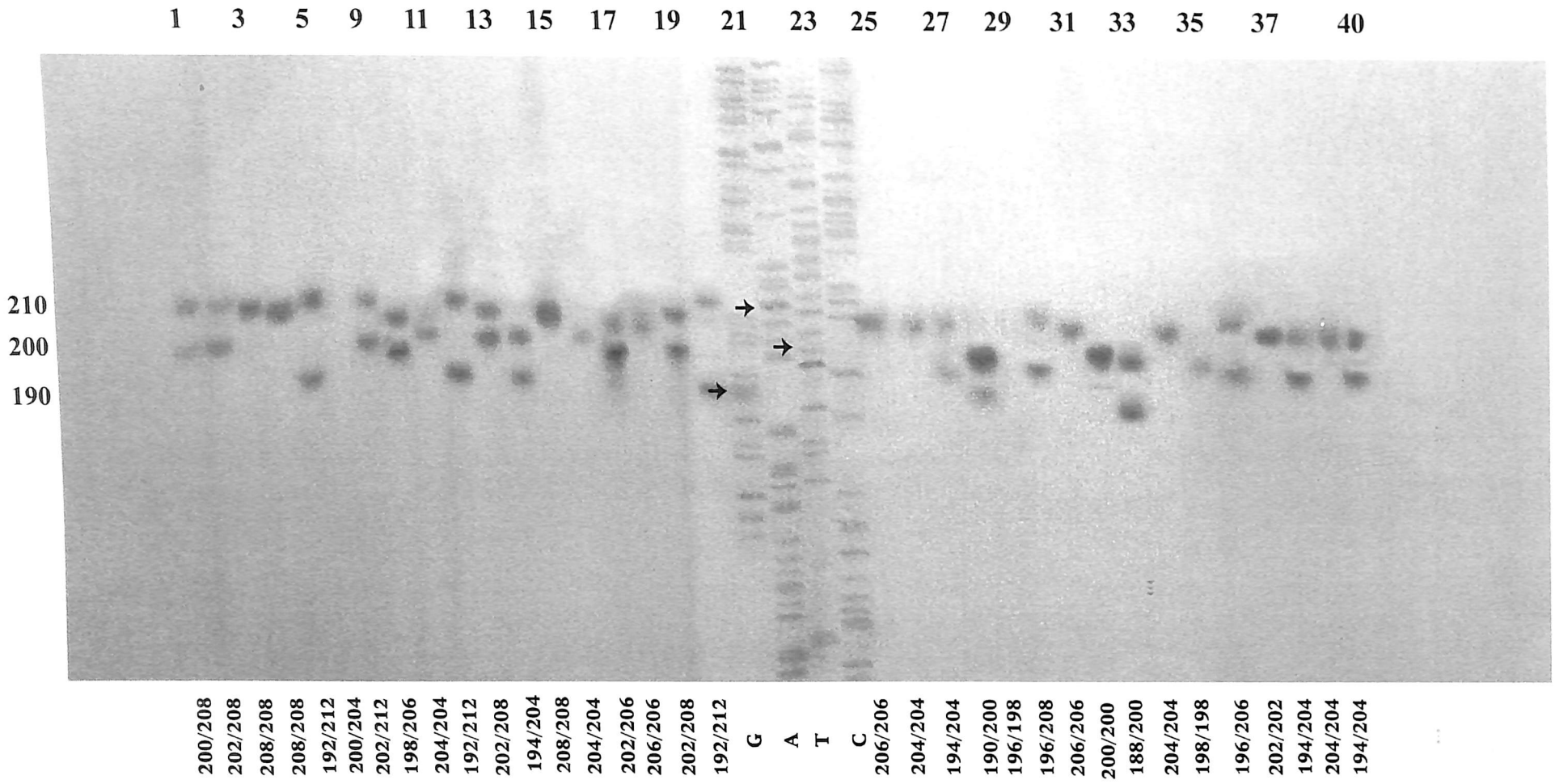

Plate.2. Autoradiograph of polymorphism at ILSTS096 locus in Vechur cattle of Kerala

Lanes 1-20; samples

21-24; G,A,T,C sequences of M13 DNA

25-40; samples

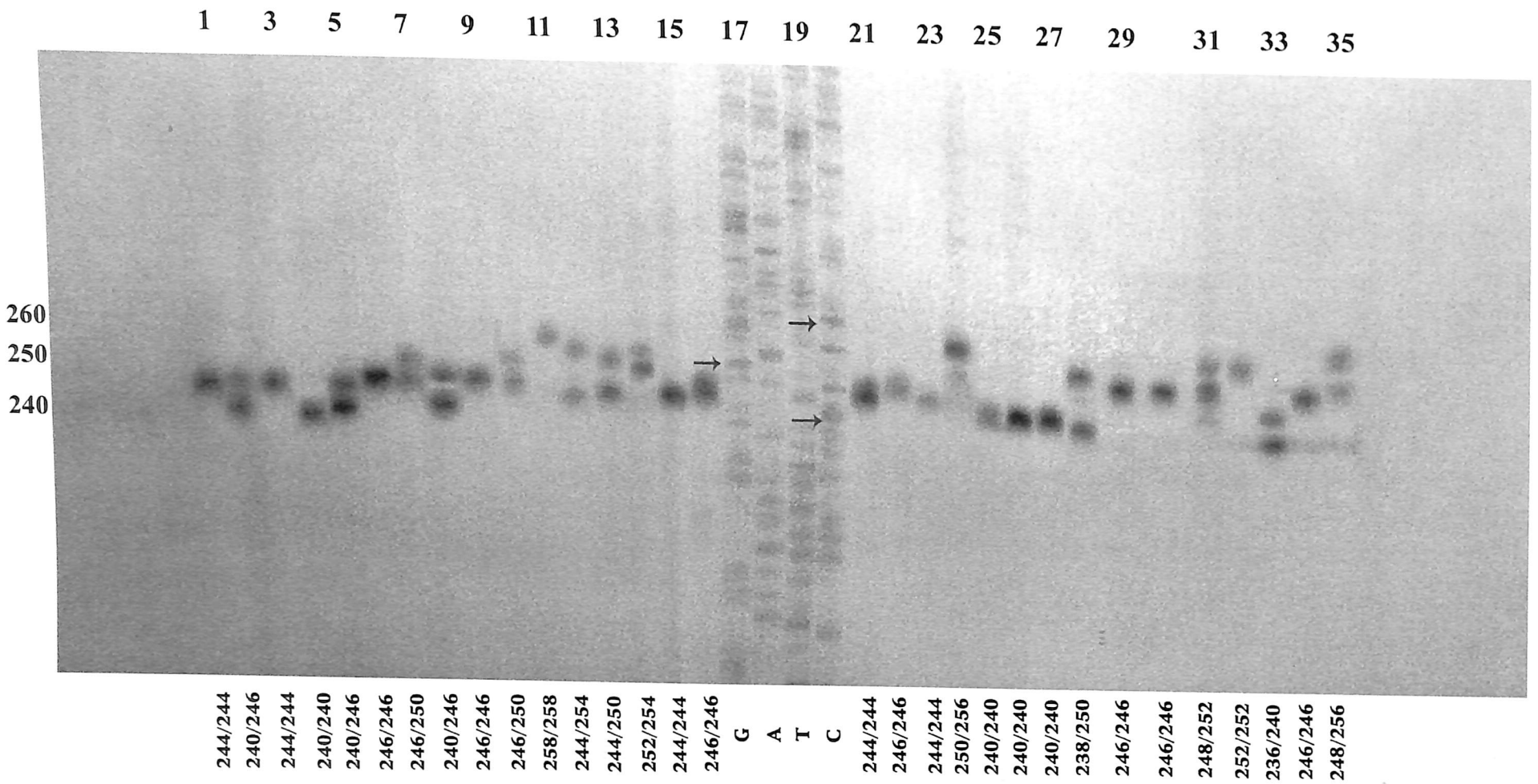

Plate.3. Autoradiograph of polymorphism at BL41 locus in Vechur cattle of Kerala

Lanes 1-16; samples

17-20; G,A,T,C sequences of M13 DNA

21-35; samples

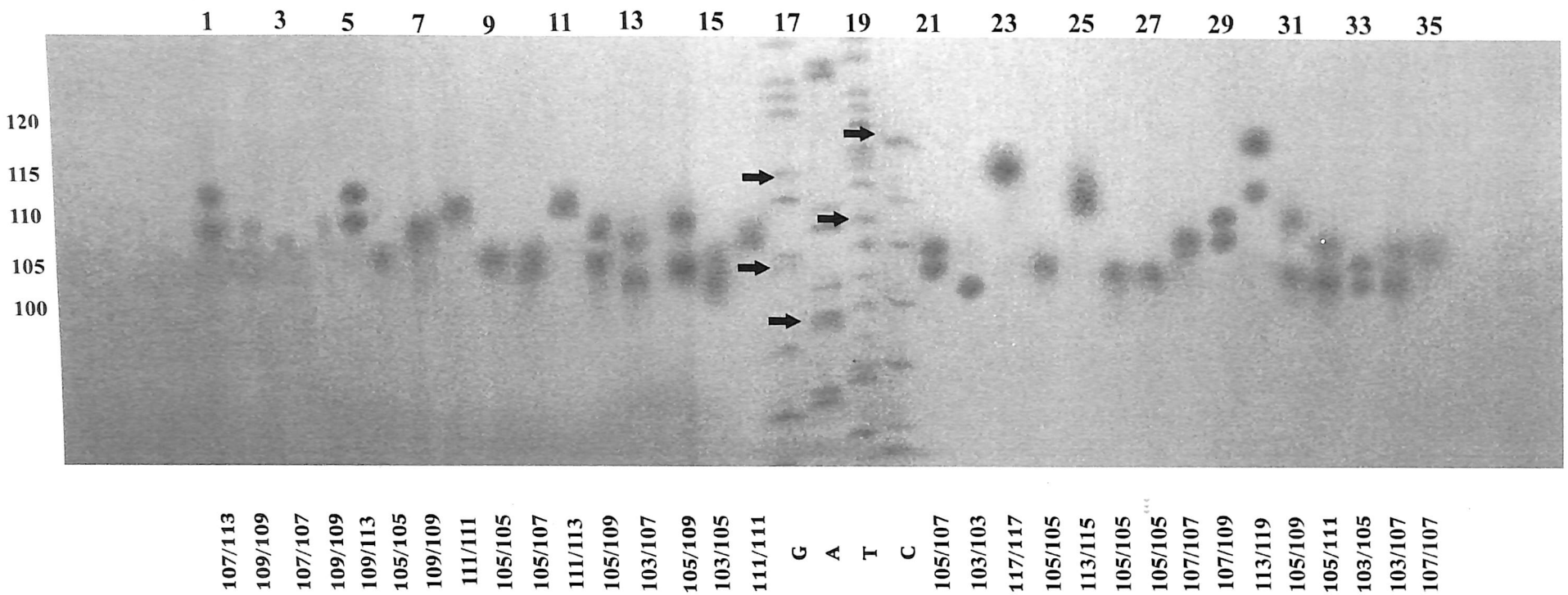

Plate.4. Autoradiograph of polymorphism at BM1508 locus in Vechur cattle of Kerala

Lanes 1-16; samples

17-20; G,A,T,C sequences of M13 DNA

21-35; samples

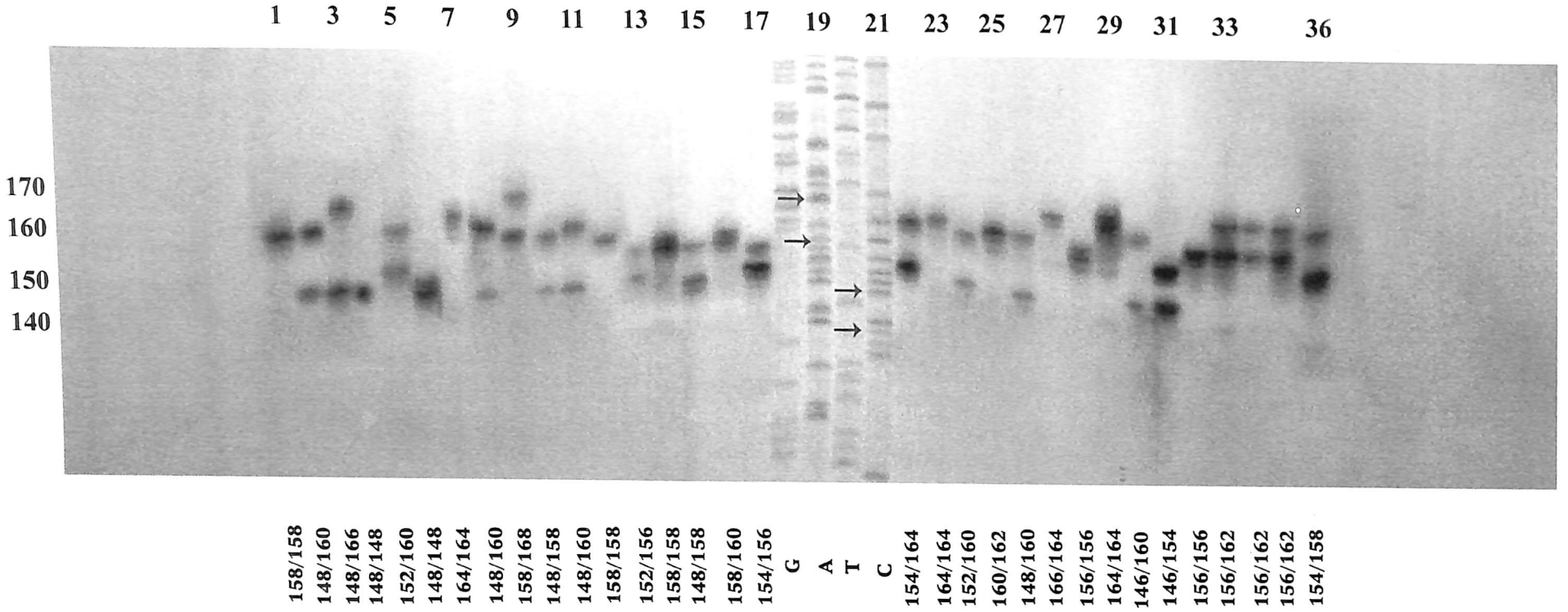

Plate.5. Autoradiograph of polymorphism at BM4305 locus in Vechur cattle of Kerala

Lanes 1-17; samples

18-21; G,A,T,C sequences of M13 DNA

22-36; samples

Discussion

5. DISCUSSION

Genetic marker technologies such as marker assisted selection, parentage identification and gene introgression can be applied to livestock selection programmes. Genetic markers can bring higher returns to the breeding programme by improving the milk yield with utilization of these markers in selection (Brascamp *et al.*, 1993). In marker assisted selection, genetic markers are used to identify specific regions of the chromosomes where the genes affecting the quantitative traits are located and this information is used in selection programmes to identify the individuals with favourable combination of QTL (Davis and DeNise, 1998). MAS has the potential to increase the selection intensity and to reduce the generation interval. This beneficial effect of MAS can be achieved by genotyping the animal at the very early stage of development irrespective of the sex.

Microsatellites are short (1-6 bp) simple tandemly repeated nucleotide sequences present as multiple copies in the genome (Litt and Luty, 1989). Weber and May (1989) reported the use of polymerase chain reaction to detect and type polymorphic DNA markers with improved sensitivity and speed.

5.1 PCR PARAMETERS

PCR amplification was carried out on the genomic DNA extracted from the blood of the Vechur cattle using five microsatellite markers viz., HUJII77, ILSTS096, BL41, BM4305 and BM1508. The PCR conditions were optimized for each of the five markers studied. Erlich *et al.* (1991) reported that the stringency of primer hybridization is increased by higher annealing temperature and lower $MgCl_2$ concentration and the concentration of other constituents like enzyme and primers and other conditions like annealing time, extension time and number of cycles also affect the specificity of the PCR.

5.1.1 $MgCl_2$

Concentration of $MgCl_2$ varied for the five sets of primers used in the study. Concentration of $MgCl_2$ in the PCR mix was optimized at 1.25m*M* per 10µl reaction for the markers ILSTS096, BM4305 and BM1508. Kemp *et al.* (1995) used 1.5m*M* concentration of $MgCl_2$ in a 5µl reaction for PCR amplification of the ILSTS096 locus. In the present study higher concentrations of $MgCl_2$ (1.5m*M* in 10µl reaction) was used in the PCR amplification of HUJII77 and BL41 locus. Shalom *et al.* (1994) reported the use of 1.5m*M* $MgCl_2$ in the PCR for the amplification of the HUJII77 marker locus.

5.1.2Annealing Temperature

The annealing temperature varies depending upon the markers used. Annealing temperature was optimized at 58°C for the markers HUJII77, ILSTS096 and BM4305. Kemp *et al.* (1995) used an annealing temperature of 55°C for the amplification of the ILSTS096 locus. Shalom *et al.* (1994) reported that annealing temperature of 55°C can be used for the amplification of the HUJII77 locus. In the present study PCR amplification of the BL41 locus was carried out at an annealing temperature of 60°C. Bishop *et al.* (1994) reported an annealing temperature of 58°C for the amplification of both BM4305 and BL41 locus. Amplification of the BM1508 locus was carried out at 55°C. The annealing temperature of most of the markers used in this study was higher than that mentioned in the literature. The reason for this is the apparent reduction in the number of non specific products produced at a higher annealing temperature. This was especially noted in case of the marker BL41 where the use of annealing temperature lower than that of 60°C was found to produce more number of non specific products. According to Erlich *et al.* (1991) higher annealing temperature reduced the misincorporation rate to less than 10^{-5} nucleotides per cycle.

5.1.3 Template DNA, Primers and Taq DNA polymerase

The concentration of other constituents of the PCR namely the template DNA, dNTP, forward and reverse primers and Taq DNA polymerase did not show any variation for the five markers studied except for BL41 which varied in the concentration of dNTP. The concentration of both the forward and reverse primers was optimized at 5p*M* in 10µl reaction for all the five marker studied. The optimum concentration of Taq DNA polymerase was found to be 0.3U per 10µl reaction.

5.1.4 dNTP

The concentration of dNTP was optimized at 250µ*M* each per10µl reaction for BL41 and the other four markers were optimized at a concentration of 200µ*M* each per 10µl reaction. The possible explanation for the higher concentration of dNTP required is that BL41 marker has a higher product size when compared to the other four markers used in the study and low concentration of dNTP can inhibit the PCR amplification. The use of limiting dNTP concentrations may influence the fidelity of DNA replication by Taq DNA polymerase (Esposito *et al.*, 1998).

5.2 NUMBER, SIZE AND FREQUENCIES OF ALLELES

5.2.1 HUJII77

Fourteen alleles were obtained at this locus ranging in size from 193-225 bp. The allele of size 221 bp had the highest frequency at this locus. Shalom *et al.* (1994) reported eleven alleles for this marker ranging in size from 187 to 213 bp. The increase in the number of alleles obtained than that mentioned in the literature may be because this study was conducted in *Bos indicus* cattle. There were no earlier reports of use of this marker in *Bos indicus* cattle.

5.2.2 ILSTS096

Twelve alleles were identified at the ILSTS096 locus in Vechur cattle ranging in size from 188-212 bp. According to Kemp *et al.* (1995) the alleles obtained at the ILSTS096 locus ranged in size from 192 to 208 bp. There were no previous reports of use of this marker in *Bos indicus* cattle.

5.2.3 BL41

In the present study eleven alleles were identified at the BL41 locus ranging in size from 238 to 258 bp. Bishop *et al.* (1994) reported eight alleles at the Bl41 locus in cattle ranging in size from 258 to 240 bp. The allele of size 250 bp was found to have the highest frequency at this locus (0.21).

5.2.4 BM1508

The BM1508 locus was found to be the least polymorphic of the five markers studied. Nine alleles were identified at this locus ranging in size from 103 to 119 bp. Nine alleles were reported for this marker ranging in size from 99-115 bp in earlier studies (Stone *et al.*, 1995). This study may be the first report of use of this marker in *Bos indicus* cattle.

5.2.5 BM4305

In the present study on Vechur cattle, eleven alleles were obtained at the BM4305 locus ranging in size from 146-168 bp. In their study on genetic linkage map, Bishop *et al.* (1994) identified eight alleles ranging in size from 148-168 bp. The 158 bp size allele was found to have the highest frequency (0.21). The use of this marker in *Bos indicus* cattle was not yet reported.

5.3 STATISTICAL ANALYSIS

5.3.1. Heterozygosity

An important characteristic of a genetic marker is its heterozygosity, which is the probability that a random individual is heterozygous for that marker. When untyped individuals are present in a pedigree, the allele frequencies will be equal but if equality is assumed, it tends to lead to a strong false evidence for linkage (Ott, 1992).

In the present study, heterozygosity was estimated from the degree of polymorphism of each marker in a panel of fifty unrelated individuals. The estimates of heterozygosity obtained were 0.899, 0.862, 0.868, 0.875 and 0.840 for the markers HUJII77, ILSTS096, BL41, BM4305 and BM1508 respectively. Of the five markers studied, heterozygosity was highest for HUJII77 (0.899) and the lowest for BM1508 (0.840). Barendse *et al.* (1994) reported a heterozygosity of 0.86 at HUJII77 marker locus for *Bos taurus* cattle which is lesser than that obtained in the present study. The reason for this increase in heterozygosity is the higher number of alleles obtained at this locus for *Bos indicus* cattle when compared to *Bos taurus* cattle. A similar molecular work was carried out in Vechur cattle using three different markers and the direct and unbiased heterozygosity obtained in this study were 0.92, 0.85, 0.88 and 0.93, 0.86, 0.89 respectively at the DRB3, ETH131 and HEL6 locus (Anilkumar, 2003).

5.3.2. Polymorphic Information Content (PIC)

PIC gives the informativeness of a marker. It is the probability that a given offspring of a parent carrying the rare allele at the index locus will allow the deduction of the parental genotypes at the marker loci (Botstein *et al.*, 1980). In the present study, the PIC values obtained were 0.890, 0.847, 0.854, 0.863 and 0.820 for the markers HUJII77, ILSTS096, BL41, BM4305 and BM1508 respectively. According to Botstein *et al.* (1980), PIC of more than 0.5 is considered to be highly

informative. All the five markers used in the study was found to be highly polymorphic with PIC value greater than 0.8. According to Weber (1990) PIC greater than 0.8 is obtained for sequences having 24 or more repeats and the informativeness of the marker was found to increase with increasing average number of repeats. A similar work was carried out in Vechur cattle using three different markers and the PIC value obtained in this study was 0.92, 0.85 and 0.88 respectively at the DRB3, ETH131 and HEL6 locus (Anilkumar, 2003).

5.4 PRODUCTION PARAMETERS OF THE SEVEN SIRE FAMILIES

5.4.1 Milk fat percentage

In the present study the average fat% of milk collected from a population of 23 Vechur cows was 4.9±0.77. The average milk fat% of the milk samples collected every week over a period of 44 weeks for a population of 15 Vechur cows was 5.95±0.12 and 6.62±0.13 for morning and evening milk respectively (Venkatachalapathy and Iype, 1998). Joshi *et al.* (2005) reported the average milk fat percentage of different *Bos indicus* breeds of cattle namely Hariana, Bachaur, Ongole and Malvi as 4.51±0.15, 5.5±0.31, 3.79±0.02 and 4.28±0.03 respectively. Milk fat percentage of Vechur cattle is higher when compared to these *Bos indicus* cattle.

In this study involving seven families, the V43 and V88 families were found to be significantly different for fat percentage. It would be beneficial to select the V43 and V88 ancestors as the mean fat percentage of the V43 family (5.58±0.42) and the V88 family (5.42±0.27) was found to be significantly higher than that of the population mean (4.93±0.16).

5.4.2 Milk protein percentage

The mean protein percentage estimated in the present study of a population of 16 Vechur cows was 3.12±0.06. The mean protein percentage did not differ

significantly in the five sire families studied. The small sample size used in this study may be the reason for the lack of significant difference among the five families for protein percentage.

5.4.3 Total Solids

The average value of total solids of milk of 16 Vechur cows in the present study was estimated as 13.75±0.19 percent. The average percentage of total solids of morning and evening milk of 15 Vechur cows was estimated as 14.79±0.13 and 15.53±0.12 respectively (Venkatachalapathy and Iype, 1998). The five families did not differ significantly for total solids in milk. This may be due to the small sample size used in the study. Hence it is advocated to conduct this study in a large population.

5.4.4 Solids Not Fat (SNF)

In the present study the average SNF estimated from the milk of 16 Vechur cows was 8.88±0.11 percent. Venkatachalapathy (1996) gave the average percentage of Solids Not Fat in a study conducted on 15 Vechur cows as 8.84±0.12 and 8.92±0.14 for morning and evening milk respectively. Joshi *et al.* (2005) reported the average SNF of milk of Nimari, Ongole, and Umblachery as 7.95±0.05, 8.68±1.30 and 7.89±0.03 respectively. The SNF content of milk of Vechur did not show much variation from the milk of these indigenous breeds of cattle. The SNF of milk of Vechur did not show any significant difference for the five families studied.

5.4.5 Age at first calving (AFC)

In the present study the average AFC obtained for a population of 41 Vechur cows was 1084.65±50.86 days. Abraham (2001) estimated the average AFC in Vechur and Kasargode cows as 1080±33.55 days and 1147.5±68.0 days respectively. Joshi *et al.* (2005) in his review reported the average AFC of Gir, Sahiwal, Red sindhi and Hariana as 1530.2±60.1, 1267.7±20.4, 1290.7±64.2 and 1566.8±3.8 days

respectively. Least Square Means of AFC in Gir estimated in a study conducted on 56 animals were 1345.90±26.6 days (Umrikar *et al.*, 1990). Vinoo *et al.* (2005) reported a mean AFC of 52.95±2.21 months in a study conducted at four Ongole cattle breeding farms. In the present study the V43, V44, V88 and V117 families were found to differ significantly for AFC. The mean AFC of V43 family (1294.50±67.52 days) was found to be greater than that of the population mean (1084.65±50.86 days). Hence it is recommended to select against the V43 ancestor for improving the AFC. The mean AFC of V88 (980.00±30.23 days), V117 (961.83±57.73 days) and V44 (878.66±17.86 days) families were found to be significantly lower than that of population mean (1084.65±50.86 days). The mean AFC of the V44 family was the lowest and was significantly different from the other two families. Hence it is advisable to select the V44 ancestor to reduce the AFC.

5.4.6 Peak Yield

The average peak yield of a population of 39 Vechur cows was estimated as 1.87±0.14 kgs in the present study. The mean peak yield given by Sennayam *et al.* (1987) in a study involving 334 Tharparkar cows was 6.45±0.09 kgs. Qureshi *et al.* (1993) estimated the mean peak yield from the records of 101 Rathi cows as 8.10±0.22 kgs. Girija (1994) reported a mean peak yield of 2.17±0.11 kgs in a study involving 28 dwarf cattle. According to Joshi *et al.* (2005) the average peak yield of some of the indigenous milch cattle breeds ranged from 10-12kgs per day even though some cows of the Gir, Sahiwal, Tharparkar, Red Sindhi and Kankrej produced record peak yields of above 20kgs per day. The peak milk yield of Vechur cattle was found to be lower than that of these indigenous cattle breeds. The peak yields estimated for the seven families did not differ significantly.

5.4.7 Days to attain Peak Yield

In the present study the average number of days required to attain peak yield in a population of 39 Vechur cows was estimated as 27.10±1.96 days. The average days to attain peak yield estimated from the records of 101 Rathi cows were

35.94±1.88 days (Qureshi *et al.*, 1993). According to Girija (1994) the mean number of days to attain peak yield in a study involving 28 dwarf cattle was estimated as 23.23±1.70days. The mean days to attain peak yield of Vechur cows was found to be almost same as that of the indigenous cattle. The mean days to attain peak yield did not show any significant difference for the seven families studied.

5.4.8 Intercalving Period

In the present study the average intercalving period obtained for a population of 23 Vechur cows was 425.75±13.34 days. In a study of production traits of 472 Tharparkar cows, the calving interval obtained was 470.32±7.06 days (Sennayan *et al.*, 1987). Umrikar *et al.* (1990) reported a mean calving interval of 606.8±21.00 days in a study conducted on 55 Gir cows. The intercalving period of the Vechur cow obtained in the present study was found to be slightly lower than that of the Gir and Tharparkar cattle. The intercalving period of the seven families studied did not show any significant difference. This may be due to the fact that the present study was conducted in a small population. Hence it is advocated to conduct this study in a large population so that selection can be applied effectively for reducing the intercalving period.

5.5 ALLELIC MEANS OF PRODUCTION PARAMETERS FOR DIFFERENT FAMILIES

5.5.1 HUJII77 locus

The animals with the allele of size 223 bp at the HUJII77 locus were having a mean fat percentage of 6.3±0.11 which was found to be significantly higher than mean fat percentage of those animals without this allele. The alleles of size 205, 209 and 221 bp having a mean fat percentage of 4.52±0.15, 4.22±0.12 and 4.5±0.13 respectively were significantly lower than that of the individuals not having these alleles. Mosig *et al.* (2001) reported that the marker HUJII77 was associated with

significant association for protein percentage. Rodriguez-Zas *et al.* (2002a) found that marker BL41 was associated with variation in milk yield and fat percentage. The frequency of this allele in the population was found to be 0.09. Hence it is advisable to select against this allele to improve the milk fat percentage of the population.

At the BL41 locus animals with the allele having a size of 238 bp was having a mean AFC of 1541.00±229.49 days. The mean AFC of this allele was found to be significantly greater than that for individuals without this allele. There were no earlier reports indicating association between this marker and AFC. The frequency of this allele in the population was 0.06; selection can be applied against this allele to prevent its frequency from increasing in the population.

5.5.4 BM4305

Allelic mean of fat percentage for the allele of size 158 bp at the BM4305 locus was significantly lower than the mean of those not having this allele. Mosig *et al.* (2001) reported association of BM4305 marker with protein percentage and milk yield on chromosome 4. No published reports were available showing association between this marker and QTL affecting fat percentage and AFC. Selection against this allele will be effective in bringing up the fat percentage of milk as they occur with a higher frequency of 0.21 in the population.

5.5.5 BM1508

The animals with the allele of size 113bp at the BM1508 locus had a significantly lower mean than the mean of those individuals without this allele. Heyen *et al.* (1999) reported the association of marker BM1508 with milk fat percentage. The frequency of this allele in the population was 0.16. Therefore selection can be performed against this allele in order to improve the milk fat percentage in the Vechur cattle population

the QTL for protein percentage on chromosome 4. The marker HUJII77 was associated with changes in milk yield and protein percentage (Rodriguez-Zas *et al.*, 2002a). There were no earlier reports of linkage existing between the marker HUJII77 and QTL affecting fat percentage.

According to Heyen *et al.* (1999) the HUJII77 marker is located on the same chromosome as the ILSTS096 and BL41 and these two markers have strong association with milk fat percentage. This may be the reason why the HUJII77 marker alleles influence the milk fat percentage. Selection can be applied for 223 bp size allele to improve the milk fat percentage as the frequency of this allele (0.04) was low in the population. It is advisable to select against the 209 and 221 bp size alleles in this population as these alleles are present in the population with a moderate frequency of 0.13 and 0.17 respectively. It is not necessary to select against the 205 bp size alleles as the frequency of this allele (0.03) is low in the population.

5.5.2 ILSTS096 locus

The allelic mean fat percentage for animals having 208 bp allele at the ILSTS096 locus was 4.96±0.17. Heyen *et al.* (1999) found that the marker ILSTS096 located on BTA3 was associated with milk protein percentage. According to Mosig *et al.* (2001) the marker ILSTS096 was associated with QTL influencing milk yield, protein yield, protein percentage, fat yield and fat percentage. The mean fat percentage of individuals having the 208 bp size allele was found to be significantly greater than that of individuals without this allele. As this allele is having a high frequency of 0.25 the selection for this allele may not be effective.

5.5.3 BL41 locus

The animals with allele of size 252 bp having a mean fat percentage of 4.50 ±0.12 was found to be significantly lower than that of individuals not having this allele. Heyen *et al.* (1999) reported that the markers BL41 and ILSTS096 showed

5.6 EVALUATION OF ANCESTORS

Even though the sire V43 can be selected for improving the fat percentage of the Vechur population, the mean AFC of this sire family was found to be greater than the population mean indicating selection against this sire. Selection can be applied for the sire V88 as the mean fat percentage of this sire is significantly greater than that of the population mean. The V88 sire was also found to have alleles of size 223 bp at HUJII77 locus and 208 bp at the ILSTS096 locus which has a positive influence on fat percentage.

The AFC of the sire family V88 and the sire family V117 were found to be significantly lower than that of the population mean. Hence selection can be applied for this ancestors but the V117 sire have the alleles of size 113 bp at the BM1508 locus which has a negative influence on fat percentage. The AFC of the dam family V44 was also significantly lower than that of the population mean. The mean AFC of this family was found to be the lowest of the seven families studied. Selection can be applied for the V44 dam but it was found to have the alleles of size 221 bp and 252bp at the HUJII77 and BL41 locus respectively which had a negative influence on fat percentage.

The V87 sire family did not differ significantly from population mean for fat percentage or for AFC. This sire was also found to have the alleles of size 205bp at the HUJII77 locus and 238 bp at the BL41 locus which had a negative influence on fat percentage and AFC respectively. Hence it is not preferable to apply selection on this sire for improving fat percentage or for reducing the AFC. The V009 family did not differ significantly either for fat percentage or for AFC from population mean. This dam was found to have the 252 bp size allele which had a negative influence on fat percentage. Hence it is not advisable to select for dam. Selection should not be applied for the V83 sire as this sire was found to contain the alleles of size 221 bp, 252bp and 158 bp at the HUJII77 locus, BL41 locus and BM4305 locus respectively which has a negative influence on fat percentage.

If we consider the two economic traits with significant effect together namely AFC and fat percentage, selection of V88 will be beneficial in having significant higher milk fat percentage and lower AFC in comparison to population mean. There is a possibility of improving the fat percentage and to reducing the AFC of the Vechur cattle maintained at the Vechur Conservation Centre by adopting line breeding of V88 sire.

Summary

6. SUMMARY

The DNA samples prepared for the study consists of a panel of 50 unrelated samples from Vechur cattle and another panel of 33 combination of ancestors and offspring of Vechur cattle. The DNA was extracted from both blood and semen using phenol : chloroform method. Good quality DNA was obtained from both the whole blood and semen samples which appeared as a single band on agarose gel electrophoresis.

Five microsatellite markers viz. HUJII77, ILSTS096, BL41, BM4305 and BM1508 were selected based on their polymorphisity and association with the QTL influencing milk production. Primers were custom synthesized and five markers showing higher degree of polymorphism were chosen for the study. The unrelated genomic DNA samples of the Vechur cattle were typed using these five microsatellite markers. The forward primer of each marker was endlabeled at the 3'end with γ ^{32}P ATP using DNA endlabeling kit.

The PCR conditions were standardized for each marker. A 10 µl PCR reaction contained 50ng of template DNA, 1µl 10X PCR buffer, 1.25-1.5m*M* $MgCl_2$ depending upon the primer, 200 µ*M* of dNTP and 0.3U of Taq DNA polymerase. The markers ILSTS096, BM1508 and BM4305 were standardized at 1.25m*M* of $MgCl_2$ and the markers HUJII77 and BL41 were standardized at 1.5m*M* of $MgCl_2$. The PCR products were checked on 1% agarose gel and then resolved on 6% denaturing polyacrylamide gel. The alleles for each marker locus appeared as a double/single bands. The size of the bands was determined by comparison with the sequenced products of M13 phage DNA run in the centre of the gel.

Fourteen alleles were identified at the HUJI77 locus with its size ranging from 193-225 bp. Twelve alleles ranging in size from 188-212 bp were obtained at the ILSTS096 locus. At the BL41 locus, eleven alleles were obtained ranging in size from 238-258 bp. Eleven alleles were identified at the BM4305 locus ranging in size

from 146-168 bp. The BM1508 locus was found to be the least polymorphic of the five markers studied with nine alleles ranging in size from 103-119 bp.

The heterozygosity value indicates the usefulness of the markers in the study of genetic polymorphism. Direct count heterozygosity values of 0.899, 0.862, 0.868, 0.875 and 0.840 were obtained for the markers HUJII77, ILSTS096, BL41, BM4305 and BM1508 respectively. Unbiased heterozygosity values of 0.932, 0.899, 0.909, 0.917 and 0.889 were obtained for the HUJII77, ILSTS096, BL41, BM4305 and BM1508 markers respectively. PIC value ranged from 0.82 for BM1508 locus to 0.89 for HUJII77 locus.

Genomic DNA of the ancestors and progenies were genotyped for these five microsatellite markers. Data regarding the AFC, peak yield, days to attain peak yield and intercalving period was obtained from the records maintained at the Vechur Conservation Centre, Mannuthy. Milk fat percentage, protein percentage, SNF and total solids were estimated from the milk samples collected from the Vechur cows. Milk fat percentage was estimated by the Gerbers method. Total solids of milk was determined by Gravimetric method. Micro Kjeldhal method was used to determine the milk protein percentage. SNF was determined from the difference between the total solids and fat percentage of milk.

Family means for the different production parameters like milk fat percentage, protein percentage, total solids, SNF, peak yield, days to attain peak yield and intercalving period were determined for daughter of each ancestor. The family means were found to differ significantly for fat percentage and AFC. The other parameters like protein percentage, total solids, SNF, peak yield, days to attain peak yield and intervcalving period did not differ significantly for the families studied.

The fat percentage of the V88 and the V43 families were found to differ significantly from the fat percentage of the other families studied. The mean fat percentage of V88 and V43 family were 5.42±0.27 and 5.58±0.42 respectively and

they were found to be significantly greater than population mean. The V43, V44, V88 and V117 families were found to differ significantly for AFC. The mean AFC (1294.50±67.52 days) of V43 family was found to be significantly greater than the population mean (1084.65±50.86 days). The V44, V88 and V117 families had a mean AFC significantly lower than that of the population. The mean AFC (878.66±17.86 days) of V44 family was significantly lower than that of V88 and V117 families.

The allele of size 223 bp at the HUJII77 locus having a mean fat percentage of 6.3±0.11 was found to be significantly higher than mean fat percentage of those animals without this allele. Though significance was observed, there are no established reports of linkage between HUJII77 marker and QTL associated with fat percentage. The alleles of size 205, 209 and 221 bp were found to have significantly lower effect on fat percentage when compared to the effect of those individuals not having this allele. Selection can be applied for the 223 bp size allele whereas it is advisable to select against 209 and 221 bp size alleles in the population in order to improve the milk fat percentage.

The allelic effect of 208 bp size allele at the ILSTS096 locus was found to be significantly greater for fat percentage than the effect of those without this allele. The selection for this allele having a high frequency of 0.25 in the population will not be effective.

The animals with allele of size 252 bp at the BL41 locus had a mean fat percentage significantly lower than that of the individuals not having this allele. As the population contains a moderate frequency of this allele, it is advisable to select against this allele. At the BL41 locus another allele of size 238 bp was found to have an allelic mean of AFC significantly higher than that for individuals not having this allele. Selection can be applied against this allele to prevent its frequency from increasing in the population.

The allele of size 158 bp at the BM4305 locus was found to be significantly lower than the mean of those not having this allele. Though there are no published reports of linkage existing between this marker and QTL affecting fat percentage, selection against this allele is necessary as this allele is present in high frequency in the population.

The allele of size 113 bp at the BM1508 locus had a significantly lower mean for fat percentage than the mean of those individuals without this allele. It is advisable to select against this allele to increase the milk fat percentage in the population.

Of the seven ancestors studied, selection for V88 sire will be beneficial because the mean fat percentage of this sire is significantly higher and the mean AFC is significantly lower than the population mean. There is a possibility of improving the fat percentage and to reducing the AFC of the Vechur cattle maintained at the Vechur Conservation Centre, Mannuthy by adopting line breeding of V88 sire.

References

REFERENCES

Abraham, B.L. 2001. Association of bodyweight and milk production in Vechur and other dwarf Desi cattle of Kerala. MVSc. Thesis, Kerala Agricultural University, Thrissur, p. 109

Andersson, L., Bohme, J., Rask, L. and Peterson, P.A. 1986. Genomic hybridization of bovine class II major histocompatability genes: Extensive polymorphism of DQ_α and DQ_β genes. *Anim. Genet.* 17: 95-112

Anilkumar, K. 2003. Delineation of random amplified polymorphic DNA markers in crossbred cattle. PhD thesis, Kerala Agricultural University, Thrissur, p. 154

AOAC. 1990. *Official method of analysis*. Thirteenth edition. Association of official analytical chemists. Washington, D.C. p. 587

Apparao, K.B.C., Bhat, P.P. and Bhat, P.N. 1994. A rapid and simple procedure for isolation of genomic DNA from blood for RFLP studies in livestock animals. *Indian J. Anim. Sci.* 64: 460-462

Aravindakshan, T.V., Nainar, A.M. and Nachimuthu, K. 1998. Extraction of DNA from cattle white blood cells. *Cherion* 27: 80-84

Arezi, B., Xing, W., Sorge, J.A and Hogrefe, H.H. 2003. Amplification efficiency of thermostable DNA polymerases. *Anal. Biochem.* 321:226-235

Arranz, J.J., Coppieters, W., Berzi, P., Cambisano, N., Grisart, B., Karim, L., Marcq, F., Moreau, L., Mezer, C., Riquet, J., Simon, P., Vanmanshoven, P., Wagenaar, D. and Georges, M. 1998. A QTL affecting milk yield and composition maps to bovine chromosome 20. *Anim. Genet.* 29: 107-115

Ashwell, M.S., Da, Y., van Tassel, C.P., vanRaden, P.M., Miller, R.H. and Rexroad, Jr.C.F. 1998. Detection of putative loci affecting milk production and composition, health and type traits in a United States Holstein population. *J. Dairy Sci.* 81: 3304-3314

Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., van Tassell, C.P., Da, Y., vanRaden, P.M., Ron, M., Weller, J.I. And Lewin, H.A. 2004. Detection of quantitative trait loci affecting milk production, health and reproductive traits in Holstein cattle. *J. Dairy Sci.* 87: 468-475

Ashwell, M.S., van Tassell, C.P., Sonstegard, T.S. 2001. A genome scan to identify quantitative trait loci affecting economically important traits in a US Holstein population. *J. Dairy Sci.* 84: 2535-2542

Bahnak, B.R., Wu, Q.Y., Coulombel,L., Drovet, L., Kerbiriou-Nabias, D. and Meyer, D. 1988. A simple and efficient method for isolating high molecular weight DNA from mammalian sperm. *Nucleic Acids Res.* 16: 1208

Barendse, W., Armitage, S.M., Kossareck, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li. L., Neibergs, H.L., Zang, N., Grosse, W.M., Weiss, J., Creighton, P., Mc Carthy, F., Ron, M., Teale, A.J., Fries, R., Mc Graw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E. and Hetzel, D.J.S. 1994. A genetic linkage map of the bovine genome. *Nature Genet.* 6:227-235

Biggin, M.D., Gibson, T.J. and Hong, G.F. 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. *Proc. Natl. Acad. Sci. USA.* 80: 3963-3965

Bishop, M.D., Hawkins, G.A. and Keefer, C.L. 1995. Use of DNA markers in animal selection. *Theriogenology* 43: 61-70

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D., Yoo, J. and Beattie, G.W. 1994. A genetic linkage map for cattle. *Genetics* 136: 619-639

Botstein, D., White, R.D., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331

Bovenhuis, H. and Weller, J.I. 1994. Mapping and analysis of Dairy cattle quantitative trait loci by maximum likelihood methodology using milk protein genes as genetic markers. *Genetics* 137: 267-280

Bovenhuis, H., van Arendonk, J.A.M., Davis, G., Elsen, J.M., Haley, C.S., Hill, W.G., Baret, P.V., Hetzel, D.J.S. and Nicholas, F.W. 1997. Detection and mapping of quantitative trait loci in farm animals. *Livest. Prod. Sci.* 52: 135-144

Brascamp, E.W., van Arendonk, J.A.M and Groen, A.F. 1993. Economic appraisal of the utilization of genetic markers in dairy cattle breeding. *J. Dairy Sci.* 76: 1204-1213

Curi, R.A. and Lopes, C.R. 2002. Evaluation of nine microsatellite loci and misidentification paternity frequency in a population of Gyr Breed bovines. *Braz. J. Vet. Res. Anim. Sci.* 39: 129-135

Davis, G.P. and deNise, S.K. 1998. The impact of genetic markers on selection. *J. Anim. Sci.* 76: 2331-2339

Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K. and Mattick, J.S. 1991. Touchdown PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res.* 19: 4008

Eckert, K.A. and Kunkel, T.A. 1990. High fidelity DNA synthesis by the Thermus aquaticus DNA polymerase. *Nucleic Acids Res.* 18: 3739-3744

Erlich, H.A., Gelfand, D. and Sninsky, J.J. 1991. Recent advances in the Polymerase Chain Reaction. *Science* 252: 1643-1651

Esposito, D.L., Palmirotta, R., Veri, M.C., Mammarella, S., D'Amico, F., Curia, M.C., Aceto, G., Crognale, S., Creati, B., Constantini, R.M., Battista, P. and Cama, A. 1998. Optimized PCR labeling in mutational and microsatellite analysis. *Clin. Chem.* 44: 1381-1387

Freyer, G., Sorensen, P., Kuhn, C., Weikard, R. and Hoeschele, I. 2003. Search for pleiotropic QTL on chromosome BTA6 affecting yield traits of milk production. *J. Dairy Sci.* 86: 999-1008

Georges, M. 2001. Recent progress in livestock genomics and potential impact on breeding programmes. *Theriogenology* 55: 15-21

Georges, M., Nielsen, D., Mackinnon, M., Mishra, A., Okimoto, R., Pasquino, A.T., Sargeant, L.S., Sorensen, A., Steele, M.R., Zhao X., Womack J.E. and Hoeschele, I. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* 139: 907-920

Girija, C.R. 1994. Characterisation and evaluation of the dwarf cattle of Kerala. PhD thesis, Kerala Agricultural University, Thrissur, p. 110

Gyllensten, U.B. and Erlich, H.A. 1988. Generation of single-stranded DNA by the Polymerase Chain Reaction and its application to direct sequencing of the HLA-DQA locus. *Proc. Natl. Acad. Sci. USA.* 85: 7652-7656

Hai-Guo, J., Guo-li, Z., Yang, C. and Ming-Xing. 2003. Study on genetic variation of 4 microsatellite DNA markers and their relationship with somatic cell counts in cow milk. *Asian-Aust. J. Anim. Sci.* 16: 1535-1539

Heyen, D.W., Weller, J.I., Ron, M., Band, M., Beever, J.E., Feldmesser, E., Da, Y., Wiggans, G.R., VanRaden, P.M. and Lewin, H.A. 1999. A genome scan for QTL influencing milk production and health traits in dairy cattle. *Physiol. Genom.* 1: 165-175

IS: 1224. 1977. Determination of fat by Gerber's method. Part. I. Milk (First revision). Indian Standards Institution, New Delhi, pp. 18

IS: 1479. 1961. Determination of total solids in milk (Gravimetric method). Methods of Tests for dairy Industry: Part-II – Chemical analysis of milk. Indian Standards Institution, New Delhi, pp. 6

Jang, G.W., Cho, K.H., Kim, T.H., Oh, S.J., Cheong, I.C. and Lee, K.J. 2005. Association of candidate genes with production traits in Korean dairy proven and young bulls. *Asian-Aust. J. Anim. Sci.* 18: 165-169

Jeanpierre, M. 1987. A rapid method for purification of DNA from blood. *Nucleic Acids Res.* 15: 961

Joshi, K.B., Singh, A. and Mukherjee, S. 2005. Genetic improvement of indigenous cattle for milk and draught: A review. *Indian J. Anim. Sci.* 75: 335-348

Kappes, S.M., Keele, J.W., Stone, R.T., McGraw, R.A., Sonstegard, T.S., Smith, T.P., Lopez-Corrales, N.L., and Beattie, C.W. 1997. A second-generation linkage map of the bovine genome. *Genome Res.* 7: 235–249

Kemp, S.J., Hirshida, A.O., Wambugu, J. Rink, A., Longeri, M.L., Ma, R.Z., Da, Y., Lewin, H.A., Barendse, W. and Teale, A.J. 1995. A panel of polymorphic bovine, ovine and caprine microsatellite markers. *Anim. Genet.* 26: 299-306

Kuhn, C., Freyer, G., Weikard, R., Goldammer, T. and Schwerin, M. 1999. Detection of a QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6. *Anim. Genet.* 30: 333-340

Kuhn, C., Freyer, G., Weikard, R., Goldammer, T. and Schwerin, M. 1999. Detection of a QTL for milk production traits in cattle by application of a specifically developed marker map of BTA6. *Anim. Genet.* 30: 333-340

Lande, R. and Thompson, R. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* 124: 743-756

Li, C., Wang, Z., Liu, B., Yang, S., Zhu, Z., Fan, B., Zhao, M.Y.S. and Li, K. 2004. Evaluation of the genetic relationship among ten Chinese indigenous pig breeds with twenty-six microsatellite markers. *Asian-Aust. J. Anim. Sci.* 17: 441-444

Lipkin, E., Mosig, M.O., Darvasi, A., Ezra, E., Shalom, A., Friedmann, A. and Soller, M. 1998. Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling using dinucleotide microsatellite markers: Analysis of milk protein percentage. *Genetics* 149: 1557-1567

Litt, M. and Luty, J.A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401

Meuwissen, T.H.E. and van Arendonk, J.A.M. 1992. Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. *J. Dairy Sci.* 75: 1651-1659

Miller, S.A., Dybes, D.D. and Polesky, M.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1216

Mosig, M.O., Lipkin E., Khutoreskaya, G., Tchourzyna, E., Soller, M., Friedmann, A. 2001. A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. *Genetics* 157: 1683-1698

Mukesh, M., Sodhi, M., Bhatia, S. and Mishra, B.P. 2004. Genetic diversity of Indian native cattle breeds as analysed with 20 microsatellite loci. *J. Anim. Breed. Genet.* 121: 416-424

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. and Erlich, H. 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology.* 51: 263-273

Nadesalingam, J., Plante, Y., Gibson, J.P. 2001. Detection of QTL for milk production on Chromosomes 1 and 6 of Holstein cattle. *Mamm. Genome.* 12: 27-31

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590

Ng-Kwai-Hang, K.F., Zadwohny, D., Hayes, J.F. and Kuhnlein, U. 1991. Identification of k-Casein genotype in Holstein Sires: A comparison between analysis of milk samples from daughters and direct analysis of semen samples from sires by Polymerase Chain Reaction. *J. Dairy Sci.* 74:2410-2415

Noor, M.A.F., Kliman, R.M., and Machado, C.A. 2001. Evolutionary history of microsatellites in the Obscura group of Drosophila. *Mol. Biol. Evol.* 18: 551-556

Oh, Y. and Mao. L. 1999. Microsatellite protocols. : *PCR Applications-protocol for functional genomics.* (eds. Innis, M.A., Gelfand, D.H. and Sninsky, J.J.). Academic Press, New York, pp. 355-363

Oliver, R.A., Brown, P., Spooner, R.L., Joosten, I. and Williams, J.L. 1989. The analyses of antigen and DNA polymorphism with the bovine major histocompatability complex: The class I antigens. *Anim. Genet.* 20: 31-41

Olsen, H.G., Gomez-Raya, L., Vage, D.I., Olsaker, I., Klungland, H., Svendsen, M., Adnoy, T., Sabry, A., Klemetsda, G., Schulman, N., Kramer, W., Thaller, G., Ronningen, K. and Lien, S. 2002. A genome scan for quantitative trait loci affecting milk production in Norwegian dairy cattle. *J. Dairy Sci.* 85: 3124-3130

Ott, J. 1992. Strategies for characterizing highly polymorphic markers in human gene mapping. *Am. J. Hum. Genet.* 51: 283-290

Pandey, A.K., Tantia, M.S., Kumar, D., Mishra, B., Chaudhary, P. and Vijh, R.K. 2002. Microsatellite analysis of three poultry breeds of India. *Asian-Aust. J. Anim. Sci.* 15: 1536-1542

Plante, Y., Gibson, J.P., Nadesalingam, J., Mehrabani-Yeganeh, H., Lefebvre, S., Vandervoort, G. and Jansen, G.B. 2001. Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. *J. Dairy Sci.* 84: 1516-1524

Qureshi, M.I., Jain, R.D. and Juwekar, A.V. 1993. A note on peak yield and persistency of first lactation yield in Rathi cattle. *Cherion* 22: 8-10

Rodriguez-Zas, S.L., Southey, B.R., Heyen, D.W. and Lewin, H.A. 2002a. Detection of a quantitative trait loci influencing dairy traits using a model for longitudinal data. *J. Dairy Sci.* 85: 2681-2691

Rodriguez-Zas, S.L., Southey, B.R., Heyen, D.W. and Lewin, H.A. 2002b. Interval and composite interval mapping of somatic cell score, yield, and components of milk in dairy cattle. *J. Dairy Sci.* 85: 3081-3091

Ron, M., Band, M., Yanai, A. and Weller, J.I. 1994. Mapping quantitative trait loci with DNA microsatellites in a commercial dairy cattle population. *Anim. Genet.* 25: 259-264

Ron, M., Feldmesser, E., Golik, M., Tager-Cohen, I., Kliger, D., Reiss, V., Domochovsky, R., Alus, O., Seroussi, E., Ezra, E. and Weller, J.I. 2004. A complete genome scan of the Israeli Holstein population for Quantitative trait loci by a daughter design. *J. Dairy Sci.* 87: 476-490

Ron, M., Heyen, D.W., Weller, J.I., Band, M., Feldmesser, E., Pasternak, H., Da, Y., Wiggans, G.R., VanRaden, P.M., Ezra, E. and Lewin, H.A. 1998. Detection and analysis of a locus affecting milk concentration in the US and Israeli dairy cattle populations. *Proceedings of 6th World Congress on Genetics and Applied Livestock Production, January 11-16, 1998,* Armidale. pp. 422-425

Ron, M., Kliger, D., Feldmesser, E., Seroussi, E., Ezra, E. and Weller, J.I. 2001. Multiple quantitative trait locus analysis of bovine chromosome 6 in the Israeli Holstein population by daughter design. *Genetics* 159: 727-735

Ruane, J. and Colleau, J.J. 1996. Marker assisted selection for a sex-limited character in a nucleus breeding population. *J. Dairy Sci.* 79: 1666-1675

*Rubinsztein, D.C., Amos, W., Leggo, J., Goodburn, S., Jain, S., Li, S.H., Margolis, R.L., Ross, C.A. and Ferguson-Smith, M.A. 1995. Microsatellite evolution – evidence for directionality and variation in rate between species. *Nature Genet.* 10: 337-343

Saiki, R.K., Gelfand, A.H., Stoffel, S., Scharf, S.J. Higuchi.R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer- directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science* 239: 487-491

Sambrook, J., Fritish, E.T. and Maniatis, T. 1989. *Molecular cloning : A laboratory manual.* Second edition. Cold Spring Harbor Laboratory Press, New York. p.1886

Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA*. 74: 5463-5467

*Schulman, N.F. and Dentine, M.R. 2005. Linkage disequilibrium and selection response in two-stage marker-assisted selection of dairy cattle over several generations. *J. Anim. Breed. Genet.* 122: 110

Segev, D.L., Saji, M., Phillips, G.S., Westra, W.H., Phillips, G.S., Westra, W.H., Takiyama, Y., Piantadosi, S., Smallridge, C., Nishiyama, P.H., Udelsman, R. and Zeiger, M.A. 1998. Polymerase chain reaction - based microsatellite polymorphism analysis of follicular and hurthle cell neoplasms of the thyroid. *J. Clin. Endocrinol. Metab.* 83: 2036-2042

Sennayan, S., Shanmugasundaram, S. and Thiagarajan, M. 1987. A study on some production traits in Tharparkar cows. *Cherion* 16: 70-75

Shalom, A., Mosig, M.O., Barendse, W., Friedmann, A. and Soller, M. 1994. Dinucleotide repeat polymorphism at the bovine HUJ246, HUJII77, HUJVI74 and HUGI75 loci. *Anim. Genet.* 25: 56

*Snedecor, G.W. and Cochran, W.G. 1985. *Statistical methods*. Seventh edition. The Iowa State University Press, USA, p. 313

Soller, M. 1990. Genetic mapping of the bovine genome using deoxyribonucleic acid level markers to identify loci affecting quantitative traits of economic importance. *J. Dairy Sci.* 73: 2628-2646

Sonstegard, T.S., Van Tassell, C.P. and Ashwell, M.S. 2001. Dairy cattle genomics: Tools to accelerate genetic improvement? *J. Anim. Sci.* 79: 307-315

*Spelman, R. and Garrick, D. 1997. Utilization of marker assisted selection in a commercial dairy cow population. *Livest. Prod. Sci.* 47: 139-147

Spelman, R. J. and Garrick, D.J. 1998. Genetic and economic responses for within family marker assisted selection in dairy cattle breeding schemes. *J. Dairy Sci.* 81: 2942-2950

Stella, A., Lohuis, M.M., Pagnacco, G. and Jansen, G.B. 2002. Strategies for continual application of marker-assisted selection in an open nucleus population. *J. Dairy Sci.* 85: 2358-2367

*Stone, R.T., Pulido, J.C., Duyk, G.M., Kappes, S.M., Keele, J.W. and Beattie, C.W. 1995. A small-insert bovine genomic library highly enriched for microsatellite repeat sequence. *Mamm. Genome.* 6: 714-724

Stuber, C.W., Moll, R.H., Goodman, M.M., Shaffer, H.E. and Weir, B.S. 1980. Allozyme frequency changes associated with selection for increased grain yield in maize (Zea mays L.). *Genetics* 95: 225

Tabor, S. and Richardson, C.C. 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA.* 84: 4767-4771

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17: 6463-6471

Trommelen, G.J.M., Dass, D.N.H.G., Vijg, J. and Uitterlinden, A.G. 1993. Identity and paternity testing of cattle: application of a deoxyribonucleic acid profiling protocol. *J. Dairy Sci.* 76: 1403-1411

Umrikar, U.D., Natarajan, N. and Thangaraju, R. 1990. Factors influencing performance traits of Gir and Jersey X Gir crossbred cattle. *Cherion* 19: 131-138

Van Hooft, W.F., Hanotte, O., Wenink, P.W., Groen, A.F., Sugimoto, Y., Prins, H.H.T. and Teale, A. 1999. Applicability of bovine microsatellite markers for population genetic studies on African buffalo (Syncerus caffer). *Anim. Genet.* 30: 214-220

van Tassell, C.P., Ashwell M.S. and Sonstegard, T.S. 2000. Detection of putative loci affecting milk, health and conformation traits in a US Holstein population using 105 microsatellite markers. *J. Dairy Sci.* 83: 1865-1872

Velmala, R., Vilkki, J., Elo, K. and Maki-Tanila, A. 1995. Casein haplotypes and their association with milk production traits in the Finnish Ayrshire cattle. *Anim. Genet.* 26: 419-425

Velmala, R.J., Vilkki, H.J., Elo, K.T., De Koning, D.J., Maki-Tanila, A.V. 1999. A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. *Anim. Genet.* 30: 136-143

Venkatachalapathy, R.T. 1996. Milk protein polymorphism and milk composition in Vechur cattle. MVSc. Thesis, Kerala Agricultural University, Thrissur, p. 104

Venkatachalapathy, R.T. and Iype, S. 1998. Fat, total solids and solids-no-fat percentage of milk in Vechur cattle. *Indian J. Anim. Sci.* 68: 570-573

Viitala, S.M., Schulman, N.F., de Koning, D. J., Elo, K., Kinos, R., Virta, A., Virta , J., Maki-Tanila A. and Vilkki, J. H. 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. *J. Dairy Sci.* 86: 1828-1836

Vinoo, R., Rao, N.G., Gupta, B.R. and Babu Rao. 2005. Genetic study on productive and reproductive traits of Ongole cattle. *Indian J. Anim Sci.* 75: 438-441

*Weber, J.L. 1990. Informativeness of human $(dC\text{-}dA)_n(dG\text{-}dT)_n$ polymorphisms. *Genomics* 7: 4-30

Weber, J.L. and May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396

Weller, J.I., Golik, M., Seroussi, E., Ezrat, E. and Ron, M. 2003. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein Population. *J. Dairy Sci.* 86: 2219-2227

Weller, J.I., Kashi ,Y., Soller, M. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *J. Dairy Sci.* 73: 2525-2537

White, T.J., Arnheim, N. and Erlich, H.A. 1989. The Polymerase Chain Reaction. *Trends. Genet.* 5: 185-188

* Orginals not consulted

Annexures

ANNEXURE I

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

40% Acrylamide

Acrylamide	380g
N,N – Methylene bisacrylamide	20 g
Water to	1000 ml

1.5% Agarose

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

10% Ammonium persulphate

APS	100 mg
Water to	1 ml

6% Denaturing Acrylamide Gel

0.5 X TBE Gelmix	-	60 ml
TEMED	-	0.125 ml
10% APS	-	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 up to 100 ml. Stored at room temperature after filtration and autoclaving.

Ethidium Bromide (10 mg/ml)

100 mg ethidium bromide dissolved in 10 ml distilled water. Solution stored at 4°C in 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 waterbath. Hydroxyquinolone added to a final concentration of 0.1%. 0.5*M* Tris HCl (pH 8.0) added to molten phenol in equal volume. Mixture stirred for 30 min on a magnetic stirrer and contents transferred into a separating funnel. Lower phenolic phase collected, mixed with equal volume of 0.1*M* Tris HCl (ph 8.0) and stirred again for 30 min. The phenolic phase 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 dark bottle at 4°C.

Phosphate Buffered Saline (PBS, pH 7.4)

Sodium chloride (NaCl)	138 m*M*	8.0647 g
Sodium Dihydrogen Phosphate ($NaH_2PO_42H_2O$)	4 m*M*	0.6240 g
Disodium hydrogen phosphate ($Na_2HPO_412H_2O$)	6 m*M*	2.1488 g

Contents weighed and mixed with 800 ml distilled water on a magnetic stirrer and pH adjusted to 7.4 using dilute HCl. Final volume made upto 1000 ml.

RBC lysis buffer

Ammonium chloride	150 m*M*	8.0235 g
Potassium chloride	10 m*M*	0.7455 g
EDTA	0.1 m*M*	0.0372 g

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

Sodium acetate (3 *M*, pH 5.5)

Dissolved 40.824 g of sodium acetate in 70 ml 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 upto 100 ml. Solution filtered and stored at room temperature.

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

Sodium chloride	75 m*M*	4.383 g
EDTA	35 m*M*	9.306 g

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

Sodium dodecyl sulphate (SDS) 20%

SDS 20 g
Distilled water to make up to 100 ml.
Stirred, filtered and stored at room temperature.

Sperm lysis buffer

Contents	Stock solution	For making 100 ml
0.5% SDS	10%	5 ml
10 m *M* Tris (pH 8.0)	1 *M*	1 ml
2% Mercaptoethanol	100%	2 ml
10 m *M* EDTA (pH 8.0)	0.5 *M*	2 ml
100 m *M* Nacl	5 *M*	2 ml

All reagents except mercaptoethanol added and volume made upto 98 ml. Heated to 65°C just before use and added mercaptoethanol.

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 EDTA (TBE) buffer (pH 8.3) 10X

Tris base	108.0 g
Boric acid	55.0 g
EDTA	9.3 g

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

0.5X TBE Gel mix

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 m*M*	8.18 g
Potassium chloride	0.5 m*M*	0.0373 g
Tris base	0.25 m*M*	0.0303 g

Dissolved in 900 ml 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 m*M*	1.2114 g
EDTA	0.1 m*M*	0.3722 g

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

Tris 1*M* (pH 8.0)

Tris base	121.14 g

Distilled water up to 1 litre. pH adjusted to 8.0, filtered and stored at room temperature.

ANNEXURE II

SOURCES OF IMPORTANT CHEMICALS AND ENZYMES USED IN THIS STUDY

(A) CHEMICAL

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.	-Finzymes
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	

(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

PCR	Polymerase Chain Reaction
DNA	Deoxy Nucleic Acid
SSLP	Simple Sequence Length Polymorphisms
PAGE	Polyacrylamide Gel Electrophoresis
PIC	Polymorphic Information Content
BTA	*Bos taurus* Autosome
QTL	Quantitative Trait Loci
MAS	Marker Assisted Selection
AFC	Age at First Calving
SNF	Solids Not Fat
EDTA	Ethylene Diamine Teraacetic Acid
DTT	Dithiothretiol
TEMED	N, N, N, N Tetramethylethylenediamine
APS	Ammonium Persulphate
PNK	Polynucleotide Kinase
H	Heterozygosity
cM.	Centimorgan
µl	microlitres
µg	microgram
mg	milligram
m*M.*	millimolar
cm	centimeter
nm	nanometer
mCi	millicurie
bp	basepairs
rpm	Revolutions per minute
SDS	Sodium Dodecyl Sulphate
dNTP.	Deoxy Nucleotide Triphosphate

ddATP.	Dideoxy Adenosine Triphosphate
ddCTP	Dideoxy Cytosine Triphosphate.
ddGTP.	Dideoxy Guanosine Triphosphate
ddTTP.	Dideoxy Thymidine Triphosphate

MARKER ASSISTED SELECTION FOR MILK PRODUCTION TRAITS IN VECHUR CATTLE

SHYMAJA UTHAMAN

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

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

ABSTRACT

A study was conducted in Vechur cattle of Kerala to study the association of microsatellite marker alleles with the milk production and composition traits and to evaluate seven ancestors using microsatellite markers. Blood and semen was used as the source of DNA in this study. DNA samples were extracted from fifty unrelated animals and thirty three ancestors and progenies. Five microsatellite markers with established linkage to QTL controlling milk production traits like milk yield, fat percentage and protein percentage were used. The DNA samples obtained from the fifty unrelated Vechur cattle were typed using these five microsatellite markers. The five markers were found to be highly polymorphic with the PIC value ranging from 0.82 at the BM1508 locus to 0.89 at the HUJII77 locus. Data regarding the peak yield, days to attain peak yield, AFC and intercalving period were collected from the Vechur Conservation Centre, Mannuthy. Milk samples collected from the Vechur Conservation Centre were used to determine the milk fat percentage, protein percentage, SNF and total solids.

The mean fat percentage and AFC were found to differ significantly in different families. Statistical analysis revealed that the V43 and V88 families had a mean fat percentage significantly greater than the population mean. The mean AFC of V43 family was also found to be significantly greater than the population mean. The V44, V88 and V117 families had a mean AFC significantly lower than that of population mean. The V44 family had the lowest mean AFC and was also significantly different from the V88 and V117 families.

Microsatellite analysis was carried out for the thirty three combinations of ancestors and progeny using the same five markers. The allelic effect of microsatellite markers in the families was studied for fat percentage and AFC. The allelic mean for fat percentage of 223 bp size allele at the HUJII77 locus was found to be significantly higher when compared to those animals without this allele even though there were no earlier reports indicating linkage between this marker and QTL affecting fat percentage. The allelic mean of fat percentage for 205, 209 and 221 bp

at the HUJII77 locus was significantly lower when compared to those of animals without this allele.

The mean of the animals with 208 bp allele at the ILSTS096 locus was found to be significantly greater for fat percentage when compared to the mean of those without this allele. There are earlier published reports indicating linkage between this marker and QTL affecting fat percentage. Selection for this allele will not be effective as it is already present in high frequency in the population.

The 252 bp allele at the BL41 locus had an allelic mean for fat percentage significantly lower than that for animals without this allele. The 238 bp size allele of this locus had an allelic mean for AFC significantly greater than that for animals without this allele. Earlier reports establish linkage between this marker and QTL affecting fat percentage but not AFC. Selection can be applied against this allele to prevent its frequency from increasing in the population.

The allelic mean of 158 bp allele at the BM1508 locus had a mean fat percentage significantly lower than that for the animals without this allele. There are earlier reports of linkage of this marker with QTL affecting protein percentage but not fat percentage. Selection against this allele is necessary as this allele is present in high frequency in the population.

Animals with 113 bp allele had an allelic mean of fat percentage significantly lower than that for the animals without this allele. This marker has linkage with QTL affecting milk fat percentage. It is advisable to select against this allele to increase the milk fat percentage in the population.

Even though the V44 sire can be selected for improving the fat percentage, the mean AFC of this sire was found to significantly greater than that of the population mean hence it is not preferred.

The sire V88 can be selected for improving the fat percentage as well as reducing the AFC. There is a possibility to improve the Vechur cattle maintained at the Vechur Conservation Centre, Mannuthy for fat percentage and AFC by adopting line breeding for V88 sire.