EVALUATION OF MICROSATELLITE MARKERS FOR SELECTION OF CROSSBRED CATTLE

NAICY THOMAS

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

DECLARATION

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

Mannuthy
19.08.05

NAICY THOMAS

CERTIFICATE

Certified that this thesis, entitled **"EVALUATION OF MICROSATELLITE MARKERS FOR SELECTION OF CROSSBRED CATTLE"** is a record of research work done independently by **Dr. Naicy Thomas**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Mannuthy
19.08.05

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

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. Naicy Thomas**, a candidate for the degree of Master of Veterinary Science in Animal Genetics and Breeding, agree that this thesis entitled **"EVALUATION OF MICROSATELLITE MARKERS FOR SELECTION OF CROSSBRED CATTLE"** may be submitted by **Dr. Naicy Thomas**, in partial fulfilment of the requirement for the degree.

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

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

Dr. Stephen Mathew
Associate Professor
Centre for Advanced Studies in
Animal Genetics and Breeding
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. Sisilamma George
Associate Professor and Head
Department of Biochemistry
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

External Examiner
(Dr. C.S. Nagarajan)
Associate Professor
Dept. of ABG
Veterinary College
Hebbal, Bangalore-24

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NAICY THOMAS

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Introduction

1. INTRODUCTION

The tropical *Bos indicus* cattle are poor in milk production and their improvement was attempted through various strategies. The introduction of *Bos taurus* genes through crossing the native cows with exotic bulls was tried in India, as early as 1845. Later on introgression of taurus genes was accepted as one of the quickest means of improvement in production traits of tropical dairy cattle. But for sustaining and improving the quantum jump in milk production, stringent selection methods are necessary.

The milk production of the crossbred cattle in Kerala has remained more or less stagnant during the past few decades. Experts believe that problems with bull selection are the major hot spot. It is the need of the hour to introduce new technologies to overcome the present plateuing of the production of dairy cattle in the state.

Traditional selection methods in dairy cattle include pedigree selection and progeny testing. But the former lacks precision and the latter is time consuming. Hence researchers were trying to reduce the generation interval in the progeny testing programmes by incorporating various technological advances into the selection process.

Development of DNA technologies has made it possible to uncover a large number of genetic polymorphisms at the DNA level. These polymorphisms are used as genetic markers for evaluation of the phenotypic variability and the subsequent application of this knowledge can be used to improve the efficiency of dairy cattle selection processes. Of the different molecular marker systems, microsatellites are important with the advantages of extensive distribution, codominant inheritance, specificity and high polymorphic information content. Studies have established that some of these markers are linked to quantitative traits. The important applications of molecular markers in conventional breeding programmes include linkage mapping of Quantitative Trait Loci (QTL), marker assisted selection (MAS) and marker assisted introgression.

Once the linkage between a QTL and a marker locus is established, it is possible to recognize which of the alternate QTL allele defined by the marker, is inherited by an individual. This information can be used for family specific or breed specific selection of the breeding stock.

MAS enables the selection of superior genotypes based on allelic information of marker loci. One of the best examples of the application of MAS within a population is the selection of young bulls for sex limited traits, prior to their introduction for progeny testing programme. It also reduces the burden of maintaining a group of unwanted bulls, which are to be culled at a later time. MAS complements rather than replace the conventional breeding systems, leading to increased rate of genetic improvement through high selection intensity, high accuracy and reduced generation interval. Thus MAS is the process of using the results of DNA testing to assist in the selection of individuals to become parents in the next generation.

In the present study, possibility of using the information of the allele frequency, genotype frequency, heterozygosity and polymorphic information content of selected polymorphic microsatellite markers in the selection of crossbred cattle was studied. The association of different production traits, viz, 305 day milk yield, peak yield, days to attain peak yield, protein percentage, fat percentage, total solids, solids not fat (SNF) and age at first calving, with the alleles of the selected microsatellite markers in individual sire families was studied by genotyping the sires and their progenies. This information will prove useful for selecting sires possessing superior genotypes for production traits in future breeding programmes.

Review of Literature

2. REVIEW OF LITERATURE

2.1 MICROSATELLITE ANALYSIS

2.1.1 Microsatellites

Weber and May (1989) reported the presence of polymorphic $(CA)_n$ and $(GT)_n$ blocks representing a vast new pool of potential genetic markers. They opined that use of PCR to detect these polymorphisms offers improved sensitivity and speed compared with standard hybridization/blotting technique.

According to Litt and Luty (1989) codominant mendelian inheritance of allelic fragments of $(TG)_n$ microsatellites is very useful for linkage studies in humans.

Tautz (1989) used PCR to demonstrate simple sequence length polymorphisms (SSLP) in different species and suggested that this might be usefully exploited for identity testing, population studies, linkage analysis and genome mapping.

Lowe *et al.* (1990) designed a computer program which rapidly scans nucleotide sequences which select all possible pairs of oligonulceotides, suitable for use as primers to direct efficient DNA amplification by the PCR. This facilitates rapid selection of effective and specific primers from long gene sequences.

According to Rubinsztein *et al.* (1995) microsatellites evolved directly at different rates in closely related species and hence could be used as the genetic markers of choice for a wide range of applications like genome mapping and forensic testing.

Zhivotovsky and Feldman (1995) concluded that number of microsatellite loci required for construction of phylogenetic trees with reliable branch length might be several hundreds. This must have contributed to the frequent failure to predict the time of divergence obtained from molecular data.

Noor *et al.* (2001) cautioned the use of microsatellite allele sizes for phylogenic analysis or to infer divergences between populations. They concluded from the study on evolutionary history of micorsatellites in drosophila that multiple alleles of microsatellites come from the polymorphism in the identified repeat array and not solely from flanking region variation.

2.1.2 Genomic DNA Isolation

2.1.2.1 Genomic DNA isolation from blood

The protocol involving Proteinase K digestion and phenol-chloroform extraction followed by ethanol precipitation is an efficient method for isolation of genomic DNA from whole blood (Andersson *et al.*, 1986; Oliver *et al.*, 1989; Sambrook *et al.*, 1989).

Modification in phenol-chloroform method for extraction of DNA from white blood cells was described in several studies (Chithra, 2002; Anilkumar, 2003; Suprabha, 2003; Mathew, 2004; Preethy, 2004).

2.1.2.2 Genomic DNA isolation from semen

Genomic DNA can be isolated from semen by mercaptoethanol – Proteinase K treatment followed by phenol-chloroform extraction method (Andersson *et al.*, 1986; Trommelen *et al.*, 1993; Anilkumar and Suprabha, 2003).

2.1.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction is one of the most powerful techniques in molecular biology used for in vitro amplification of DNA and use of a thermostable DNA polymerase (Taq) has allowed sequence specific and highly sensitive detection and amplification of individual DNA sequences from very small initial quantities of DNA (Mullis *et al.*, 1986; Saiki *et al.*, 1988).

The standard PCR reaction mixture consists of PCR buffer (50 m*M* KCl, 10 m*M* Tris HCl, 1.5 m*M* $MgCl_2$ and 0.1 per cent gelatin), four dNTPs each at a concentration of 1.25 m*M*, 10 p*M* each primer one and two, template DNA, 2.5

units of Taq DNA polymerase and water to a final volume of 100 µl (Sambrook *et al.*, 1989).

2.1.4 Markers Linked to QTL

2.1.4.1 ILSTS096

Kemp *et al.* (1995) described that the microsatellite marker ILSTS096 with a $(CA)_{15}$ dinucleotide repeat was located on BTA with a relative position of 27.411 cM and heterozygozity of 59 with a size range of 192-208 bp. The polymorphic information content observed for this marker was 0.71. This was also published by Ihara *et al.* (2004) in the genetic map of the cattle genome based on 3802 microsatellites.

Heyen *et al.* (1999), in his genome scan for QTL affecting milk production and health traits detected that the microsatellite marker ILSTS096 was located on BTA3 affects milk yield, fat yield, fat percentage, protein yield and protein percentage. Rodriguez-Zas *et al.* (2002a) confirmed the effect of ILSTS096 on protein percentage in milk.

2.1.4.2 HUJII77

According to Shalom *et al.* (1994) the microsatellite marker HUJII77 was located on BTA3 with a relative position of 81.331 cM, with a size range of 187-213 bp and this marker has dinucleotide GT repeat sequences, which can be represented as $(GT)_2TT(GT)_{15}$ with 8 alleles and a heterozygosity of 86%. This was also confirmed by Ihara *et al.* (2004) in the genetic map of the cattle genome based on 3802 microsatellites.

In a genome scan for QTL affecting milk production and health traits in dairy cattle Heyen *et al.* (1999) found that the microsatellite marker HUJII77 affected protein percentage of milk. Rodriguez-Zas *et al.* (2002a) detected the effects of the marker HUJII77 on milk yield and protein percentage and suggested the linkage with a pleiotropic QTL.

Vallejo *et al.* (2003) studied the genetic diversity and background linkage disequilibrium in the North American Holstein cattle population using 54 Microsatellites and reported that the marker HUJII77 had 6 alleles in the sample population with a heterozygozity of 70.

2.1.4.3 BL41

The microsatellite marker BL41 is located on BTA 3 with a relative position of 43.292 cM and heterozygosity of 54 with a size range of 236-258 bp (Bishop *et al.*, 1994; Grosz *et al.*, 1997; Ihara *et al.*, 2004).

In a genome scan for QTL affecting milk production traits and health traits Heyen *et al.* (1999) found out that BL41 had effects on fat percentage and protein percentage of milk. Rodriguez-Zas *et al.* (2002a) detected the effects of the same marker on milk yield also, in addition to the milk fat and protein percentages.

2.1.4.4 BM1508

The microsatellite marker BM1508 is located on BTA14 with a relative position of 17.846 cM and heterozygosity of 42 with a size range of 99-115 bp (Stone *et al.*, 1995; Ihara *et al.*, 2004).

Heyen *et al.* (1999) reported that the microsatellite marker BM1508 located on BTA14 had effect on milk fat percentage.

2.1.4.5 BM4305

The microsatellite marker BM4305 is located on BTA14 with a relative position of 83.309 cM and heterozygosity of 69 with a size range of 148-168 bp (Bishop *et al.*, 1994; Ihara *et al.*, 2004).

The effect of BM4305 on protein percentage of milk was detected by Ashwell *et al.* (1998). Heyen *et al.* (1999) detected that the same marker affected milk yield also.

2.1.5 DNA Sequencing

Sanger *et al.* (1977) introduced the di-deoxy method for sequencing the DNA based on the ability of a DNA polymerase to extend a primer annealed to the template that is to be sequenced, until 2'-3' di-deoxy nucleotides are incorporated as chain terminators.

Biggin *et al.* (1983) showed that the use of deoxy adenosine 5'-(α-[^{35}S] thio) triophosphate as the label incorporated in dideoxy nucleotide sequence reactions increase the sharpness of the bands on an autoradiograph and so increases the resolutions achieved. dATP (α-^{35}S) is an analogue of dATP in which sulfur atom replaces a non-bridge oxygen atom on the α-phosphate and decay characteristics of ^{35}S are well suited to use in the dideoxy sequencing method.

Tabor *et al.* (1987) conducted a DNA sequence analysis with a modified bacteriophage T7 DNA polymerase and in the sequencing reaction they used the DNA of vector M13 mp8.

2.1.6 Endlabeling and Polyacrylamide Gel Electrophoresis

Tautz (1989) endlabeled 5' end of the primers using γ^{32}P-ATP and polynucleotide kinase and the PCR products were mixed with an equal volume of formamide dye solution and were resolved on a 6% denaturing acrylamide gel. He opined that minor bands visible after autoradiography were PCR artifacts which represented slippage events, which occured during PCR replication process.

Esposito *et al.* (1998) suggested that the two most common methods used for PCR labeling were endlabeling of primers or incorporation of a labeled nucleotide during PCR amplification. After incorporation of radioactivity, the radio labeled PCR products were mixed with one volume of formamide buffer, subjected to denaturing electrophoresis in 5% polyacrylamide gel containing 8 mol/L urea; gels were dried and autoradiographied for 1-4 days at room temperature.

Segev *et al.* (1998) conducted a PCR based microsatellite analysis in which one primer was end-labeled with T_4 kinase and γ^{32}P ATP. PCR was carried out in a

total volume of 10 µl containing 5-20 ng genomic DNA, 4 ng labeled primers, 20 ng of unlabelled primers in 35 cycles. Each cycle consisted of denaturing at 94°C for one minute, annealing at 55-60°C for one minute. PCR products were resolved on 6% urea polyacrylamide gels after addition of formamide. Gels were dried and autoradiographied.

Polyacrylamide gels are more commonly used for microsatellite analysis because they offer better band resolution and higher sensitivity for bands, when used with labeled PCR products (Oh and May, 1999).

Nadesalingam *et al.* (2001) used $\gamma^{32}P$ end-labeled forward primer for the PCR and the products were loaded in 6% denaturing (7M) polyacrylamide gels alongside and M13 sequencing ladder. After electrophoresis, the gels were fixed, air dried and exposed to X-ray films. Allele size of microsatellites were scored against the M13 sequence ladder.

2.2 GENOME MAPS

Barendse *et al.* (1994) constructed a cattle genetic linkage map, which marks about 90 per cent of the expected length of cattle genome. Less than 50 cM distance was found in male and female genetic maps. This physical map in which 114 microsatellites were assigned would be important for localizing QTL and would provide a basis for further mapping.

Bishop *et al.* (1994) integrated 172 microsatellite markers into a skeletal map of bovine genome. They also merged bovine cytogenetic and linkage maps by physically assigning informative microsatellites to the chromosomes.

A bovine linkages map constructed with 1280 polymorphic DNA markers, of which 627 were new, was presented by Kappes *et al.* (1997). This map provides sufficient marker density for genomic scans of populations with segregating QTL and subsequent implementation of MAS mating schemes.

Ihara *et al.* (2004) developed a comprehensive genetic map on the basis of more than 880,000 genotypes with a potential genetic resolution of 0.8 cM at 95 per

cent confidence level. They incorporated 2,325 microsatellites into the second generation genetic map by linkage analysis. This new map would accelerate fine mapping and positional cloning of genes for economically important traits in cattle.

2.3 QUANTITATIVE TRAIT LOCI (QTL) FOR MILK PRODUCTION TRAITS

Weller *et al.* (1990) introduced grand daughter design for Marker-QTL linkage in dairy cattle. They compared this design with daughter design as a function of QTL effect, heritability of the trait and number and structure of animals tested. In the grand daughter design the power increased with increased number of grand sires, sons per grand sire, daughter per son and gene effect, whereas decrease with increased heritability. In the case of daughter design the magnitude of effect of QTL had the greatest effect on power followed by effect of heritability. Number of sons affected power was more than the number of grand sires.

Georges *et al.* (1993) identified close linkage between a microsatellite marker, TGLA116 and the gene causing weaver disease in cattle. They confirmed the observations of Hoeschile and Meinert (1990).

The first scientific journal report of detection of QTL effects in dairy cattle with DNA microsatellites was presented by Ron *et al.* (1994). They observed a significant effect on milk yield and protein with paternal alleles for locus D21S4. They concluded that this information could be made use of in the rate of genetic gain for protein production.

Georges *et al.* (1995) found QTL for milk production traits on chromosomes 1, 6, 9, 10 and 20 within American Holstein cattle by exploiting progeny testing using 159 autosomal microsatellites and suggested that the identification of QTL segregating in elite dairy cattle populations was the first step towards the application of MAS for milk production. They stated that in conjunction with the already published bovine maps (Barendse *et al.*, 1994 and Bishop *et al.*, 1994) this marker set will give very adequate genome coverage for further mapping studies in cattle.

A QTL affecting milk yield and milk composition was confirmed on bovine chromosome 20 by a grand daughter design comprising 1152 sons for 6 microsatellite markers (Arranz *et al.*, 1998).

Ashwell *et al.* (1998) found strong evidence of associations between three microsatellite markers BM 415, BM 6425 and BM 719 and QTL for protein percentage and productive herd life in two US Holstein families.

In a study on selective DNA pooling on milk protein percentage, significant associations of five markers were observed (Lipkin *et al.*, 1998). They opined that this methodology could make genome-wide mapping of QTL accessible to moderately sized breeding organizations.

Ron *et al.* (1998) used DNA microsatellites to detect individual loci affecting economically important quantitative traits in dairy cattle by a grand daughter design. It was determined by maximum likelihood that the QTL had a substitution effect of about 0.28 per cent in fat and probably located 10-20 cM from the marker CSSM66.

Ashwell and van Tassel (1999) suggested that chromosome 14 may have a QTL affecting protein percentage of milk, chromosome 2, 21 and 23 might have QTL affecting productive life and chromosome 4, 5, 14 and 23 have those affecting udder type traits in dairy cattle.

A genome scan was conducted with an estimated coverage of 255 cM (85%) for 174 genetic markers. Marker effects on fat percentage were found on chromosome 3 and 14. The QTL identified by this study would be useful for MAS (Heyen *et al.*, 1999).

Kuhn *et al.* (1999) reported a QTL with effect on both milk fat yield and milk protein yield on BTA 6 in cattle.

Velmala *et al.* (1999) suggested the existence of two QTL on chromosome 6 with an effect on milk production traits like protein percentage, milk yield and fat yield in Finnish Ayrshire cattle.

van Tassel *et al.* (2000) identified association between microsatellite markers and QTL for milk production, conformation and productive life traits in Holstein grand sire families using genotypic data from 105 markers.

In another study, Ashwell *et al.* (2001) updated the analysis of 105 marker genotypes for different production and conformation traits.

Results of a study by Mosig *et al.* (2001) confirmed the effectiveness of selective DNA pooling using milk samples for QTL mapping in dairy cattle.

A significant QTL for fat and protein yield and fat percentage on chromosome 1 and QTL effects on milk yield and protein percentage representing one or two QTL on chromosome 6 were described by Nadesalingam *et al.* (2001). Estimates of QTL effects on breeding value ranged from 340-640 kg for milk, 15.6 – 28.4 kg for fat and 14.4 – 17.6 kg for protein.

Plante *et al.* (2001) genotyped sons of 6 Holstein sires and found the presence of QTL affecting milk, fat and protein yields on chromosome 20 and 26 and another QTL affecting fat and protein percentage on chromosome 3.

Ron *et al.* (2001) studied 9 Israeli Holstein families with 2978 daughters for QTL effects on chromosome 6 for milk production traits by a daughter deviation. The hypothesis of two QTL affecting production traits was verified by multiple regression analysis. A third QTL close to position 0 cM was also identified in this study with a different profile of effects.

By an autosomal genome scan for QTL effects on milk production Olsen *et al.* (2002) suggested QTL for milk yield, protein percentage, fat percentage, fat yield and protein yield on chromosome 3, 5, 6, 11, 13, 18 and 20. The position of the QTL for percentage traits on chromosome 6 was estimated to 16 cM.

According to Pareek *et al.* (2002) selective DNA pooling provided a rapid and efficient analysis of large number of individuals and could be best exploited in

genome wide detection of linked loci with small and large QTL effects. This could be applied to a moderately sized halfsib family of around 500 animals.

Based on a longitudinal linkage analysis approach, Rodriguez-Zas *et al.* (2002a) described that the marker HUJII77 was associated with a change in milk yield and protein percentage. They suggested that the marker may be associated with a QTL with pleiotropic effects or multiple QTL in the region. This was confirmed by the same authors later (2002b).

Weller *et al.* (2002) used a modified granddaughter design to obtain QTL allele frequencies/genotypes. They discovered a substitution effect of 0.4 phenotypic standard deviation for protein percentage.

Freyer *et al.* (2003) confirmed the pleiotropic QTL for fat and protein yields on chromosome 6. The QTL position at 68 cM was bracketed by markers TGLA 37 and FBN 13 and the pleiotropic QTL accounted for 22-25% variation in both the traits.

Viitala *et al.* (2003) used a granddaughter design to genotype Finnish Ayrshire cattle with 150 markers to construct a 2764 cM male linkage map. They used whole genome scan of Finnish Ayrshire cattle which generally confirmed the results of QTL from previous studies of Holstein Friesian cattle.

Identification of putative QTL affecting female fertility, milk production and somatic cell score was done by Ashwell *et al.* (2004). These results also provided evidence of QTL affecting protein and fat percentage on BTA 3 and BTA 6, affecting fat percentage alone on BTA 14 and affecting protein percentage alone on BTA 20.

Khatkar *et al.* (2004) conducted meta-analysis of published reports on dairy cattle QTL. The analysis indicated two consensus regions affecting milk yield on chromosome 6 at 19 cM and 87 cM explaining 4.2 and 3.6% genetic variance of milk yield.

In an extensive study with 5221 cows of 11 Israeli Holstein families, Ron *et al.* (2004) used 73 microsatellites with maximum spacing between markers of 53 cM.

2.4 MARKER ASSISTED SELECTION (MAS)

Lande and Thompson (1990) integrated traditional methods of selection on phenotypes with molecular genetics. They derived selection indices using molecular markers and thus increasing the efficiency of selection substantially. Lande and Thompson opined that increasing selection efficiency and the sample sizes necessary to achieve them depended on genetic parameters and the selection scheme.

According to Soller (1990), the advent of DNA level markers virtually removed the lack of suitable genetic markers to map loci affecting quantitative traits through linkage. For mapping QTL showing considerable variations in milk production within population, analysis of progeny groups within sires will be most effective.

Meuwissen and van Arendonk (1992) discovered that predictions of the within family variance of the grand offspring by the markers, amounted to a maximum of 13.3 per cent. They enlisted four steps for the use of genetic markers in the animal breeding namely, selection of the genetic markers, establishment of linkage maps of the markers, detection of association between marker and QTL and use of marker – QTL associations in breeding programmes.

The first necessary step in the application of MAS is the location of individual loci affecting economic traits (ETL) using genetic linkage between ETL and the genetic markers (Brascamp *et al.*, 1993).

By examining the benefits of Marker Assisted Selection (MAS) through simulation of an adult multiple ovulation embryo transfer nucleus breeding scheme, Ruane and Colleau (1996) found that with the favourable allele at an initial frequency of 0.5, MAS substantially increased responses at the QTL, but reduced

the polygenic responses. The experiment also indicated that MAS for sex-limited traits are more advantageous compared to a trait measured in both the sexes.

Spelman and Garrick (1997) evaluated two within family MAS schemes genetically and economically using stochastic simulation for a locus. They concluded that use of reproductive technologies on bull dams was imperative to prevent gains from MAS being eroded by loss of polygenic selection differential.

According to Davis and deNise (1998), MAS rely on three phases namely, the detection phase in which QTL are located and their effects on phenotypes are measured, the evaluation phase where the markers are evaluated in commercial populations and the implementation phase where markers are combined with phenotypic and pedigree information in genetic evaluation for predicting the genetic merit of individuals within the population.

Montaldo and Mezo-Herrera (1998) reviewed the references on markers and MAS and concluded that molecular pedigree and phenotypic information would be integrated in future in the selection process. The use of molecular techniques offers new opportunities and challenges for building and using more predictive and efficient statistical models for livestock improvement.

According to Georges (2001) molecular knowledge of economically important traits made it possible to move favourable alleles from exotic stock into commercial populations by marker assisted introgression, thereby increasing the genetic variance. This in turn had the potential to increase selection intensity and reduce generation interval.

Economic trait loci analysis by way of determining allele frequency and contribution of phenotype can be accurately used for selection. This type of complementing selection process with molecular markers greatly improves the genetic gain while reducing the cost of generating the progeny test data in dairy industry (Sonstegard *et al.*, 2001).

Abdel-Azim and Freeman (2002) compared a QTL – assisted selection scheme (QAS) and a QTL – free selection scheme (QFS), and found that the accuracy of evaluation under the QAS was higher than QFS in all pathways of selection of young bulls and first lactation cows. QAS scheme had a higher accuracy of 0.049 and 0.185 than QFS scheme respectively.

Gianola *et al.* (2003) described phenotype marker associations using multi-level hierarcheal linear models. They reviewed the advantages and disadvantages of several methods which were incorporating molecular informations into predictors of genetic merit in improvement programmes.

2.5 STATISTICAL ANALYSIS

2.5.1 Heterozygosity

Nei (1978) defined heterozygosity per locus as the mean population heterozygosity over all structural loci in the genome. The statistical formula for arriving at population heterozygosity at a locus was also given as $1-\Sigma P_i^2$, where P_i was the frequency of i^{th} allele at a locus in a population.

The formula derived by Ott (1992) was used to find out the heterozygosity of microsatellite markers in several studies (Anilkumar, 2003; Chen *et al.*, 2004; Chu *et al.*, 2004; Preethy, 2004).

Pandey *et al.* (2002) estimated the genetic variation by calculating the observed/direct count and unbiased heterozygosities for microsatellite loci in three breeds of poultry.

2.5.2 Polymorphic Information Content (PIC)

PIC value can be calculated by using the formula,

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

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 (Botstein *et al.*, 1980). PIC values for microsatellite markers were calculated by this formula in several studies (Anilkumar, 2003; Hai-Guo *et al.*, 2003; Chen *et al.*, 2004; Preethy, 2004; Jang *et al.*, 2005).

Materials and Methods

3. MATERIALS AND METHODS

3.1 SOURCE OF SAMPLES

Blood was used as a source of DNA from crossbred cows maintained at the University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi. Five milliliter of venous blood was collected into 15 ml sterile disposable polypropylene tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant (1 mg/ml of blood). DNA samples from 100 genetically unrelated animals were used to find out the PIC of the selected markers. For microsatellite analysis of quantitative traits, 117 animals belonging to different sire families were genotyped and animals belonging to ten sire families were selected for the study.

Semen was used as a source of DNA in the case of bulls. Frozen semen of bulls from ICAR Progeny Testing Scheme and fresh semen of bulls of Kerala Agricultural University Bull Station, Mannuthy were used for DNA extraction. Five frozen semen straws, each containing 30 million sperms were used for extraction of DNA from every bull sampled. In the case of fresh semen about 1-2 ml containing approximately 300-500 million sperms was obtained on the day of collection itself and transported to the laboratory in chilled condition.

3.2 ISOLATION OF GENOMIC DNA

3.2.1 Extraction from Whole Blood

DNA was extracted by modifications in the phenol-chloroform protocol (Andersson *et al.*, 1986). For extraction of DNA from blood, five milliliter of blood was mixed with RBC lysis solution (150m*M* NH_4Cl, 10 m*M* KCl, 0.1 m*M* EDTA) in chilled condition. The mixture was frequently mixed for about 10 minutes. It was centrifuged to remove the supernatent. The step was repeated till the supernatent was clear. The pellet was washed twice with 6 ml of Tris Buffered Saline (TBS – 140 m*M* NaCl, 0.5 mM KCl, 0.25 mM Tris) and resuspended it in 5 ml of Saline EDTA buffer (SE – 75 m*M* NaCl, 35 m*M* EDTA). To this cell suspension 25 µl of Proteinase K (25 mg/ml) and 0.25 ml 20% SDS were added. Mixed well and incubated at 50°C for a minimum of three hours. Then 300 µl of

5 *M* NaCl was added to the solution directly. Added an equal volume of saturated phenol (pH 7.8) and mixed thoroughly by inversion for 10 min and centrifuged at 4000 rpm for 15 minutes. The aqueous phase containing DNA was collected in fresh tubes. The same procedure was repeated using an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1) and chloroform : isoamyl alcohol (24:1). The DNA was precipitated from the supernatent by adding chilled isopropanol and one tenth volume of 3*M* Sodium acetate (pH 5.5). The precipitated DNA was washed in 70% ethanol and dissolved in 0.5 ml of Tris-EDTA buffer (TE – 10 m*M* Tris, 0.1 m*M* EDTA) and stored at –20°C.

3.2.2 Extraction from Semen

A modified phenol-chloroform protocol was used for the isolation of DNA from semen (Andersson *et al.*, 1986). The undiluted fresh semen of concentration 300-500 million/ml or frozen semen (5 straws of 30 million each) was used for isolation of DNA. The sample was washed twice with 10 ml Phosphate Buffered Saline (PBS – 135 m*M* NaCl, 4 m*M* NaH_2PO_4). Pellet was resuspended in 2 ml of PBS by vortexing. To this added 6 ml of sperm lysis buffer (10 m*M* Tris, 50 m*M* NaCl, 10 m*M* EDTA, 1 per cent SDS, 2 per cent mercaptoethanol), mixed well and incubated at 55°C in water bath for 30 minutes with occasional mixing. Added 100 µl of Proteinase K (20 mg/ml) and incubation was continued at 55°C for overnight. After digestion, phenol-chloroform extraction of DNA was carried out as described earlier.

Yield and purity of isolated DNA samples were estimated by using spectrophotomer. The optical density (OD) values at 260 nm and 280 nm were taken for each diluted DNA sample.

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

Purity of the isolated DNA was established from the ratio of OD values. The quality was also assessed by running the samples in one per cent Agarose gel. Presence of sharp band without smearing or additional bands ensured good quality DNA extraction.

3.3 ANALYSIS OF QUANTITATIVE TRAITS

Milk samples were collected from 117 animals maintained at the University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi and analysed for total solids, fat (IS: 1224, 1977), protein (AOAC, 1990, N* 6.38), and solids not fat (SNF). Data regarding milk yield, age at first calving, peak yield and days to attain peak yield of each animal were collected from the records maintained at University Livestock Farm, Mannuthy and Cattle Breeding Farm, Thumburmuzhi.

3.4 PCR ANALYSIS

3.4.1 Selection of Primers

A set of microsatellite markers with close linkage to QTL were selected from various literatures. The primers for their markers were custom synthesised. The markers were typed for their polymorphism. Five markers viz. ILSTS 096, HUJII77, BL41, BM1508 and BM4305 were chosen for the study. The sequences of the forward and reverse primers for each locus were as follows.

Locus		Primer sequence (5'-3')
ILSTS096	F	GTGACCTGGAGAAGTTTTCC
	R	ACCACGCTCTGACTTGTAGC
		(Kemp *et al.*, 1995; Ihara *et al.*, 2004)
HUJII77	F	TCCATCAAGTATTTGAGTGCAA
	R	ATAGCCCTACCCACTGTTTCTG
		(Shalom *et al.*, 1994; Ihara *et al.*, 2004)
BL41	F	CCTCTGCCATCTTTATTCCG
	R	AAGATCAACTTATTCCTCACAGTG
		(Bishop *et al.*, 1994; Grosz *et al.*, 1997; Ihara *et al.*, 2004)
BM1508	F	CAGGTGTACAGCAAACTGAATC
	R	CGTCAAAACATTCGTTCAGG
		(Stone *et al.*, 1995; Ihara *et al.*, 2004)

BM4305	F	CCAAGACATGAAAGCAATCTG
	R	CTCTAGGTACATCCATGTTGCA
		(Bishop *et al.*, 1994; Ihara *et al.*, 2004)

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

3.4.2 Incorporation of Radioactivity: Endlabeling of Primers

For visualizing the PCR products by autoradiography, forward primer of each marker was radio-labelled at the 5' end with γ^{32}P-ATP. The reaction was carried out with the DNA endlabeling Kit 1 (Genei).

The procedure followed for end-labeling was as follows:

10X Polynucleotide Kinase (PNK) buffer	-	1 µl
Forward primer (200 pM/µl)	-	1 µl
T_4 Polynucleotide Kinase (5 u/µl)	-	0.5 µl
γ^{32}P -ATP (10 mCi/ml)	-	1 µl
Nuclease free water	-	6.5 µl

The mixture was incubated at 37°C for 30 minutes. The final volume was made upto 40 µl. One microlitre of this endlabeled primer was used for every 10 µl of PCR.

3.4.3 PCR Conditions

For each microsatellite locus PCR conditions were standardised separately. Each reaction was carried out in a 10 µl volume. PCR was done with 10X PCR buffer (100 m*M* Tris – pH 8.3, 500 m*M* KCl), 1 µl of endlabeled forward primer (5 pM), 5 pM of reverse primer and 0.3 units of Taq DNA polymerase. Concentration of $MgCl_2$ and dNTP were standardised for each set of primers separately. The

reaction mixture was mixed well and subjected to amplification in a thermal cycler (Techne Flexigene).

Each PCR cycle had an initial denaturation at 94°C for three minutes followed by 35 cycles each consisting of denaturation at 94°C for one minute, annealing at 55°C-58°C according to the primer sets used for one minute and extension at 72°C for one minute. This was followed by a final extension for five minutes at 72°C. The samples were then cooled down to 4°C and stored at –20°C till further analysis.

3.5 SEQUENCING M13 BACTERIOPHAGE DNA

To determine the allele size of markers, comparison with a sequencing ladder is necessary. Single stranded M13 phage DNA was sequenced using the DNA Sequencing Kit Version 2.0 (M/s Amersham Biosciences Corporation, USA). Reactions were as follows.

1. Preparation of annealing mixture

M13 phage DNA (0.2 µg/µl)	-	5 µl
5X Sequenase reaction buffer	-	2 µl
Forward primer (0.5 p*M*/µl)	-	1 µl
Distilled water	-	2 µl

The mixture was centrifuged, incubated at 65°C for two minutes and then slowly cooled to room temperature over 15-20 minutes and placed on ice.

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

3. Dilution of labeling mix

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

5X labeling mix	-	0.5 µl
Water	-	2.0 µl

4. Dilution of enzyme

The enzyme (sequenase version 2.0) was diluted eight fold with sequenase enzyme dilution buffer.

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

5. Labeling reaction

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

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

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

3.5 µl of labeling reaction mixture was transferred to each termination tubes (G, A, T, C) mixed well and incubated at 37°C for 5 minutes. The reaction was stopped by addition of 4 µl of stop solution provided in the sequencing kit and stored at –20°C.

3.6 ELECTROPHORESIS

3.6.1 Denaturing Polyacrylamide Gel Electrophoresis

The radio-labeled PCR products were fractionated using 6 per cent denaturing polyacrylamide gels. Denaturing poly acrylamide gel electrophoresis (PAGE) was performed on the vertical sequencer (Consort) as described by Biggin *et al.* (1983).

3.6.1.1 Casting the Gel

The glass plates (41 x 33 cm, 39 x 33 cm) were cleaned with soap solution, dried and wiped thoroughly with alcohol. The plates were assembled with 0.35 mm spacers in between and the sides and bottom were sealed with sealing tape.

The gel was prepared by mixing 60 ml of 0.5X Tris Borate EDTA (TBE) gel mix (6 per cent Acrylamide, 6 *M* urea, 0.5X TBE) and 125 µl each of 10 per cent ammonium persulphate solution (APS) and N,N,N',N', Tetra Methyl Ethylene Diamine (TEMED) in a beaker. The mixture was poured between the glass plates avoiding air bubbles. The plates were clamped and the shark toothed comb was inserted on top with the toothed surface facing upwards. One hour was allowed to set the gel and then the tapes were removed, plates were assembled in the sequencer. 1X TBE buffer (pH 8.3) (0.045 *M* Tris-borate, 0.001 *M* EDTA) was used as tank buffer. The comb was removed, wells were cleaned with buffer and comb was reinserted in opposite direction to form sample-loading wells.

3.6.1.2 Loading of Samples

3.5 µl of formamide loading buffer (0.02 per cent Xylene Cyanol, 0.02 per cent Bromophenol Blue, 10 m*M* EDTA, 98 per cent deionised formamide) was added to the PCR products, mixed well, denatured at 95°C for 5 minutes and cooled immediately on ice. 3.5-4 µl each of this mixture was loaded into each well. Sequenced products of M13 DNA were loaded in 4 wells (G, A, T, C).

3.6.1.3 Polyacrylamide Gel Electrophoresis

The gels were electrophoresed at 35 W for 1.5-3 hours according to the PCR product size, maintaining a temperature around 45-50°C. The bromophenol blue acted as the indicator of the mobility of DNA fragments and had a mobility equivalent to a 25 base fragment and the Xylene Cyanol dye had a mobility equivalent to an approximately 100 base fragment.

3.7 AUTORADIOGRAPHY

After electrophoresis, the glass plates were separated. The gel adhering to one of the plates was transferred to a filter paper. After marking the position of the first well, the gel was covered with a Klin film and dried in a gel drier at 82°C. The gel was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2 cm) in a cassette (Kiran Hyper Cassette) with an intensifying screen. The X-ray film was developed depending on the intensity of radioactive signal. Developing was done by transferring the film serially into 1X developer solution (Kodak) for three to five minutes, distilled water for one minute (washing) and finally into fixer solution (Kodak) for six to ten minutes. The developed film was washed thoroughly in running water and dried.

3.8 MICROSATELLITE TYPING

The number of alleles for each marker was counted and their size was determined by comparing with M13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in the order. The allele sizes were determined corresponding to the G, A, T and C bands. The allele frequency was worked out.

3.9 STATISTICAL ANALYSIS

3.9.1 Heterozygosity (H)

3.9.1.1 Direct Count Heterozygosity

The usefulness of a marker depends on its heterozygosity. Heterozygosity was calculated by the method of Ott (1992).

$$H = 1 - \sum_{i=1}^{k} P_i^2$$

Where, P_i is the frequency of i^{th} allele at a locus.

3.9.1.2 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.

3.9.2 Polymorphic Information Content (PIC)

PIC values for the markers were calculated as

$$PIC = 1 - [\sum_{i=1}^{n} P_i^2] - [\sum_{i=1}^{n-1} \sum_{j=i-1}^{n} 2 P_i^2 P_j^2]$$

Where P_i and P_j are the frequencies of i^{th} and j^{th} alleles respectively (Botstein *et al.*, 1980).

3.9.3 Analysis of Economic Traits in the Population

One way analysis of variance was done to find out the effect of sire families on eight economic traits, viz, milk yield, fat percentage, protein percentage, SNF, total solids, peak yield, days to attain peak yield and AFC, using the method of Snedecor and Cochran (1985).

3.9.4 Analysis of Allelic Effects on Economic Traits

The economic traits with significant difference between families were analysed for allelic effects. All the alleles of the sires were included in the analysis. Large sample test (Z test) for the comparison of means of allele containing population with that of the population without the allele was done by the method suggested by Snedecor and Cochran (1985).

Results

4. RESULTS

4.1 ISOLATION OF GENOMIC DNA

Blood, semen and milk samples for the study were collected from the University Livestock Farm, Mannuthy, Cattle Breeding Farm, Thumburmuzhi, ICAR Progeny Testing Scheme and Kerala Agricultural University Bull Station, Mannuthy. DNA was isolated from whole blood, fresh and frozen semen samples using modified phenol-chloroform extraction procedures. The DNA obtained was of good quality. The ratio of optical densities at 260 nm and 280 nm was taken as the measure of the purity of the DNA isolated. Single, clear band without shearing on agarose gel electrophoresis indicated the presence of good quality high molecular weight DNA.

4.2 PCR CONDITIONS

A panel of microsatellites was selected, the primers were custom synthesised. The standardisation of PCR conditions was done with the DNA samples of the genetically unrelated animals. Based on the polymorphism of the marker loci five markers were selected, viz., ILSTS096, HUJII77, BL41, BM1508 and BM4305. The reaction components, temperature and the time standardised for each microsatellite marker used in the study are presented in Table 4.1 to 4.5. Forward primers of the microsatellite loci used in the study were endlabeled with γ^{32}P ATP using the enzyme T_4 polynucleotide kinase (PNK), prior to PCR.

4.3 MICROSATELLITE TYPING ON GENETICALLY UNRELATED ANIMALS

The selected microsatellite markers were typed on genetically unrelated animals to find out allelic number, allelic size, allelic frequency, genotype frequency, PIC, direct count heterozygosity and unbiased heterozygosity. Autoradiographs of polymorphism at the selected microsatellite loci are showed in Plates. 1 to 5.

4.3.1 Allelic Number, Allelic Size, Allelic Frequency and Genotype Frequency

The PCR products were fractionated by denaturing polyacrylamide gel electrophoresis and visualised by autoradiography. The numbers of each allele were counted manually and their size was obtained by the comparison with the sequence of M13 DNA run alongside the samples in the gel. The allelic frequencies and genotypic frequencies were calculated separately.

4.3.1.1 ILSTS096

Twelve alleles with a size ranging from 188-212 bp were detected for ILSTS096 locus with 35 genotypes. The allelic sizes and frequencies are presented in Table 4.6. Genotypes and genotype frequencies are presented in Table 4.7. The highest frequency was noted for the genotype 200/200 (0.132).

4.3.1.2 HUJII77

HUJII77 had thirteen alleles with a size range of 193-221 bp with 36 genotypes. The allelic sizes with their frequencies and genotypes with their frequencies are presented in Table 4.8 and 4.9 respectively. Interestingly around 40 percentage of the animals were homozygous at the locus.

4.3.1.3 BL41

Fourteen alleles were detected with a size range of 232-266 bp with 36 genotypes. The allelic sizes with their frequencies are presented in Table 4.10. Genotypes and genotype frequencies are presented in Table 4.11. The highest frequency of 0.267 was noted for the allele 242.

4.3.1.4 BM1508

BM1508 had seven alleles with a size range of 103-115 bp. The size and frequency of alleles are presented in Table 4.12. The allele 113 had the highest frequency of 0.429 followed by the allele 107 (0.340). Seventeen genotypes were

detected and the frequencies are presented in Table 4.13. The genotype 113/113 had the highest frequency of 0.244 followed by 107/107 (0.231).

4.3.1.5 BM4305

Twelve alleles were detected with a size range of 146-168 bp with 37 genotypes. The different alleles detected with their frequencies and genotype frequencies are presented in Table 4.14 and 4.15 respectively. The highest frequency noted for the allele 158 (0.241) and the genotype frequency for 158/158 (0.101).

4.3.2 Direct Count Heterozygosity

Direct count heterozygosity was calculated by the method of Ott (1992). Direct count heterozygosity values obtained for each microsatellite marker are presented in Table 4.16.

4.3.3 Unbiased Heterozygosity

Unbiased heterozygosity was calculated using the formula of Pandey *et al.* (2002) and the values obtained are presented in Table 4.16.

4.3.4 Polymorphic Informaton Content (PIC)

The level of informativeness of the microsatellite markers can be measured as PIC. PIC was calculated by the method of Botstein *et al.* (1980). The PIC values are presented in Table 4.16. The highest PIC value was obtained for the microsatellite marker ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630).

4.4 MICROSATELLITE TYPING ON SELECTED DAIRY CATTLE POPULATION

Ten sire families (halfsib progenies with their sires) were genotyped using the five selected microsatellite markers, viz., ILSTS096, HUJII77, BL41, BM1508 and BM4305.

4.4.1 Allelic Number, Allelic Size, Allelic Frequency and Genotype Frequency

The PCR products were fractionated by denaturing polyacrylamide gel electrophoresis and visualised by autoradiography. The number of alleles, allelic size, allelic frequency and genotype frequency were as follows.

4.4.1.1 ILSTS096

Eleven alleles were observed with a size range of 188-208 bp. The highest frequency was observed for the allele 198. The size and frequency of alleles are presented in Table 4.17. Thirty three genotypes were noted and their frequencies are presented in Table 4.18. The highest frequency was observed for the genotype 196/198.

4.4.1.2 HUJII77

HUJII77 had thirteen alleles with a size range of 193-217 bp with 23 genotypes. The highest frequency was observed for the allele 209 and the genotype 209/211. The size and frequency of the alleles and the genotype frequencies are presented in Table 4.19 and 4.20 respectively.

4.4.1.3 BL41

Ten alleles were detected with a size range of 236-266 bp with 23 genotypes. The allelic sizes with their frequencies are presented in Table 4.21 and genotypic frequencies are presented in Table 4.22. The highest frequency was observed for the allele 246 and the most common genotype was 246/246.

4.4.1.4 BM1508

BM1508 had seven alleles with a size range of 103-115 bp. The size and frequency of alleles are presented in Table 4.23. Eighteen genotypes were detected and the frequencies are presented in Table 4.24. The most common allele and genotype were 109 and 105/109 respectively.

4.4.1.5 BM4305

Ten alleles were detected with a size range of 146-168 bp with 30 genotypes. The different alleles detected with their frequencies and genotype frequencies are presented in Table 4.25 and 4.26 respectively. The highest frequency was observed for the allele 160 and the genotypes 158/160 and 160/162.

4.5 RELATIONSHIP BETWEEN ECONOMIC TRAITS AND MICROSATELLITE LOCI POLYMORPHISMS IN DIFFERENT SIRE FAMILIES

Names of the bulls and their daughter averages for milk production traits and AFC are presented in Table 4.27 and for milk composition traits are presented in Table 4.28. The population averages for different economic traits are presented in Table 4.29.

4.5.1 305 Day Milk Yield

The population average for 305 day milk yield was 2070.5±59.1 kg. Among the sire families the highest milk yield was recorded for the family of Deven (2480.2±123 kg) and the lowest for the family of Hakkim (1860.3±289 kg). Analysis of variance for effect of sire families on 305 day milk yield is presented in Table 4.30, which showed no significant difference between the sire families.

4.5.2 Milk Fat Percentage

The average milk fat percentage of the population was 3.738±0.0788. Analysis of variance for milk fat percentages for the ten sire families is presented in Table 4.31. A significantly lower fat percentage was observed for the families of Deva (3.13±0.2), 250 (2.83±0.2) and Hakkim (3.58±0.4).

4.5.3 Milk Protein Percentage

The population average for milk protein percentage was 2.781±0.0286. The highest protein percentage was recorded for the family of Oscar (2.97±0.14) and the

lowest for the family of Deva (2.62±0.08). Analysis of variance for protein percentages (Table 4.32) showed no significant difference among the sire families.

4.5.4 Percentage of Total Solids in Milk

The population average for total solids percentage in milk was 12.191±0.097. The highest total solids percentage was observed for the family of Dipesh (12.71±0.5) and the lowest for the family of the bull, 250 (11.18±0.3). Analysis of variance for total solids percentages (Table 4.33) showed no significant difference among the ten sire families.

4.5.5 SNF Percentage

The population average for SNF percentage was 8.456±0.0053. Among the ten sire families the analysis of variance (Table 4.34) revealed no significant difference for SNF percentage.

4.5.6 Peak Yield

Names of the bulls and their daughter averages for peak yields are presented in Table 4.27. The highest peak yield of 13.8±1.9 kg was recorded for the family of Dara and the lowest for the family of Dipesh (10.9±0.5 kg). But the analysis of variance for peak yield (Table 4.35) did not show any significant difference among the ten sire families. The population average for the peak yield was 11.858±1.141 kg.

4.5.7 Days to Attain Peak Yield

The population average for days to attain peak yield was 24.304±1.88 days. The highest daughter average for the days to attain peak yield was for the family of Dara (50.4±14.1 days) and the lowest was for the family of Deva (15.5±4.19 days). The analysis of variance for days to attain peak yield is presented in Table 4.36, which revealed no significant difference among the families.

4.5.8 Age at First Calving

The population average for AFC was 1077.43±11.55 days. Analysis of variance (Table 4.37) revealed a significantly higher AFC for the families of Dipesh (1145±52 days), Onkar (1233±73 days) and Gopal (1226±84 days).

4.5.9 Relationship of Sire Families with Economic Traits

Genotypes of the ten sires at different microsatellite loci are presented in Table 4.38.

4.5.9.1 Deva

The bull Deva had a significantly lower daughter average for milk fat percentage. The daughter average for milk yield was 2188.7±144 kg. The family had significantly lower AFC and lower fat percentage of milk.

4.5.9.2 Oscar

The daughter average for milk fat percentage of the family of Oscar was significantly higher than three other families. The family of Oscar showed a significantly lower AFC (975±95 days) than that of the families of Dipesh, Onkar and Gopal.

4.5.9.3 Bull no. 250

The lowest milk fat percentage was observed for the family of the bull 250 (2.83±0.2), which showed no significant difference from the families of Deva and Hakkim and had significant difference from the other seven sire families. AFC was significantly lower than three other families.

4.5.9.4 Debu

The lowest AFC (956±49 days) was recorded for the family of Debu. The milk fat percentage of this family was significantly higher than that of the families of Deva, Hakkim and Bull no. 250.

4.5.9.5 Deven

The highest 305 day milk yield was recorded for the family of Deven (2480.2±123), among the ten sire families. The daughter average for milk fat percentage was 4.03±0.2, which was significantly higher than that of the families of Deva, Hakkim and Bull no. 250.

4.5.9.6 Hakkim

The lowest 305 day milk yield was recorded for the family of Hakkim (1860.3±289 kg). The daughter average for milk fat percentage was 3.58±0.4, which had no significant difference from the families of Deva and Bull no. 250 and had a significant difference from other seven sire families. The family also had significantly lower AFC.

4.5.9.7 Dipesh

A higher AFC (1145±52 days) was recorded for the family of Dipesh. The fat, protein, SNF and total solids percentages in milk were higher than their population averages.

4.5.9.8 Onkar

Among the sire families, the highest AFC (1233±73 days) was observed for the family of Onkar. This was significantly higher than other seven sire families. The 305 day milk yield was 1945.7±207 kg, which was lower than the population average.

4.5.9.9 Dara

The highest daughter average for peak yield (13.8±1.9 kg) was recorded for the family of Dara, but the difference was not significant from the other sire families. The 305 day milk yield was 2284.1±322kg, which was slightly higher than the population average (2070.5±59.1 kg). A higher milk fat percentage of 3.8±0.46 was observed.

4.5.9.10 Gopal

The AFC of 1226±84 days was observed for the family of Gopal was significantly higher than other seven sire families. The family had significantly higher milk fat percentage compared to the families of Deva, Hakkim and Bull no. 250.

4.6 ALLELIC EFFECTS ON MILK FAT PERCENTAGE AND AFC

All the five microsatellite markers had significant effect on milk fat percentage. The microsatellite markers, ILSTS096, BL41 and BM4305 had shown significant effect on AFC. Z test was done to find out the allelic effects of different microsatellites on these production traits.

4.6.1 ILSTS096

The allelic averages of ILSTS096 for milk fat percentage and AFC are presented in Table 4.39. Two alleles associated with higher milk fat percentage and one allele with lower fat percentage were identified.

4.6.1.1 Allele 188

The animals having the allele 188 had a significantly lower average for milk fat percentage (2.986±0.21), when compared to the animals without this allele (3.803±0.523).

4.6.1.2 Allele204

A significantly lower fat percentage (3.29±0.19) was noted for the animals with the allele 204. The animals without this allele had a fat percentage of 3.81±0.11.

4.6.1.3 Allele 198

A significantly higher fat percentage (4.13±0.15) was observed for the animals with the allele 198, compared to the animals without this allele (3.44±0.11).

4.6.1.4 Allele 196

The average AFC for the animals with the allele 196 was 1015±70.88 days, which was significantly lower than that of the animals without this allele (1092.26±22.89 days).

4.6.2 HUJII77

The allelic averages of HUJII77 locus of milk fat percentage and AFC are presented in Table 4.40. One of the alleles showed significant effect on milk fat percentage.

4.6.2.1 Allele 205

The animals with the allele 205 showed a significantly lower milk fat percentage (3.3±0.18) when compared to animals without this allele (3.78±0.11).

4.6.3 BL41

Alleles of the microsatellite marker BL41 had significant effect on milk fat percentage and AFC. The allelic averages for these traits are presented in Table 4.41.

4.6.3.1 Allele 240

Animals with the allele 240 had significantly higher milk fat percentage (4.15±0.22), when compared to animals without this allele (3.64±0.108).

4.6.3.2 Allele 242

Animals with the allele 242 had an average milk fat percentage of 3.21±0.17, which was significantly lower than the animals without this allele (3.85±0.109).

4.6.3.3 Allele 246

The average AFC was 1003.9±24.51 days for the animals with the allele 246, which had a significant difference from the animals without this allele (1104.37±22.67 days).

4.6.4 BM1508

The allelic averages of milk fat percentage and AFC at BM1508 locus are presented in Table 4.42. Two alleles with effect on higher milk fat percentage were identified.

4.6.4.1 Allele109

The average milk fat percentage was 3.95±0.11 for the animals with the allele 109. This was significantly higher than the animals without this allele (3.45±0.162).

4.6.4.2 Allele 113

Animals with the allele 113 had a significantly higher milk fat percentage of 4.2±0.2. The animals without this allele had an average of 3.63±.108.

4.6.5 BM4305

For the marker BM4305, alleles with significant effects on milk fat percentage (two numbers) and AFC (one) were identified. The allelic averages for BM4305 locus are presented in Table 4.43.

4.6.5.1 Allele 154

The average milk fat percentage was 3.19±0.23, for the animals with the allele 154, which showed a significant difference from the animals without this allele (3.81±0.0106).

The animals with this allele had an average AFC of 970.8±22.94 days which was significantly higher than the animals without this allele (1093.346±20.37 days).

4.6.5.2 Allele 166

The animals with the allele 166 at BM4305 locus had an average milk fat percentage of 4.58±0.2, which was significantly higher than the animals without this allele (3.64±0.102).

Table 4.1 PCR conditions standardised for microsatellite locus ILSTS096

Parameter	Level
Template DNA	50ng
Forward primer concentration	5 p*M*
Reverse primer concentration	5 p*M*
dNTP concentration	200 μ*M* each
$MgCl_2$ concentration	1.25 m*M*
Taq DNA polymerase	0.3 units
Reaction volume	10 μl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing	58°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

Table 4.2 PCR conditions standardised for microsatellite locus HUJII77

Parameter	Level
Template DNA	50ng
Forward primer concentration	5 p*M*
Reverse primer concentration	5 p*M*
dNTP concentration	200 μ*M* each
$MgCl_2$ concentration	1.5 m*M*
Taq DNA polymerase	0.3 units
Reaction volume	10 μl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing	58°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

Table 4.3 PCR conditions standardised for microsatellite locus BL41

Parameter	Level
Template DNA	50ng
Forward primer concentration	5 p*M*
Reverse primer concentration	5 p*M*
dNTP concentration	250 µ*M* each
$MgCl_2$ concentration	1.5 m*M*
Taq DNA polymerase	0.3 units
Reaction volume	10 µl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing	58°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

Table 4.4 PCR conditions standardised for microsatellite locus BM1508

Parameter	Level
Template DNA	50ng
Forward primer concentration	5 p*M*
Reverse primer concentration	5 p*M*
dNTP concentration	200 µ*M* each
$MgCl_2$ concentration	1.25 m*M*
Taq DNA polymerase	0.3 units
Reaction volume	10µl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing	55°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

Table.4.5 PCR conditions standardised for microsatellite locus BM4305

Parameter	Level
Template DNA	50ng
Forward primer concentration	5 p*M*
Reverse primer concentration	5 p*M*
dNTP concentration	200 µ*M* each
$MgCl_2$ concentration	1.25m*M*
Taq DNA polymerase	0.3 units
Reaction volume	10 µl
Initial denaturation	94°C for three minutes
Denaturation for PCR cycles	94°C for one minute
Primer annealing	58°C for one minute
Primer extension	72°C for one minute
Number of cycles	35
Final extension	72°C for five minutes

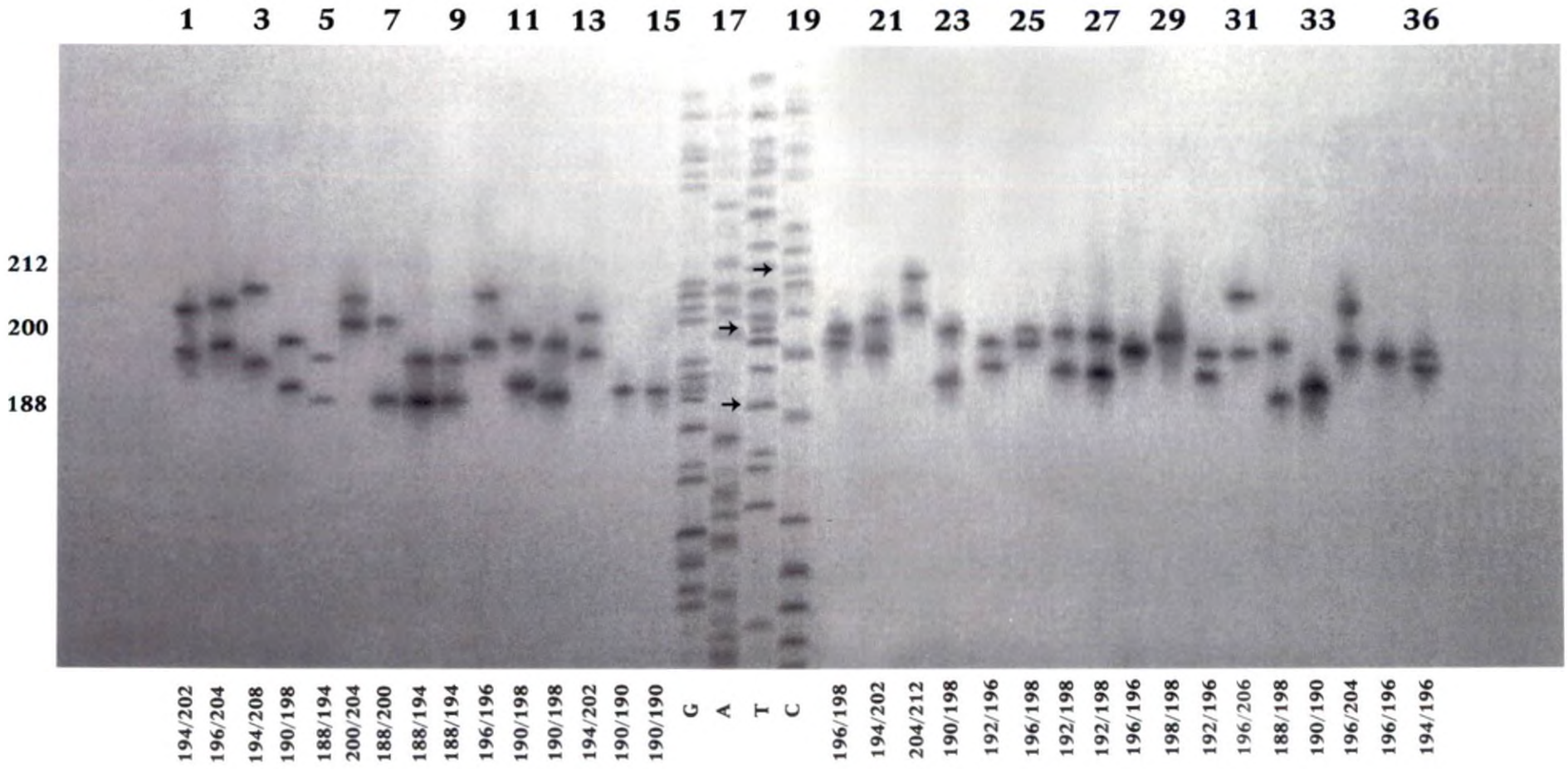

Plate.1. Autoradiograph of polymorphism at ILSTS096 locus in crossbred cattle of Kerala
Lanes 1-15; samples

16-19; G,A,T,C sequences of M13 DNA

20-36; samples

Table 4.6 Allele sizes and frequencies at ILSTS096 locus in crossbred cattle population of Kerala

Sl. No	Size in base pairs	Allelic frequency
1	188	0.046
2	190	0.026
3	192	0.086
4	194	0.066
5	196	0.105
6	198	0.191
7	200	0.191
8	202	0.013
9	204	0.079
10	206	0.086
11	208	0.105
12	212	0.007

Table 4.7 Genotypes and genotype frequencies at ILSTS096 locus in crossbred cattle population of Kerala

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	188/188	0.013	19	196/198	0.053
2	188/192	0.026	20	196/204	0.013
3	188/194	0.013	21	196/206	0.039
4	188/198	0.013	22	198/198	0.079
5	188/200	0.013	23	198/200	0.013
6	190/190	0.013	24	198/206	0.053
7	190/194	0.013	25	198/208	0.026
8	190/198	0.013	26	200/200	0.132
9	192/192	0.039	27	200/202	0.013
10	192/196	0.013	28	200/204	0.013
11	192/198	0.053	29	200/206	0.026
12	194/194	0.013	30	200/208	0.026
13	194/196	0.026	31	204/204	0.053
14	194/198	0.013	32	204/208	0.013
15	194/200	0.013	33	204/212	0.013
16	194/202	0.013	34	206/206	0.026
17	194/208	0.013	35	208/208	0.066
18	196/196	0.026			

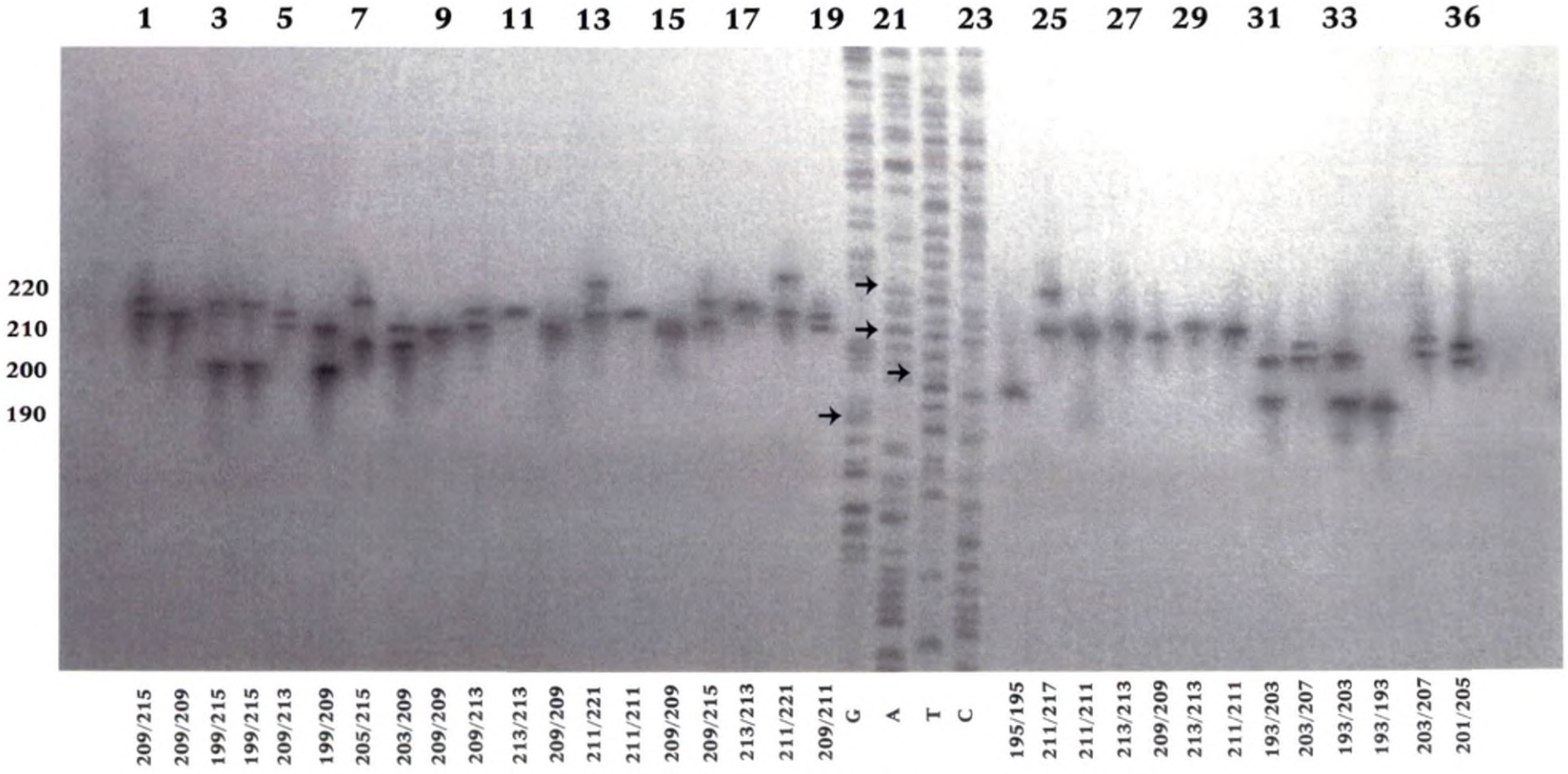

Plate.2. Autoradiograph of polymorphism at HUJII77 locus in crossbred cattle of Kerala
Lanes 1-19; samples

20-23; G,A,T,C sequences of M13 DNA

24-36; samples

Table 4.8 Allele sizes and frequencies at HUJII77 locus in crossbred cattle population of Kerala

Sl. No	Size in basepairs	Allelic frequency
1	193	0.025
2	195	0.012
3	199	0.074
4	201	0.006
5	203	0.130
6	205	0.062
7	207	0.031
8	209	0.296
9	211	0.056
10	213	0.121
11	215	0.099
12	217	0.025
13	221	0.062

Table 4.9 Genotype and genotype frequencies at HUJII77 locus in crossbred cattle population of Kerala

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	193/193	0.012	19	205/211	0.025
2	193/203	0.025	20	205/213	0.025
3	195/195	0.012	21	205/215	0.012
4	199/203	0.012	22	207/209	0.025
5	199/207	0.025	23	209/209	0.173
6	199/209	0.074	24	209/211	0.012
7	199/213	0.012	25	209/213	0.037
8	199/215	0.025	26	209/215	0.037
9	201/205	0.012	27	211/211	0.025
10	203/203	0.049	28	211/217	0.012
11	203/205	0.012	29	211/221	0.012
12	203/207	0.012	30	213/213	0.049
13	203/209	0.049	31	213/217	0.012
14	203/213	0.012	32	213/221	0.025
15	203/215	0.012	33	215/215	0.049
16	203/221	0.025	34	215/221	0.012
17	205/205	0.012	35	217/217	0.012
18	205/209	0.012	36	221/221	0.025

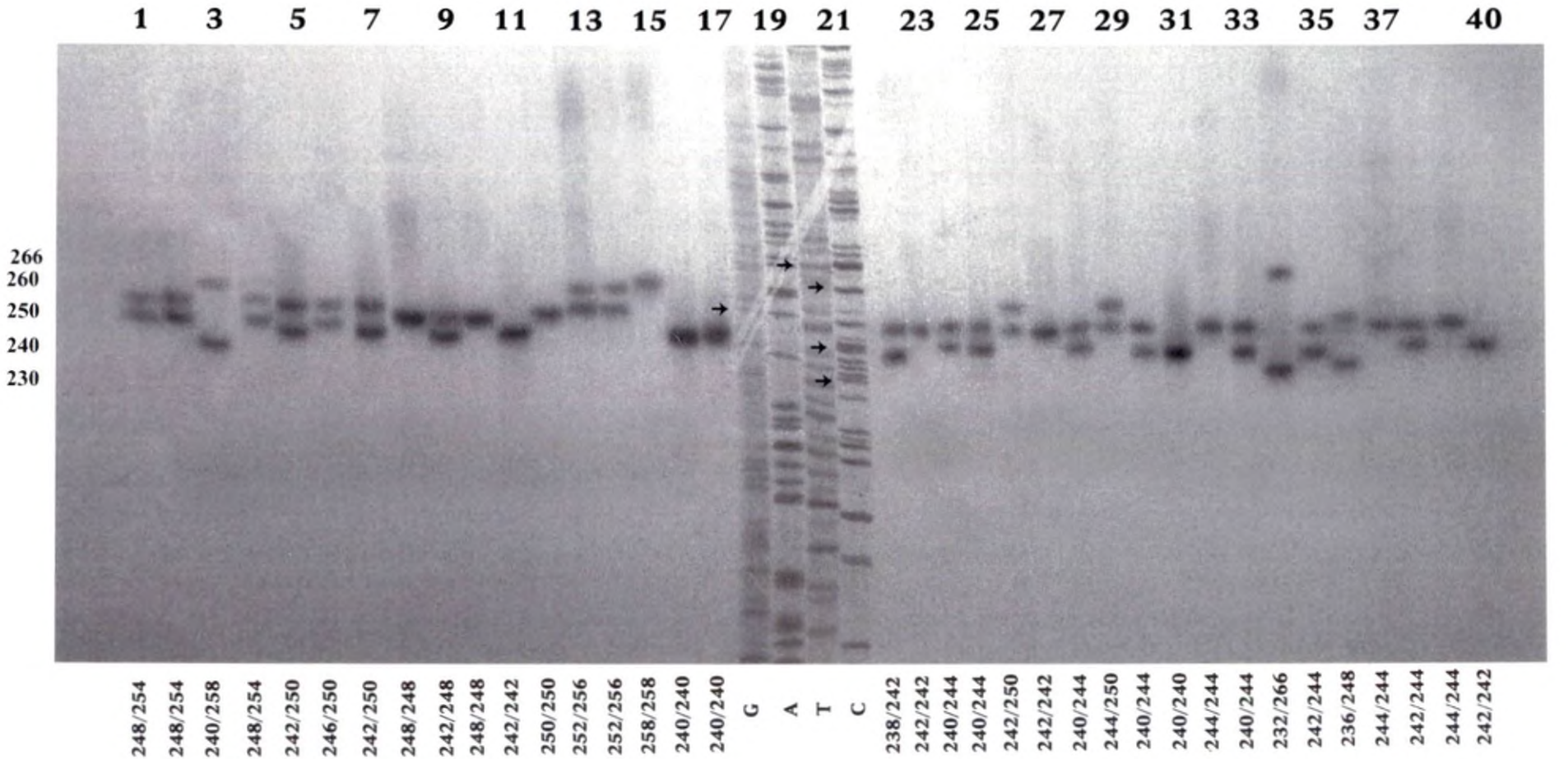

Plate.3. Autoradiograph of polymorphism at BL41 locus in crossbred cattle of Kerala
Lanes 1-17; samples

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

22-40; samples

Table 4.10 Allele sizes and frequencies at BL41 locus in crossbred cattle population of Kerala

Sl. No	Size in base pairs	Allelic frequency
1	232	0.027
2	236	0.073
3	238	0.067
4	240	0.027
5	242	0.267
6	244	0.153
7	246	0.047
8	248	0.147
9	250	0.067
10	252	0.013
11	254	0.027
12	256	0.040
13	258	0.040
14	266	0.007

Table 4.11 Genotype and genotype frequencies at BL41 locus in crossbred cattle population of Kerala

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	232/242	0.013	19	242/248	0.040
2	232/244	0.013	20	242/250	0.013
3	232/250	0.013	21	244/244	0.080
4	232/266	0.013	22	244/248	0.013
5	236/236	0.013	23	244/250	0.013
6	236/238	0.013	24	244/254	0.013
7	236/242	0.080	25	244/256	0.013
8	236/248	0.013	26	246/246	0.027
9	236/256	0.013	27	246/248	0.013
10	238/238	0.027	28	246/250	0.027
11	238/242	0.027	29	248/248	0.080
12	238/244	0.027	30	248/254	0.027
13	238/250	0.013	31	248/258	0.027
14	240/240	0.013	32	250/250	0.027
15	240/244	0.013	33	252/256	0.027
16	240/258	0.013	34	254/256	0.013
17	242/242	0.160	35	256/258	0.013
18	242/244	0.040	36	258/258	0.013

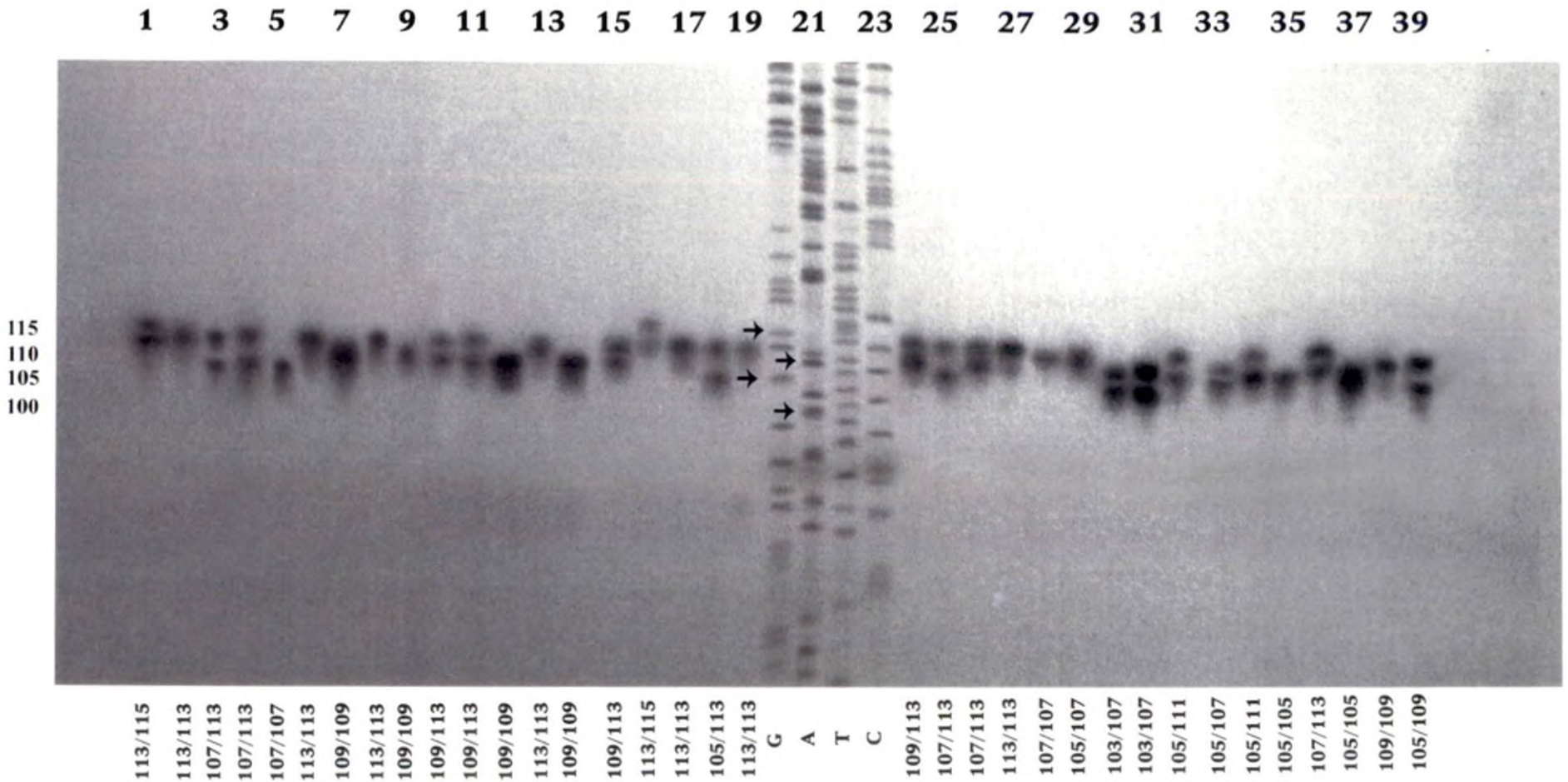

Plate.4. Autoradiograph of polymorphism at BM1508 locus in crossbred cattle of Kerala
Lanes 1-19; samples

20-23; G,A,T,C sequences of M13 DNA

Table 4.12 Allele sizes and frequencies at BM1508 locus in crossbred cattle population of Kerala

Sl. No	Size in base pairs	Allelic frequency
1	103	0.064
2	105	0.045
3	107	0.340
4	109	0.103
5	111	0.013
6	113	0.429
7	115	0.006

Table 4.13 Genotype and genotype frequencies at BM1508 locus in crossbred cattle population of Kerala

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	103/103	0.013	10	105/113	0.026
2	103/107	0.026	11	107/107	0.231
3	103/109	0.013	12	107/109	0.026
4	103/111	0.013	13	107/113	0.154
5	103/113	0.051	14	109/109	0.013
6	105/105	0.013	15	109/113	0.128
7	105/107	0.013	16	113/113	0.244
8	105/109	0.013	17	113/115	0.013
9	105/111	0.013			

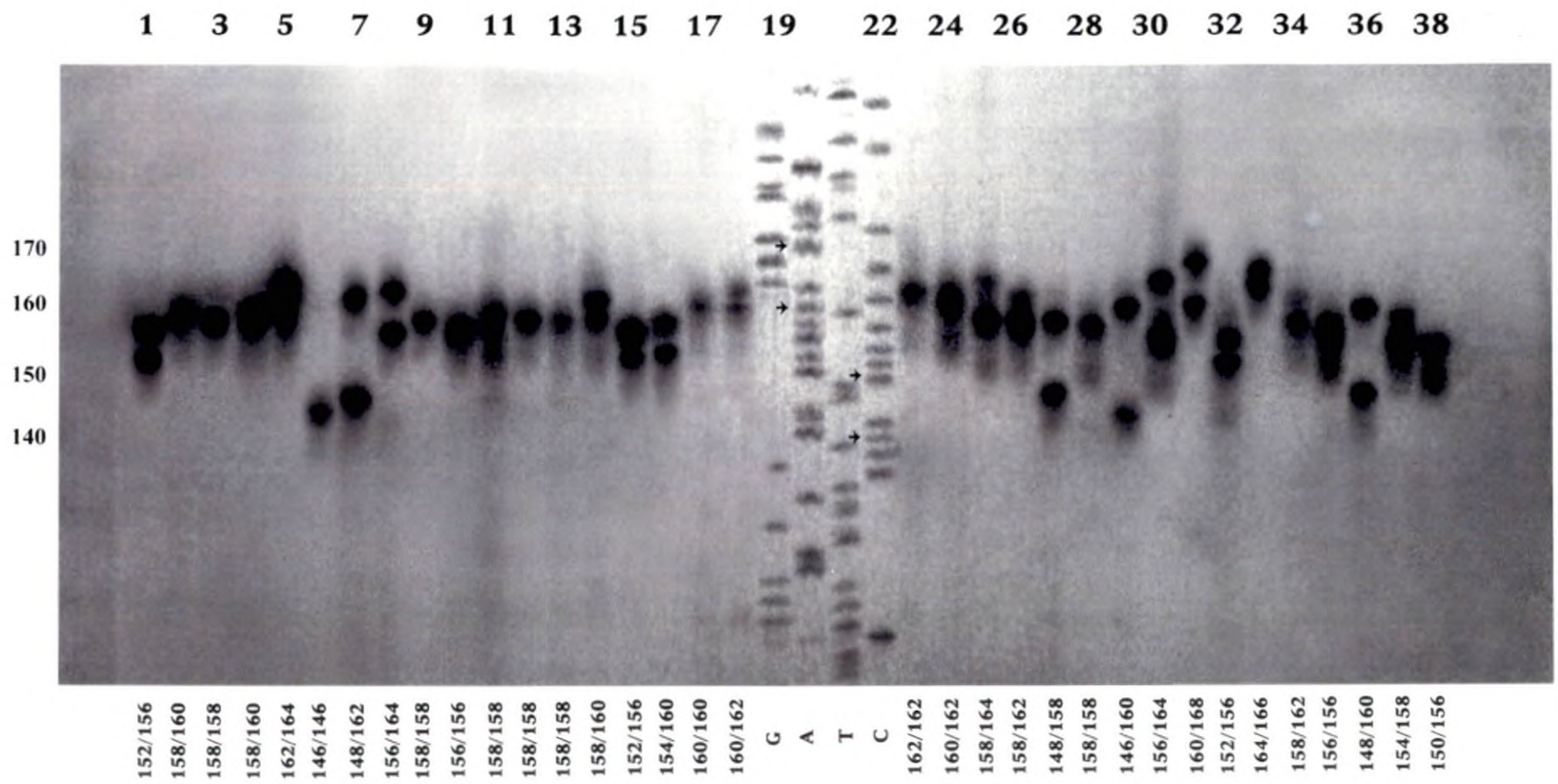

Plate.5. Autoradiograph of polymorphism at BM4305 locus in crossbred cattle of Kerala

Lanes 1-18; samples

19-22; G,A,T,C sequences of M13 DNA

23-38; samples

Table 4.14 Allele sizes and frequencies at BM4305 locus in crossbred cattle population of Kerala

Sl. No	Size in base pairs	Allelic frequency
1	146	0.057
2	148	0.032
3	150	0.013
4	152	0.006
5	154	0.070
6	156	0.158
7	158	0.241
8	160	0.146
9	162	0.108
10	164	0.101
11	166	0.063
12	168	0.006

Table 4.15 Genotype and genotype frequencies at BM4305 locus in crossbred cattle population of Kerala

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	146/146	0.013	20	156/156	0.063
2	146/154	0.013	21	156/158	0.051
3	146/156	0.013	22	156/160	0.038
4	146/160	0.013	23	156/164	0.025
5	146/162	0.013	24	158/158	0.101
6	146/164	0.025	25	158/160	0.076
7	146/166	0.013	26	158/162	0.025
8	148/156	0.013	27	158/164	0.038
9	148/158	0.013	28	158/166	0.063
10	148/160	0.013	29	160/160	0.025
11	148/162	0.025	30	160/162	0.025
12	150/156	0.025	31	160/164	0.013
13	152/156	0.013	32	160/166	0.025
14	154/154	0.025	33	160/168	0.013
15	154/156	0.013	34	162/162	0.038
16	154/158	0.013	35	162/164	0.038
17	154/160	0.025	36	164/164	0.013
18	154/162	0.013	37	164/166	0.025
19	154/164	0.013			

Table 4.16 Number of alleles, Allele sizes, Heterozygosity and Polymorphic Information Content of different microsatellite markers in crossbred cattle population

Marker	ILSTS096	HUJII77	BL41	BM1508	BM4305
Number of observations	76	80	75	78	79
Number of alleles	12	13	14	7	12
Size range of alleles (bp)	188-212	193-221	232-258	103-115	146-168
Unbiased heterozygosity	0.880	0.854	0.865	0.686	0.864
Direct count heterozygosity	0.877	0.851	0.862	0.683	0.861
PIC value	0.865	0.842	0.849	0.630	0.846

Table 4.17 Allele sizes and frequencies in ten sire families at ILSTS096 locus

Sl. No	Size in base pairs	Allelic frequency
1	188	0.073
2	190	0.040
3	192	0.065
4	194	0.145
5	196	0.129
6	198	0.266
7	200	0.081
8	202	0.056
9	204	0.097
10	206	0.032
11	208	0.016

Table 4.18 Genotype and genotype frequencies in ten sire families at ILSTS096 locus

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	188/188	0.032	18	194/204	0.048
2	188/194	0.032	19	194/206	0.032
3	188/196	0.016	20	196/198	0.113
4	188/198	0.016	21	196/200	0.016
5	188/202	0.016	22	196/202	0.032
6	190/194	0.016	23	196/204	0.032
7	190/198	0.048	24	196/208	0.016
8	190/200	0.016	25	198/198	0.097
9	192/194	0.032	26	198/200	0.032
10	192/198	0.048	27	198/204	0.016
11	192/200	0.016	28	198/206	0.032
12	192/202	0.016	29	200/202	0.016
13	192/204	0.016	30	200/204	0.032
14	194/194	0.016	31	202/204	0.016
15	194/196	0.032	32	202/208	0.016
16	194/198	0.032	33	204/204	0.016
17	194/200	0.032			

Table.4.19 Allele sizes and frequencies in ten sire families at HUJII77 locus

Sl. No	Size in base pairs	Allelic frequency
1	193	0.008
2	195	0.016
3	197	0.008
4	199	0.024
5	201	0.016
6	203	0.185
7	205	0.065
8	207	0.048
9	209	0.258
10	211	0.226
11	213	0.113
12	215	0.008
13	217	0.024

Table 4.20 Genotype and genotype frequencies in ten sire families at HUJII77 locus

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	193/203	0.016	13	205/211	0.048
2	195/203	0.016	14	207/207	0.032
3	195/211	0.016	15	207/209	0.016
4	197/209	0.016	16	207/215	0.016
5	199/203	0.032	17	209/209	0.097
6	199/211	0.016	18	209/211	0.113
7	201/203	0.016	19	209/213	0.081
8	201/205	0.016	20	209/217	0.048
9	203/203	0.081	21	211/211	0.097
10	203/205	0.065	22	211/213	0.048
11	203/209	0.048	23	213/213	0.048
12	203/211	0.016			

Table 4.21 Allele sizes and frequencies in ten sire families at BL41 locus

Sl. No	Size in base pairs	Allelic frequency
1	236	0.056
2	238	0.097
3	240	0.081
4	242	0.113
5	244	0.105
6	246	0.250
7	250	0.234
8	252	0.048
9	258	0.008
10	266	0.008

Table 4.22 Genotype and genotype frequencies in ten sire families at BL41 locus

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	236/236	0.016	13	242/242	0.048
2	236/242	0.016	14	242/244	0.016
3	236/244	0.032	15	242/250	0.097
4	236/246	0.032	16	244/244	0.065
5	238/238	0.048	17	244/246	0.016
6	238/240	0.016	18	246/246	0.145
7	238/246	0.081	19	246/250	0.016
8	240/240	0.016	20	246/258	0.016
9	240/244	0.016	21	250/250	0.113
10	240/246	0.048	22	250/252	0.081
11	240/250	0.032	23	250/266	0.016
12	240/252	0.016			

Table 4.23 Allele sizes and frequencies in ten sire families at BM1508 locus

Sl. No	Size in base pairs	Allelic frequency
1	103	0.113
2	105	0.185
3	107	0.097
4	109	0.323
5	111	0.185
6	113	0.081
7	115	0.016

Table 4.24 Genotype and genotype frequencies in ten sire families at BM1508 locus

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	103/103	0.016	10	107/109	0.016
2	103/105	0.016	11	107/111	0.065
3	103/107	0.065	12	109/109	0.097
4	103/109	0.065	13	109/111	0.048
5	103/111	0.048	14	109/113	0.113
6	105/105	0.032	15	109/115	0.016
7	105/107	0.048	16	111/111	0.065
8	105/109	0.177	17	111/113	0.032
9	105/111	0.065	18	113/115	0.016

Table 4.25 Allele sizes and frequencies in ten sire families at BM4305 locus

Sl. No	Size in base pairs	Allelic frequency
1	146	0.081
2	148	0.032
3	154	0.105
4	156	0.081
5	158	0.202
6	160	0.218
7	162	0.129
8	164	0.105
9	166	0.040
10	168	0.008

Table 4.26 Genotype and genotype frequencies in ten sire families at BM4305 locus

Sl. No.	Genotype	Genotype frequency	Sl. No.	Genotype	Genotype frequency
1	146/156	0.032	16	156/160	0.048
2	146/158	0.065	17	156/162	0.032
3	146/160	0.016	18	158/158	0.048
4	146/162	0.016	19	158/160	0.081
5	146/164	0.016	20	158/162	0.016
6	146/166	0.016	21	158/164	0.065
7	148/158	0.016	22	158/166	0.032
8	148/160	0.032	23	160/160	0.065
9	148/162	0.016	24	160/162	0.081
10	148/164	0.048	25	160/164	0.032
11	148/166	0.016	26	160/166	0.032
12	150/160	0.032	27	162/162	0.032
13	154/154	0.016	28	162/164	0.016
14	154/156	0.032	29	162/166	0.016
15	154/160	0.016	30	162/168	0.016

Table 4.27 Names of the bulls and their daughter averages for 305 day milk yield, Peak yield, Days to attain Peak yield and Age at First Calving (AFC)

Sl. No	Bull	No. of progenies	305 day milk yield(kg)	Peak yield (kg)	Days to attain PeakYield	AFC (days)
1	Deva	12	2188.7±144	11.2±0.5	15.5±4.19	1043±18 [a]
2	Oscar	4	2073.6±850	11.1±1.1	18.75±3.4	975±95 [a]
3	250	6	1961.6±291	13.4±0.6	27.0±9.40	1002±25 [a]
4	Debu	7	2111.8±131	12.3±1.6	18.0±3.30	956±49 [a]
5	Deven	6	2480.2±123	12.6±0.8	22.0±4.32	1079±29 [a]
6	Hakkim	4	1860.3±289	12.13±1	17.0±2.10	1011±32 [a]
7	Dipesh	7	2221.5±387	10.9±0.5	24.0±7.10	1145±52 [b]
8	Onkar	6	1945.7±207	12.5±0.2	26.0±5.70	1233±73 [b]
9	Dara	5	2284.1±322	13.8±1.9	50.4±14.1	1076±57 [a]
10	Gopal	5	2059±325.5	13.1±1.1	26.0±5.30	1226±84 [b]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.28 Names of the bulls and their daughter averages for Fat %, Protein % SNF % and Total solids %

Sl. No	Bull	No. of progenies	Fat %	Protein %	SNF %	Total solids %
1	Deva	12	3.13±0.2 [b]	2.62±0.08	8.40±0.20	11.52±0.28
2	Oscar	4	4.4±0.20 [a]	2.97±0.14	8.44±0.24	12.5±0.300
3	250	6	2.83±0.2 [b]	2.71±0.96	8.34±0.30	11.18±0.24
4	Debu	7	4.25±0.3 [a]	2.92±0.10	8.20±0.30	12.43±0.40
5	Deven	6	4.03±0.2 [a]	2.78±0.20	8.36±0.16	12.39±0.11
6	Hakkim	4	3.58±0.4 [b]	2.83±0.17	8.50±0.40	12.07±0.80
7	Dipesh	7	4.2±0.28 [a]	2.87±0.07	8.52±0.29	12.71±0.50
8	Onkar	6	4.03±0.3 [a]	2.77±0.18	8.43±0.31	12.46±0.54
9	Dara	5	3.8±0.46 [a]	2.73±0.07	8.30±0.27	12.02±0.70
10	Gopal	5	3.7±0.34 [a]	2.64±0.14	8.28±0.12	11.96±0.30

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.29 Averages for milk production and milk composition traits in crossbred cattle population

Sl. No.	Parameter	Population average
1	305 day milk yield(kg)	2070.5± 59.1
2	Peak yield (kg)	11.858±1.141
3	Days to attain Peak Yield	24.304±1.88
4	Age at First Calving (days)	1077.43±11.55
5	Fat %	3.738±0.0788
6	Protein%	2.781±0.0286
7	SNF%	8.456±0.0053
8	Total solids%	12.191±0.097

Table 4.30 Analysis of variance for effects of sire groups on 305 day milk yield

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	1160996.5	128999.61	0.3879101^{NS}
Error	47	15629864	332550.29	
Total	56	16790860.5		

NS-non significant ($p<0.05$)

Table 4.31 Analysis of variance for effects of sire groups on milk fat %

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	15.1701	1.685566	3.969169*
Error	50	21.23324	0.424665	
Total	59	36.40334		

*-significant ($p<0.05$)

Table 4.32 Analysis of variance for effects of sire groups on milk protein %

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	0.769965	0.085552	1.03951 NS
Error	50	4.114999	0.082300	
Total	59	4.884964		

NS-non significant ($p<0.05$)

Table 4.33 Analysis of variance for effects of sire groups on total solids %

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	14.531227	1.6145808	1.5477191 NS
Error	50	52.160008	1.0432002	
Total	59	66.691235		

NS-non significant ($p<0.05$)

Table 4.34 Analysis of variance for effects of sire groups on SNF %

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	0.540409	0.060045	0.13661 ^{NS}
Error	50	21.97702	0.43954	
Total	59	22.517429		

NS-non significant ($p<0.05$)

Table 4.35 Analysis of variance for effects of sire groups on peak yield

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	54.67573	6.075081	1.05996 ^{NS}
Error	52	298.034	5.731422	
Total	61	352.70973		

NS-non significant ($p<0.05$)

Table 4.36 Analysis of variance for effects of sire groups on days to attain peak yield

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	4316.2665	479.58517	0.8222786 ^{NS}
Error	52	30328.443	583.23929	
Total	61	34644.7095		

NS-non significant ($p<0.05$)

Table 4.37 Analysis of variance for effects of sire groups on age at first calving

Source	Degrees of Freedom	Sum of squares	Mean Sum of Squares	F value
Between sires	9	497705.1	55300.57	3.581902*
Error	52	802822	15438.88	
Total	61	1300527.1		

*-significant ($p<0.05$)

Table 4.38 Genotypes of the sires at different microsatellite loci

Sl. No.	Sire	Marker Name				
		ILSTS096	HUJII77	BL41	BM1508	BM4305
1	Deva	196/204	203/205	242/250	103/111	158/160
2	Oscar	194/198	203/209	240/246	109/113	156/158
3	250	188/200	211/211	238/246	105/111	154/164
4	Debu	188/198	205/211	238/246	103/109	154/162
5	Deven	192/198	209/213	246/252	109/113	146/160
6	Hakkim	196/202	213/221	240/246	109/111	160/162
7	Dipesh	198/206	207/209	250/252	109/111	146/158
8	Onkar	194/198	207/209	238/250	103/109	158/160
9	Dara	196/198	203/211	236/244	105/109	160/162
10	Gopal	198/200	211/213	240/244	105/111	148/166

Table 4.39 Effects of ILSTS096 alleles of sires on AFC and milk fat percentage of offsprings

Sl. No.	Allele	Average Age at First Calving (days)	Average Milk Fat Percentage
1	188	1077.57±61.01 [a]	2.986±0.21 [a]
2	192	1104.40±0.300 [a]	4.013±0.30 [b]
3	194	1088.50±27.65 [a]	3.710±0.18 [b]
4	196	1015.07±70.88 [b]	3.443±0.21 [b]
5	198	1060.73±32.00 [a]	4.130±0.15 [c]
6	200	1072.60±62.32 [a]	3.860±0.18 [b]
7	202	1115.40±71.32 [a]	3.675±0.26 [b]
8	204	1026.38±25.63 [a]	3.290±0.19 [a]
9	206	1146.75±64.00 [a]	4.200±0.34 [b]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.40 Effects of HUJII77 alleles of sires on AFC and milk fat percentage of offsprings

Sl. No.	Allele	Average Age at First Calving (days)	Average Milk Fat Percentage
1	203	1112.88±35.80 [a]	3.48±0.16 [b]
2	205	1057.25±56.54 [a]	3.30±0.18 [a]
3	207	1090.50±79.90 [a]	4.00±0.34 [b]
4	209	1087.77±31.67 [a]	3.85±1.44 [b]
5	211	1068.00±37.02 [a]	3.56±0.22 [b]
6	213	1048.82±24.94 [a]	4.00±0.24 [b]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.41 Effects of BL41 alleles of sires on AFC and milk fat percentage of offsprings

Sl. No.	Allele	Average Age at First Calving (days)	Average Milk Fat Percentage
1	236	1040.50±40.69 [b]	3.38±0.33 [b]
2	238	1030.70±51.23 [b]	3.65±0.28 [b]
3	240	1163.44±51.98 [b]	4.15±0.22 [c]
4	242	1071.92±25.21 [b]	3.21±0.17 [a]
5	244	1121.30±44.02 [b]	3.98±0.19 [b]
6	246	1003.90±24.51 [a]	3.70±0.20 [b]
7	250	1110.00±28.50 [b]	3.60±0.15 [b]
8	252	1121.33±47.80 [b]	3.98±0.22 [b]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.42 Effects of BM1508 alleles of sires on AFC and milk fat percentage of offsprings

Sl. No.	Allele	Average Age at First Calving (days)	Average Milk Fat Percentage
1	103	1053.70±26.00 [a]	3.43±0.20 [a]
2	105	1047.65±21.84 [a]	3.68±0.21 [a]
3	109	1090.23±28.24 [a]	3.95±0.11 [b]
4	111	1124.10±31.78 [a]	3.42±0.23 [a]
5	113	1077.63±63.29 [a]	4.20±0.20 [b]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Table 4.43 Effects of BM4305 alleles of sires on AFC and milk fat percentage of offsprings

Sl. No.	Allele	Average Age at First Calving (days)	Average Milk Fat Percentage
1	146	1083.50±33.12 [b]	3.94±0.14 [b]
2	148	1162.50±80.70 [b]	3.53±0.33 [b]
3	154	970.80±22.94 [a]	3.19±0.23 [a]
4	156	1077.30±33.85 [b]	3.60±0.24 [b]
5	158	1065.80±30.80 [b]	3.79±0.16 [b]
6	160	1067.50±30.89 [b]	3.66±0.14 [b]
7	162	1107.79±45.47 [b]	3.68±0.23 [b]
8	166	1126.00±51.83 [b]	4.58±0.20 [c]

Means bearing same superscripts do not differ significantly ($p<0.05$)

Discussion

5. DISCUSSION

Mammalian genome contains large amount of repetitive DNA sequences, an increasing number of which are being identified as stretches of tandem repeat units. The repeat units ranging in size from two to six base pairs form stretches of DNA referred to as short tandem repeats or microsatellites. Short tandem repeats such as $d(A)_n$, $d(T)_n$, $(dT\text{-}dG)_n$ and $(dA\text{-}dC)_n$ have been described by several authors (Litt and Luty, 1989; Tautz, 1989; Weber, 1990).

One of the applications of molecular technology in dairy cattle breeding is the identification of regions of DNA affecting the production traits (Georges *et al.*, 1995). This can be achieved by a genetic linkage map, which clearly describes the marker-QTL associations. Detection of allelic variations at loci influencing economically important traits of cattle has become feasible due to the construction of bovine linkage maps, mostly composed of microsatellites (Barendse *et al.*, 1994; Bishop *et al.*, 1994; Kappes *et al.*, 1997; Ihara *et al.*, 2004). MAS is the process of using the results of DNA testing to assist in the selection of individuals to become parents of the next generation, by combining the genotypes and expected progeny differences of the bulls.

5.1 ALLELIC NUMBER, ALLELIC SIZE, ALLELIC FREQUENCY AND GENOTYPE FREQUENCY

5.1.1 *ILSTS096*

For ILSTS096 locus, twelve alleles with a size range of 188-212 bp were detected with 35 genotypes. This is slightly different from the product size range of 192-208 bp reported for this marker by Kemp *et al.* (1995) and Ihara *et al.* (2004). The highest frequency of 0.191 was observed for the alleles 198 and 200. The highest genotype frequency observed in the unrelated animals was for the genotype 200/200. Interestingly this genotype was absent in the selected dairy cattle population. In the case of selected dairy cattle population, the allele 212 was absent. The highest gene frequency was observed for the allele 198 and the highest genotype frequency was that of 196/198.

5.1.2 HUJII77

Thirteen alleles with a size range of 193-221 bp and 36 genotypes were observed for the microsatellite marker HUJII77 in crossbred cattle of Kerala. In the selected dairy cattle population, thirteen alleles were observed with 23 genotypes. The reduction in the number of genotypes may be due to the increased homozygosity of the selected population. In both populations, the highest frequency was observed for the allele 209. Shalom *et al.* (1994) and Ihara *et al.* (2004) reported a size range of 187-213 bp for the marker and the number of alleles observed by them was eleven.

5.1.3 BL41

Fourteen alleles were identified in the unrelated population with a size range of 232-266 bp with 36 genotypes. Bishop *et al.* (1994) detected eight alleles with a size range of 240-258 bp. Number of alleles observed in the selected dairy cattle population was ten with a size range of 236-266 bp. The highest gene frequency of 0.267 was noted for the allele 242 in unrelated population and the highest genotype frequency of 0.160 for 242/242 genotype. But in the selected sire families the highest frequency was observed for the allele 246 and the genotype 246/246. One peculiarity noted for this marker was 44 per cent animals in the genetically unrelated population and 45 per cent animals in the selected dairy cattle population were homozygous. This is far more than the expected homozygote frequencies, which requires a detailed investigation with more samples.

5.1.4 BM1508

Number of alleles observed for BM1508 was lowest and the size range was the smallest among the markers studied. Seven alleles with a size range of 103-115 bp with seventeen genotypes were observed in the genetically unrelated population. The size range and the number of alleles were similar in the selected dairy cattle population. The most common allele and the genotype in the selected population were 109 and 105/109. The size range and the number of alleles reported for this

marker by earlier studies were 99-115 bp and nine (Stone *et al.*, 1995; Ihara *et al.*, 2004).

5.1.5 BM4305

Twelve alleles were detected with a size range of 146-168 bp and 37 genotypes in the genetically unrelated population. In unrelated animals alleles 156, 158 and 160 were having higher frequencies. In the selected dairy cattle population, ten alleles were detected with the same range of unrelated population and the highest frequency was noted for the allele 160, followed by the allele 158. In a study on the construction of a genetic linkage map of cattle, Bishop *et al.* (1994) detected 8 alleles for BM4305 locus with a size range of 148-168 bp.

The earlier studies on the selected markers were conducted in exotic cattle (*Bos taurus*). The present study was conducted in crossbred cattle (*Bos indicus* X *Bos taurus*). This might be the reason for the increase in the number and the size of alleles for ILSTS096, HUJII77, BL41 and BM4305 loci.

5.2 DIRECT COUNT HETEROZYGOSITY, UNBIASED HETEROZYGOSITY AND POLYMORPHIC INFORMATION CONTENT (PIC)

The highest direct count heterozygosity was observed for the microsatellite marker ILSTS096 (0.877), followed by BL41 (0.862), BM4305 (0.861), HUJII77 (0.851) and BM1508 (0.683). The highest unbiased heterozygosity of 0.880 was observed for the microsatellite marker ILSTS096, followed by BL41 (0.865), BM4305 (0.864), HUJII77 (0.854) and BM1508 (0.686). The heterozygosity obtained for HUJII77 was found to be slightly less than the heterozygosity obtained (0.86) by Barendse *et al.* (1994) and Shalom *et al.* (1994).

According to Botstein *et al.* (1980) a marker is highly informative if its PIC is greater than 0.5. In the present study, all the five markers were highly informative. The highest PIC value was obtained for the microsatellite marker ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630).

5.3 RELATIONSHIP BETWEEN ECONOMIC TRAITS AND DIFFERENT SIRE FAMILIES OF CROSSBRED CATTLE

5.3.1 305 Day Milk Yield

The population average for 305 day milk yield was 2070.5±59.1 kg. This was similar to that obtained by Radhika (1997). Kannan *et al.* (2000) reported a mean 305 day lactation yield of 2113±54.98 kg for crossbred dairy cattle. Among the sire families the highest milk yield was recorded for the family of Deven (2480.2±123 kg) and the lowest for the family of Hakkim (1860.3±289 kg). There were no significant differences between the sire families for 305 day milk yield.

5.3.2 Milk Fat Percentage

According to Eckles *et al.* (1973) the average milk fat percentage of Holstein and Jersey cows were 3.27 and 5.22 respectively. The average milk fat percentage of crossbred dairy cattle population in the study was 3.738±0.0788. A report by Radhika (1997) stated a milk fat percentage of 3.67±0.12 and 3.96±0.152 during mid lactation in Cattle Breeding Farm, Thumburmuzhi and University Livestock Farm, Mannuthy. Since the present population included the animals from both farms, the average value is approximately equal to the average value reported by Radhika (1997). This indicates that the milk fat percentage has remained stagnant for past eight years. Sathian (2001) observed an average value of 4.64 for milk fat percentage in crossbred dairy cattle. A significantly lower fat percentage was observed for the families of Deva (3.13±0.2), Bull no.250 (2.83±0.2) and Hakkim (3.58±0.4). The milk fat percentage of the former two were below the Prevention of Food Adulteration (PFA) standards.

5.3.3 Milk Protein Percentage

The population average for milk protein percentage was 2.781±0.0286. The average milk protein percentage in crossbred dairy cattle reported by Sathian (2001) and Ally (2003) were 3.13 and 2.88±0.04 respectively. Among the families, the highest milk protein percentage was recorded for the family of Oscar (2.97±0.14)

and the lowest for the family of Deva (2.62±0.08). There were no significant differences between the families under study.

5.3.4 Percentage of Total Solids in Milk

The population average for percentage of total solids in milk was 12.91±0.097. The average total solid percentage during mid lactation in crossbred dairy cattle population, reported by Radhika (1997) was 12.20±0.058. The average total solids percentage observed by Sathian (2001) and Ally (2003) in crossbred dairy cattle were 13.23 and 12.31±0.19 respectively. In the present study, the highest total solids percentage was observed for the family of Dipesh (12.71±0.5) and the lowest for the family of the Bull no. 250 (11.18±0.3). These differences were not statistically significant.

5.3.5 SNF percentage

The population average for SNF percentage in milk was 8.456±0.0053. Similar values were observed earlier by other workers in crossbred cattle of Kerala (Radhika, 1997; Sathian, 2001; Ally, 2003). Analysis of variance revealed no significant difference among the ten sire families for the SNF percentage in milk.

5.3.6 Peak Yield

Kannan *et al.* (2000) reported an average peak yield of 11.42±0.23 kg for crossbred cattle of Kerala. In the present study, the average peak yield obtained was 11.858±1.141 kg, which was similar to the earlier report. The highest peak yield of 13.8±1.9 kg was recorded for the family of Dara and the lowest for the family of Dipesh (10.9±0.5 kg). The analysis of variance for peak yield did not show any significant difference among the ten sire families.

5.3.7 Days to attain Peak Yield

Girija (1980) reported that the days to attain peak yield in crossbred dairy cattle (Jersey X Zebu) at ULF, Mannuthy as 44.75±1.23 days. In the present study, the population average for days to attain peak yield was 24.304±1.88 days.

This low value observed in the study is likely a pointer for the management problems. The analysis of variance for days to attain peak yield revealed no significant difference among the sire families.

5.3.8 Age at First Calving

The population average for AFC was 1077.43±11.55 days. Analysis of variance revealed a significantly higher AFC for the families of Dipesh, Onkar and Gopal. In the present study, the AFC of crossbred cows varied from 956±49 days to 1233±73 days. This is in line with the observations of Hiremath (2000), which ranged from 916.5±107.44 days to 1228±75.97 days in crossbred dairy cattle. The literatures for the linkage of microsatellite markers and AFC are very meagre as the AFC is not considered to be an important economic trait in many of the developing countries.

5.4 ALLELIC EFFECTS ON MILK FAT PERCENTAGE AND AGE AT FIRST CALVING

The study on eight economic traits revealed that significant differences exist in milk fat percentage and AFC. The absence of significant differences in the other six traits may be due to the low sample size. So the allelic effects of the five microsatellite markers on milk fat percentage and AFC were found out.

5.4.1 Allelic Effects of ILSTS096

The animals having the allele 188 had a significantly lower milk fat percentage of 2.986±0.21, when compared to the animals without this allele (3.803±0.523). A similar effect was observed for the allele 204 also, with an average milk fat percentage of 3.29±0.19, while the animals without this allele had a significantly higher fat percentage of 3.81±0.11. In both the cases the allelic averages were lower than the PFA standards. A significantly higher milk fat percentage (4.13±0.15) was observed for the animals with the allele 198, compared to the animals without this allele (3.44±0.11). The strong association of ILSTS096 locus on BTA3 with milk yield, fat percentage and protein percentage was reported

by Heyen *et al.* (1999) in his genome scan for QTL affecting milk production and health traits.

In the unrelated dairy cattle population the frequency of allele 188 was 0.046 and the frequency of allele 204 was 0.079. Since both alleles are associated with low milk fat percentage, negative selection for these alleles can be done to increase the milk fat percentage. Since the frequencies of both these alleles are low, the impact of selection will be meagre. In the unrelated population, the frequency of the allele 198 was 0.191 and the selection for this allele in the population will be beneficial to improve the milk fat percentage.

The average AFC for the animals with allele 196 was 1015±70.88 days, which was significantly lower than that of the animals without this allele (1092.26±22.89 days). This is the first report indicating the association between ILSTS096 locus and AFC. Selection for the allele 196 will be beneficial for lower AFC in crossbred cattle of Kerala.

5.4.2 Allelic Effects of HUJII77

The animals with the allele 205 at HUJII77 locus showed a significantly lower milk fat percentage (3.3±0.18), compared to the animals without this allele (3.78±0.11). Heyen *et al.* (1999) detected the effect of HUJII77 locus on protein percentage and Rodriguez-Zas *et al.* (2002a) detected the effect of HUJII77 on milk yield and protein percentage. But in the present study, no significant difference was observed for milk yield and protein percentage among the sire families. Hence it was not possible to correlate the association of this marker with milk yield and milk protein percentage. The association between HUJII77 locus and fat percentage is not reported yet. This microsatellite marker is located on BTA3, in which the markers ILSTS096 and BL41 are located and both of them have strong associations with milk fat percentage (Heyen *et al.*, 1999). This may be the reason for the association shown by the alleles of HUJII77 with the milk fat percentage.

The frequency of the allele 205 in the unrelated dairy cattle population was 0.062. Since the frequency of the allele is low, the selection against this allele may not contribute much in improving the milk fat percentage.

5.4.3 Allelic Effects of BL41

Heyen *et al.* (1999) mapped the QTL for milk yield, fat yield, fat percentage and protein percentage between the markers D3S21 (BL41) and D3S34 (TGLA263). According to Rodriguez-Zas *et al.* (2002a), the BL41 locus was associated with variations in milk yield and fat percentage and a QTL near the markers ILSTS096 (16cM) and BL41 (32cM) might be responsible for the significant association between them and changes in these parameters.

The allele 240 had a frequency of 0.027 in the population and it was observed that this allele had strong association with higher milk fat percentage (4.15±0.22). The frequency of the allele 242 was 0.267 and the animals with this allele had significantly lower milk fat percentage. The frequency of the allele 240 is comparatively low and the frequency of the allele 242 in the population is very high. So selection for the allele 240 and selection against the allele 242 will have a good impact for higher milk fat percentage in progenies.

Animals with the allele 246 had a significantly lower AFC (1003.9±24.51 days), compared to animals without this allele (1104.37±22.67 days). Since the frequency of the allele 246 in the population was 0.047, the selection for this allele will be beneficial to reduce the AFC. The association between BL41 and AFC is also being reported for the first time. Since ILSTS096 and BL41 showed association with AFC, the reason can be something connected with the QTL associated with these markers.

5.4.4 Allelic Effects of BM1508

For BM1508 locus, the alleles 109 and 113 had a significantly higher milk fat percentage, compared to the animals without these alleles. Heyen *et al.* (1999) reported the marker effects of BM1508 on milk fat percentage.

The frequencies of the alleles 109 and 113 were 0.103 and 0.429 respectively. Selection for both these alleles will improve the milk fat percentage. Since the frequency of the allele 109 is very low in the population, the selection for this allele will be more beneficial for increasing the milk fat percentage compared to the allele 113, which had a very high frequency in the population studied.

5.4.5 Allelic Effects of BM4305

The average milk fat percentage was 3.19±0.23, for the animals with the allele 154, which was significantly different from the animals without this allele (3.81±0.0106). Another trend found in animals with this allele was the lower AFC of 970.8±22.94 days compared to the animals without this allele (1093.3±20.372 days). Animals with the allele 166 at the BM4305 locus had an average milk fat percentage of 4.58±0.2, which was significantly higher than the animals without this allele (3.64±0.102). The microsatellite marker BM4305 had effects on milk yield and protein percentage (Ashwell *et al.*, 1998 and Heyen *et al.*, 1999). No published reports are available for the association of BM4305 locus on BTA14 with the QTL affecting milk fat percentage and AFC.

Since the allele 154, which had a lower frequency of 0.070 in the population, showed significant association with reduced milk fat percentage and lower AFC, selection for this allele will yield a good result for AFC. But it may not be of use as it reduces the fat percentage in milk, which is not advantageous.

The allelic average for milk fat percentage is very high (4.58±0.2) for the allele 166. The allele 166 had a frequency of 0.063, which is low in the population. Hence selection for this allele can be advocated strongly for the population under study.

In the present study, three markers (ILSTS096, BL41 and BM1508), which had alleles associated with lower fat percentage in milk were also having alleles associated with lower AFC. This needs to be enquired further.

5.5 EVALUATION OF SIRES BASED ON THEIR GENOTYPE

5.5.1 Deva

The genotype of the sire Deva at ILSTS096 locus was 196/204. The allele 196 showed significant association with lower AFC, selection for this allele will yield a good result for AFC. But the other allele 204 is associated with lower milk fat percentage. So selection for this genotype is of no use as it reduces the fat percentage in milk, which is not advantageous. The genotype at the locus HUJII77 for the bull was 203/205. The allele 205 showed a significant association with decrease in the milk fat percentage. The genotype was 242/250 at the BL41 locus. The allele 242 showed a significant effect on the reduction in milk fat percentage. The genotype at the locus BM1508 was 103/111 and at the locus BM4305 was 158/160. No significant effect was observed for these alleles on milk fat percentage and AFC. Since the sire Deva had the alleles for reduction in milk fat percentage at ILSTS096, HUJII77 and BL41 loci and lacking the favourable alleles for increase in milk fat percentage the selection is not favourable. All these facts explain why this family had significantly lower milk fat percentage compared with seven other families.

5.5.2 Oscar

The sire Oscar had the allele 198 at the ILSTS096 locus, allele 240 at BL41 locus and the alleles 109 and 113 at BM1508 which had significant effect on higher milk fat percentage. The other alleles present at the different loci typed did not show any effect on the milk fat percentage and AFC.

5.5.3 Bull no. 250

The sire had the allele 188 at ILSTS096 locus and allele 154 at BM4305 locus and both have effect on reduction in fat percentage of milk. All the other alleles typed did not show any significant effect on milk fat percentage. The presence of unfavourable alleles in two loci was salient to establish the significantly lower milk fat percentage of the family.

5.5.4 Debu

The sire Debu had the allele 188 and the allele 198 at the ILSTS096 locus. The former one had significant effect on decrease in milk fat percentage and the latter had an opposite effect. The allele 205 at HUJII77 and 154 at BM4305 locus had significant effects on lower milk fat percentage. The opposite effects of the alleles for milk fat percentage make it difficult to predict the betterment of the family for the character. The allele 109 at the BM1508 locus had a positive effect on milk fat percentage. Favourable alleles for lower AFC, ie, 154 at BM4305 locus and 246 at BL41 were present.

5.5.5 Deven

The sire Deven had the favourable alleles for milk fat percentage, the allele 198 at ILSTS096 locus and the alleles 109 and 113 at the BM1508 locus. The allele 246 which had significant effect on lower AFC was present at BL41 locus. All the other alleles typed did not show any significant effect on milk fat percentage and AFC. So the selection of this sire for higher milk fat percentage and lower AFC is favourable. Favourable alleles at two loci make it possible to predict the higher milk fat percentage.

5.5.6 Hakkim

The favourable alleles for the milk fat percentage, allele 240 at BL41 locus and the allele 109 at BM1508 locus were present. Inspite of the favourable alleles at two loci, the milk fat percentage of the family was significantly lower. But the average milk fat percentage for the family was calculated from only four progenies. This may be the reason for contradictory results. The allele 246 at the BL41 locus and the allele 196 at ILSTS096 locus, which had a significantly lower AFC, were also present.

5.5.7 Dipesh

The sire has the favourable alleles for milk fat percentage, allele 198 at the ILSTS096 locus and 109 at the BM1508 locus. Hence the progenies are expected to

have higher milk fat percentage. This holds true for the family. The family of Dipesh was having significantly higher AFC. Absence of three of the alleles connected with lower AFC is indicative of this.

5.5.8 Onkar

The family of the bull was expected to have higher milk fat percentage, since the favourable alleles, 198 at the ILSTS096 locus and 109 at the BM1508 locus were present. On analysis it was found to be true. The bull Onkar was devoid of any of the alleles connected with lower AFC. On analysis of the data it was found that the family had higher AFC.

5.5.9 Dara

The bull has the allele 198 at ILSTS096 locus and the allele 109 at BM1508 locus, which have significant effect on high milk fat percentage. Phenotypically also the family had significantly higher milk fat percentage. The family had a significantly lower AFC, which was indicative of the presence of the allele 196 at the ILSTS096 locus.

5.5.10 Gopal

The allele 198 at the ILSTS096 locus, 240 at the BL41 locus and 166 at the BM4305 locus were present. All the three had significantly higher averages for milk fat percentage. The family mean observed for the bull was also significantly higher. The members of the family of Gopal were not having any of the three recommended alleles for low AFC. As expected they showed significantly higher AFC.

Summary

6. SUMMARY

A recent application of molecular technology in dairy cattle breeding is the identification of the regions of the DNA affecting the production traits. Marker Assisted Selection (MAS) is the process of using the results of DNA testing to assist in the selection of individuals to become parents of the next generation by combining the genotypes and the expected progeny differences of the bulls. MAS enables the selection of the superior genotypes based on the allelic information of the marker loci. In the present study, the possibility of using the informations of the allele frequency, genotype frequency, heterozygosity and PIC of five microsatellite markers and their association with the economically important traits for the selection of crossbred cattle were studied.

Genomic DNA was isolated using a modified phenol-chloroform extraction procedure. DNA samples from 100 genetically unrelated animals were used to find out the PIC of the markers. For the microsatellite analysis of the quantitative traits, genomic DNA was isolated from 117 animals and from this ten sire families were selected for the study. Milk samples were analysed for the estimation of fat percentage, protein percentage, SNF and total solids percentage. Data regarding milk yield, age at first calving, peak yield, days to attain peak yield of these animals were also collected.

A set of microsatellite markers viz. ILSTS096, HUJII77, BL41, BM1508 and BM4305, with close linkage to QTL affecting the economically important traits were selected, the primers were custom synthesized and optimum PCR conditions were standardised.

M13 phage DNA was sequenced by the dideoxy chain termination method using Sequenase Version 2.0 Sequencing Kit. The G, A, T, and C sequences were used as markers to find out the allele sizes.

The amplified PCR products were fractionated by denaturing polyacrylamide gel electrophoresis and visualised by autoradiography. The number of alleles and their size for each marker were determined by comparing with M13

sequencing ladder. The allelic frequencies and genotype frequencies were worked out for the unrelated population and the selected dairy cattle population separately.

ILSTS096 locus had twelve alleles with a size range of 188-212 bp and 35 genotypes in the unrelated population. The allele 212 was absent and highest gene frequency was for the allele 198 in the selected dairy cattle population.

Thirteen alleles with a size range of 193-221 bp and 36 genotypes were obtained for HUJII77 locus in the population. Interestingly around 40 percentage of the animals were homozygotes at the locus. But the number of genotypes observed in the selected dairy cattle population was 23.

Fourteen alleles were identified in the unrelated population for BL41 locus, with a size range of 232-266 bp and 36 genotypes. Only ten alleles were observed in the selected dairy cattle population. One peculiarity noted for this marker was 44 per cent animals in the genetically unrelated population and 45 per cent animals in the selected dairy cattle population were homozygous.

The number of alleles observed was seven with a size range of 103-115 bp for BM1508 locus. Seventeen genotypes were observed. The number of alleles observed for BM1508 was the lowest and the size range was the smallest among the markers.

Twelve alleles with a size range of 146-168 bp and 37 genotypes were observed for BM4305 locus in the population. Only ten alleles were detected in the selected dairy cattle population.

The highest direct count heterozygosity was observed for the microsatellite marker ILSTS096 (0.877), followed by BL41 (0.862), BM4305 (0.861), HUJII77 (0.851) and BM1508 (0.683). The highest unbiased heterozygosity of 0.880 was observed for the microsatellite marker ILSTS096, followed by BL41 (0.865), BM4305 (0.864), HUJII77 (0.854) and BM1508 (0.686).

All the five markers were highly informative, as their PIC values were more than 0.5. The highest PIC value was obtained for the microsatellite marker

ILSTS096 (0.865), followed by BL41 (0.849), BM4305 (0.846), HUJII77 (0.842) and BM1508 (0.630).

Among the eight economically important traits (milk yield, fat percentage, protein percentage, SNF, total solids, peak yield, days to attain peak yield and AFC), milk fat percentage and AFC had significant difference among the families. The families of Deva, Bull No.250 and Hakkim showed significantly lower milk fat percentage compared to the other seven families. The AFC of the families of Dipesh, Onkar and Gopal was significantly higher from the other seven sire families.

All the five microsatellite markers had significant effect on milk fat percentage. ILSTS096, BL41 and BM4305 had effect on AFC. Z test was done to find out the allelic effects of different microsatellites on milk fat percentage and AFC.

The allelic averages of milk fat percentage for the allele 188 and 204 of ILSTS096 locus were significantly lower and that of the allele 198 was significantly higher than the animals without these alleles. The average AFC for the animals with the allele 196 at ILSTS096 locus was significantly lower compared to the animals without this allele. The allelic frequencies of 188 and 204 of the marker ILSTS096 were low in the population and that of 198 was high. So selection against the alleles 188 and 204 and selection for the allele 198 can be done, but the impact of selection will be meagre. The allelic average of AFC for the allele 196 is significantly lower, and the selection for this allele is likely to be beneficial to reduce the AFC.

Animals with the allele 205 at HUJII77 locus had significantly lower milk fat percentage compared to the animals without this allele. The selection against this allele may not contribute much in improving the milk fat percentage as the frequency of this allele in the population was low.

In the present study, alleles of the microsatellite marker BL41 showed significant effects on milk fat percentage and AFC. The allelic average of milk fat percentage for the allele 240 was significantly higher and that of the allele 242 was

significantly lower. The frequency of the allele 240 was comparatively low and the frequency of the allele 242 is very high in the population. So the selection for the allele 240 and selection against the allele 242 will have good impact on milk fat percentage of the selected animals. The allelic average of AFC for the allele 246 was significantly lower and the frequency of this allele in the population is comparatively low. Hence selection for this allele will be favourable.

Animals having the alleles 109 and 113 at BM1508 locus had a higher milk fat percentage than the animals without these alleles. The highest frequency was observed for the allele 113 in the unrelated population. The frequency of the allele 109 was low. So selection for the allele 109 will be more beneficial.

For BM4305 locus, the allele 154 had effects on both lower AFC and lower milk fat percentage. Since the frequency of this allele is low in the population, the selection for this allele will yield a good result for lower AFC, but it may not be useful as it reduces the milk fat percentage, which will affect the quality of the milk. The animals with the allele 166 had the highest average of milk fat percentage. Since the frequency of this allele in the population is very low, selection for this allele will have good impact on higher milk fat percentage.

The families of Deva, Bull No.250 and Hakkim showed significantly lower milk fat percentage compared to the other seven families. The genotype of the sire Deva had three alleles at different loci (204 at ILSTS096 locus, 205 at HUJII77 locus and 242 at BL41 locus), which had significantly lower averages for milk fat percentage. All these are indicative of the fact that the family had significantly lower milk fat percentage compared with seven other families. The Bull no. 250, which had the lower family average for milk fat percentage had the allele 188 at ILSTS096 locus and allele 154 at BM4305 locus and both have effect on reduction in fat percentage of milk. All the other alleles typed for this sire did not show any significant effect on milk fat percentage. The genotypes of the sire Hakkim had the favourable alleles for the milk fat percentage, allele 240 at BL41 locus and the allele 109 at BM1508 locus. The low milk fat percentage observed for this family may be due to the experimental error resulted from the low number of progenies.

Significantly higher AFC was observed for the families of Dipesh, Onkar and Gopal among the ten sire families. These sires were lacking any of the three identified alleles for low AFC (allele 196 at ILSTS096 locus, allele 246 at BL41 locus and allele 154 at BM4305 locus).

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Annexures

ANNEXURE I

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

40% Acrylamide

Acrylamide	380g
N_IN – 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	-SRL, Bombay

(B) PRIMERS

InVitrogen (India) Pvt. Ltd.

(C) MOLECULAR MARKERS

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

(D) ENZYMES

Taq DNA polymerase	-Bangalore Genei Pvt. Ltd.
Proteinase-K	-Bangalore Genei Pvt. Ltd.
PNK	-Bangalore Genei Pvt. Ltd.

(E) KITS

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

(F) ISOTOPES

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

ANNEXURE – III

ABBREVIATIONS

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

ANNEXURE – IV

PEDIGREE DETAILS OF THE BULLS

Semen samples were obtained from BAIF, PDC and Kerala Agricultural University Bull Station, Mannuthy.

Sl.No.	Bull Name	Date Of Birth	Genetic group
1	Deva	24/12/1991	50 % HF + 50 % I
2	Deven	01/12/1995	50 % HF + 50 % S
3	Debu	03/11/1991	50 % HF + 50 % I
4	Dipesh	11/12/1994	50 %HF+ 25 % G +25%S
5	Dara	—	50 %HF+ 50 % G
6	Gopal	—	62.5%HF + 37.5 % G
7	Hakkim	—	75 % HF + 25 % I
8	Oscar	—	50 % J + 50 % I
9	Onkar	—	50 % J + 50 % I
10	Bull No.250	—	—

HF-Holstein Friesian, I- Indigenous (*Bos indicus*), G-Gir, S-Sahiwal, J-Jersey

(Data from Annual Progress report (01/01/2004-31/12/2004) on Field Progeny Testing, KAU, Mannuthy by Dr. Stephen Mathew, Associate Professor, Centre for Advanced Studies in Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy.)

EVALUATION OF MICROSATELLITE MARKERS FOR SELECTION OF CROSSBRED CATTLE

NAICY THOMAS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

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

ABSTRACT

The possibility of using the informations of the allele frequency, genotype frequency, heterozygosity and PIC of five selected microsatellite markers (ILSTS096, HUJII77, BL41, BM1508 and BM4305) and their associations with the economically important traits for the selection of crossbred cattle were studied. Among the economic traits studied milk fat percentage and AFC showed significant difference among the ten sire families and hence their associations with the selected microsatellite markers were worked out.

PCR conditions were standardised for each marker separately. The number of alleles, size range and number of genotypes identified were 12, 188-212 bp and 35 respectively for ILSTS096 locus, 13, 193-221 bp and 36 for HUJII77 locus, 14, 232-266 bp and 36 for BL41 locus, 7, 103-115 bp and 17 at BM1508 locus and 12, 146-168 bp and 37 for BM4305 locus in the unrelated crossbred dairy cattle population.

The highest direct count heterozygosity was obtained for ILSTS096 followed by BL41, BM4305, HUJII77 and BM1508 (0.877-0.683). The highest unbiased heterozygosity was obtained for ILSTS096 followed by BL41, BM4305, HUJII77 and BM1508 (0.880-0.686). All the markers were highly informative as their PIC values (0.865-0.630) were more than 0.5.

Three sire families namely, Deva, Bull No.250 and Hakkim showed significantly lower milk fat percentage and three other (Dipesh, Onkar and Gopal), showed significantly higher AFC. All the five microsatellite markers had significant effect on milk fat percentage and three of them (ILSTS096, BL41 and BM4305) showed significant effect on AFC. The allelic averages of fat percentage for the allele 188 and 204 at ILSTS096 locus, 205 at HUJII77 locus and 154 at BM4305 locus were significantly lower and that of 198 at ILSTS096 locus, 240 at BL41 locus, 109 and 113 at BM1508 locus and 166 at BM4305 locus were significantly higher. The allelic averages of AFC for the alleles 196 at ILSTS096 locus, 246 at BL41 locus and 154 at BM4305 locus were significantly lower. All these three alleles were absent in the sires of the three families having higher AFC.

The allelic frequencies of 188 and 204 of the marker ILSTS096 were low in the population and that of 198 was high. So selection against the alleles 188 and 204 and selection for the allele 198 can be done, but the impact of selection will be meagre. At the BL41 locus, frequency of the allele 240 was comparatively low and the frequency of the allele 242 is very high in the population. So the selection for the allele 240 and selection against the allele 242 will have good impact on milk fat percentage of the selected animals. Frequency of allele 246 in the population is comparatively low. Hence selection for this allele will be favourable to reduce the AFC. The frequency of the allele 109 of BM1508 is low. So selection for this allele will be more beneficial. The animals with the allele 166 at BM4305 locus had the highest average of milk fat percentage and the frequency of this allele in the population is very low, hence selection for this allele will have good impact on higher milk fat percentage.