The use of genomics for improving livestock production

By

Ntanganedzeni Olivia Mapholi-Tshipuliso

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Promoter: Dr A Kotze
Co-promoter: Mrs. K Ehlers
External co-promoter: Dr. M.D. MacNeil

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Declaration

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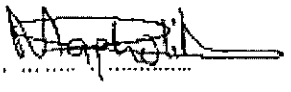
SIGNATURE: 

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THE USE OF GENOMICS FOR IMPROVING LIVESTOCK PRODUCTION

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Abbreviations

QTL	-Quantitative trait loci
BV	-Breeding value
RFLP	-Restriction Fragment Length Polymorphism
AFLP	-Amplified Fragment Length Polymorphism
SNP	-Single Nucleotide Polymorphism
EST	-Expressed Sequence Tag
USDA-ARS	-United State Department of Agriculture- Agricultural Research Service
MARC	-Meat Animal Research Centre
cDNA	-Complimentary DNA
RNA	-Ribonucleic acid
DNA	-Deoxyribonucleic acid
NCBI	-National Centre for Biotechnology Information
SD	-Standard deviation
AI	-Artificial insemination
BTA	-Bovine chromosome
CGC	-composite gene combination
GH	-growth hormone
MAS	-Marker assisted selection
MAI	-Marker assisted introgression
LE	-linkage equilibrium
LD	-linkage disequilibrium
EBV	-Estimated breeding value
BLUP	-Best linear unbiased prediction
PCR	-Polymerase chain reaction
SFA	-saturated fatty acid
MUFA	-mono unsaturated fatty acid
PUFA	-polyunsaturated fatty acid
LARRL	-Livestock and Range Research Laboratory

THE USE OF GENOMICS FOR IMPROVING LIVESTOCK PRODUCTION

Abstract

The goal of the study was to examine ways in which molecular genetics can be used to enhance the performance and sustainability of beef cattle production. A review of the literature of livestock and poultry was included to describe different approaches previously used for quantitative trait loci (QTL) studies, followed by two case studies. The first case study was to detect QTLs that affect relative amounts of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids using 328 F_2 progeny of Wagyu x Limousin F_1 derived from eight Wagyu founder bulls. The search was implemented with 217 markers covering the 29 bovine autosomes. A total of six QTLs were found which are located on five different chromosomes; on a genome-wide basis two were statistical significant and four were suggestive QTLs. On BTA2, a QTL was found that had additive effects on SFA (4 cM, F = 10.07, P = 0.04), MUFA (4 cM, F = 23.62, P < 0.01) and PUFA (11 cM, F = 20.74, P < 0.01). Two QTLs with dominance effects on MUFA were observed on BTA9 (P = 0.04; 2 QTL vs. 1 QTL). Three additional suggestive QTLs for dominant effects on the relative amounts of fatty acids were also detected. A QTL affecting the PUFA content were observed at 31 cM on BTA10 (F = 9.22; P = 0.06) and at 12 cM on BTA15 (F = 9.67, P = 0.06). Finally, a QTL affecting the MUFA content was found at 47 cM on BTA22 (F = 9.62, P = 0.08). A second case study included an experimental data that was analyzed and divided into two components: 1) to validate the pedigree expectation of genomic contributions to successive generations of backcrossing at loci unlinked to the locus being introgressed; and 2) to examine the effectiveness of the introgression strategy. Experimentally, backcrossing a self coat colour pattern into Line 1 Hereford was attempted. The two founder populations, F1 cross, two subsequent generations of backcrossing, and an intercross generation were evaluated. In total 526 were genotyped using 34 unlinked and five linked microsatellite markers. Estimated contributions of Line 1 Hereford in the F_1, B_1 and B_2 generations were 0.500, 0.750, and 0.875, compared to expected contributions based on a pedigree of 0.540, 0.746, and 0.819. In this study, the introgression was compromised because the linked markers used did not sufficiently segregate between the founder populations however more markers will be required for further research. Finally,

to integrate the knowledge gained in the preceding studies, an experiment was designed to identify QTLs that have effect on tick resistance, carcass weight and carcass quality in Nguni and Angus cattle using the F_2 design. If successful, the results of this study might lead to use of marker assisted introgression to increase resistance to tick of South African Angus and (or) add value to the carcass quality and carcass weight to Nguni.

Keywords: Quantitative trait loci, genetic markers, beef quality, fatty acids, backcrossing, Nguni

Chapter 1

Literature review

GENETIKA
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CHAPTER 1

1.1 Introduction

Agriculture plays an important role to reliably assure the availability of high-quality foods to satisfy human demand while minimizing environmental risks. Livestock production can provide a sustainable source of food and can contribute to economic growth. Worldwide, concerns about livestock production have been raised with regard to the interaction between farming practices and environmental sustainability. These issues, as well as a decreased availability of manpower in farming has resulted in the use of scientific technologies as a means of producing more and improved products (Pretty, 1998; Gagnoux, 2000; Galal *et al.*, 2000; Garnier *et al.*, 2003).

Recently, biotechnology contributed as a tool used to achieve improved livestock production. The identification of and selection for heritable traits has improved the quality of numerous animal products and the efficiency with which they are produced (Dekkers & Hospital, 2002). For example, cattle breeding have changed the cattle genome through quantitative selection for desired phenotypic traits (Fadiel *et al.*, 2005). Swine and poultry breeding industries have produced superior hybrid stocks by selecting among breeds, exploiting complimentary traits between breeds and continuing selection within the hybrids (Clutter & Schinkel, 2001). This latter strategy is facilitated by relatively short generation intervals and reproductive rates (number of offspring per female per year) that are moderate to high (de Koning *et al.*, 2003). The genetic improvement of livestock production depends on the identification of selection criteria to support the overall goal by enhancing the performance of beef cattle and to increase the sustainability of their production.

CHAPTER 1

The use of genomic tools (e.g., genome sequences, genetic maps, proteomics, protein structure modeling, bioinformatics software and databases and microarrays) and information generated for genetic improvement and the selection of animals have demonstrated the great potential to improve livestock production in agriculture (Hugo, 2006). Many quantitative genetic studies have identified techniques and databases that can be used to associate phenotypes with causal polymorphisms or markers (Bovenhuis *et al.*, 1997). These technologies hold promise for being able to lead to more accurate assessments of merit and to accelerate the genetic improvement of farm animals, especially for traits that are difficult to measure (Dekkers & Hospital, 2002).

Livestock genomics has followed in the path of the human genome enterprise (Eggen, 2003) adopting both its strategies and technologies to improve livestock production (Womack, 2005). The completed genome map for humans and to be completed genome maps for mice and several livestock species will provide a tool to accelerate an understanding of heritable traits. The amount of information currently available on the genomes of many livestock species (including cattle) has increased dramatically over the past few years (Webb, 2001). Examples of these are available on the following websites[1].

The application of genomics in animal production includes parentage identification, traceability of food and animals, marker assisted selection and marker assisted introgression. Genomics can also provide better evaluations of predicted genetic values or breeding values (BV) used by breeders, in particular when traits cannot be measured on a large scale for technical and/or economic reasons. In addition to the more

[1] www.angus.org.au/Databases/BIRX/omia; www.ncbi.nih.gov/entrez/query.fcgi?db=gene ; http://sol.marc.usda.gov/cattle ; http://pigest.genome.iastate.edu/index.html

accurate estimates of BV, the generation interval may be reduced through selection of breeding animals at a younger age where selection intensity may be increased (Elsen, 2003). The introgression of genes from novel and adapted populations into commercial stock may also improve the production efficiency and enhance sustainability (Dekkers, 2004).

1.2 Genetic maps and markers

Molecular genetic markers have been identified throughout the genomes of many species. Different types of genetic markers include: Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), microsatellites, Single Nucleotide Polymorphism (SNP) and Expressed Sequence Tags (EST). A genetic marker refers to a known DNA sequence of which the inheritance can be followed and can thus be used to describe variation. Genetic markers are landmarks along the chromosomes and can also be used to identify the loci where the gene of interest is located.

The discovery of highly polymorphic microsatellite markers for example as discovered by Litt & Luty, (1989) and other researchers facilitated the development of genomic linkage maps for several species including farm animals (Barendse *et al.*, 1994, 1997; Bishop *et al.*, 1994; Kappes *et al.*, 1997; Ihara *et al.*, 2004).

Microsatellite markers are mainly used because they generally have several alleles and, hence, the parental origin of a particular allele can be traced to determine the inheritance of a specific region of chromosomes through generations of families. They contain five

to 20 copies of a short sequence motif that is between two bp to four bp in length and is repeated in tandem (Willams, 2005). Vignal *et al.* (2002) indicated that microsatellite markers are commonly used because they are easy to analyse with simple PCR reactions followed by denaturing gel electrophoresis. They provide a high degree of information as a result of having a large number of alleles per locus. Collaboration between the Shirakawa Institute of Animal Genetics and the USDA-ARS U.S. Meat Animal Research Centre has produced a bovine genome map with an inter-marker interval of approximately 1.4 cM (W. M. Snelling, *personal communication*). This enhanced linkage map[2] provides a resource essential to precisely map QTL locations.

ESTs are small pieces of complementary DNA (cDNA) usually 200-500 nucleotides long (Fadiel *et al.,* 2005). They are useful as markers for desired fragments of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) that can be used for gene detection and positional mapping in a genome[3]. The National Centre for Biotechnology Information (NCBI) provides EST databases for many farm animals. For example, the bovine genome EST is available on ArkDB,[4] esemble pig-[5] and chicken-[6] EST databases are also available on the web.

Recently, some gene-specific markers (SNPs) have been added to bovine genetic maps to map specific target genes (Clawson *et al.*, 2004; Thue *et al.*, 2004). SNP markers are developed using randomly selected bovine ESTs with human orthologs, and added to the bovine linkage map via a two point linkage (Stone *et al.*, 2002). In addition,

[2] available at www.bovineqtlv2.tamu.edu/index.html and http://www.animalgenome.org/QTLdb
[3] http://www.ncbi.nlm.nih.gov/about/primer/est.html
[4] http://www.ncbi.nlm.nih.gov//mapview/map_search.cgi?taxid=9031
[5] http://pigest.genome.iastate.edu/index.html
[6] http://pigest.genome.iastate.edu/index.html

these markers will further refine comparative relationships between bovine linkage maps and the well-annotated human and model organism (e.g., mouse) genome sequences (Everts-van der Wind *et al.*, 2004).

In species where no maps are available, AFLP markers can be used. AFLP is a PCR based method and uses restriction fragment analysis. Extracted DNA is cut with different restriction enzymes to produce well-defined restricted fragments with sticky ends (Anderson, 2000). Double stranded linkers of approximately 20 bp with matching sticky ends are ligated on all the restriction fragments and those fragments are amplified in PCR with 20 nucleotide length primers that recognise linkers on both ends of those fragments. AFLP markers generate high levels of polymorphisms. This can be applied in gene mapping techniques and also has the ability to differentiate between individuals in a population, which makes it useful for amongst others studies on paternity and analysis of gene flow between populations. Several studies in pigs emphasize that AFLP markers can be used for genotyping to detect QTLs in animal experimental crosses (Wimmer *et al.*, 2002). The disadvantage of AFLP is that the number of steps needed to produce results can be very limited.

1.3 Mapping quantitative trait loci

Historically, the selection for traits of economic value has relied on phenotypic and/or pedigree data. Molecular genetics has now made it possible to detect and map a QTL which can be used for the genetic improvement of livestock. The development of a large number of molecular markers and interval mapping methods has paved the way for QTL mapping using inter-crosses of inbred experimental organisms (Paterson *et al.*,

1988). The identification of genes at those loci that control particular traits can be approached in several ways. The first is through the candidate gene approach and the second is through a whole genome scan (genetic mapping). The study of the structure of an acquired trait and the identification of the biochemical pathways that are involved in its expression may be particularly useful for monogenic traits and in a candidate gene approach. The identification of a gene that controls a similar phenotype in another species may also suggest a potential equivalent candidate gene that could be considered in the species of interest. The candidate gene approach in addition thus requires relevant information regarding the trait in the other species.

For more complex traits where several genes are likely to contribute to the variability, the genetic mapping approach can be used (Williams, 2005). Unfortunately, many of the economically important traits in livestock production are not monogenic but are affected by genes at several different loci. Knowledge of the location of these loci can provide markers linked to genes causing this variation in traits of economic importance. These markers could then be used in breeding programmes to assist with the selection for these traits. Genes that control QTLs are mapped using segregating genetic markers to track the inheritance of chromosomal regions within families and to associate marker genotypes with phenotypic information from individuals expressing the trait (Georges *et al.*, 1995). Thus, linkage maps of genomic markers (i.e., microsatellites or SNPs) are a necessary prerequisite to the genome-wide searches for loci where genes that affect the production related traits are located. All experimental approaches for QTL mapping localize genes that control particular traits within fairly broad chromosomal regions (Georges *et al.*, 1995).

CHAPTER 1

In livestock species, several experimental designs based on different family structures have been used to map QTLs. Statistical power (probability of detecting an effect, if one exists) in QTL mapping depends on several factors including the experimental design, the size of the QTL effect, the marker type, the number of markers, and the sample size (Bovenhuis *et al.*, 1997). There are four experimental designs that have been used for QTL mapping. Two of these designs are used for dairy cattle breeding while the others are used in beef cattle breeding

1.3.1 Dairy cattle

The majority of studies in dairy cattle have been conducted using either the daughter design (Figure 1) or the granddaughter design (Figure 2). In the daughter design, sires are genotyped to identify heterozygous markers spread across the genome. The daughters of those sires are then genotyped for the identified markers and their phenotypes are recorded. In the granddaughter design, the marker genotype is determined for the sons of heterozygous sires and the quantitative trait value measured on the daughters of the sons. Weller *et al.* (1990) found that the power to detect a QTL is influenced by the number of families included in the experiment and by the size of the individual families. For example, in the daughter design the power to detect ($P < 0.01$) a QTL effect of 0.2 SD was 0.76 for an experiment with five sires and 800 daughters each compared to 0.56 for an experiment with 20 sires and 200 daughters per sire. The power of the granddaughter design increases with the number of sons per grandsire. Similar to the daughter design, greater power was obtained if many sons of relatively few grandsires were assayed in the granddaughter design. In general, the power was greater for the

granddaughter design than for the daughter design, given the same number of marker assays. For example, an experiment with 4000 assays per marker (as above) could have 20 grandsires with 200 sons each and easily achieve power > 0.80, unless the QTL effect or the number of daughters per sire was very small. The granddaughter design generally requires half as many marker assays for the equivalent power as the daughter design. Thus, in time and materials costs for an experiment, the granddaughter design would be more economical compared to the daughter design.

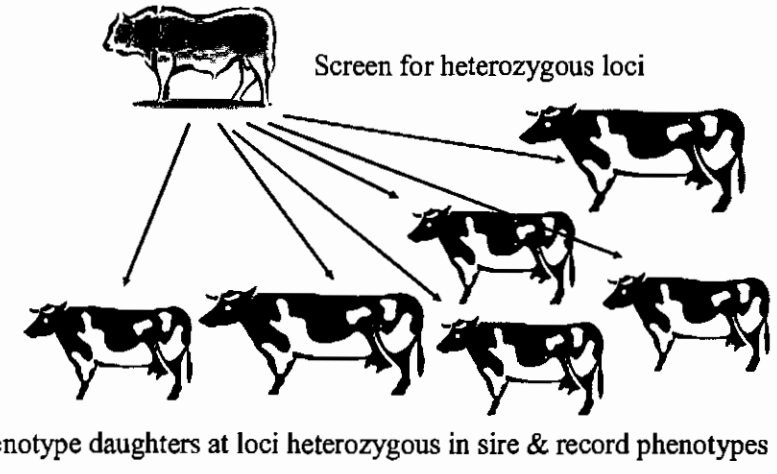

Figure 1: Schematic representation of the daughter design.

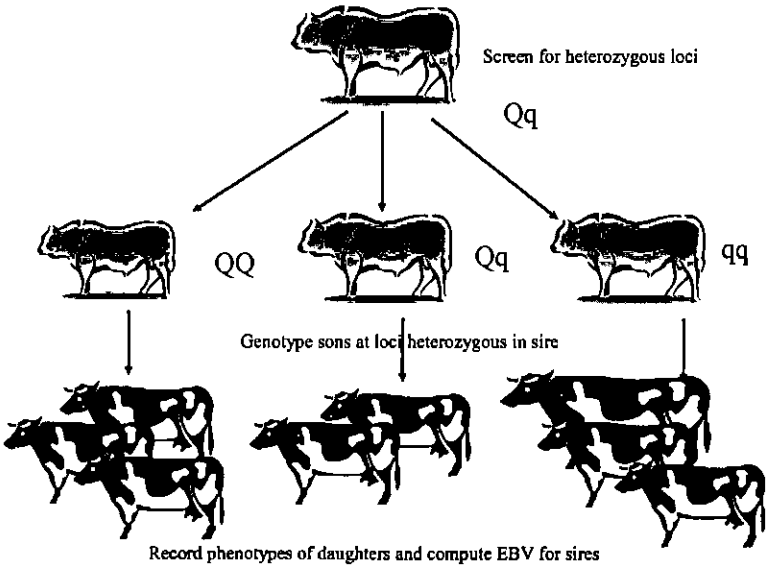

Figure 2: Schematic representation of the granddaughter design.

The daughter design may be most practical in countries where breeding is by artificial insemination (AI), the number of tested bulls used each year is low, herds are large and the distance between herds is very small. The granddaughter design is more practical in countries where selected sires have many progeny-tested sons. For example, as of 1990, the U.S. Holstein population had 46 sires with more than 50 sons born between 1975 and 1982 whose genetic evaluations are based on at least 20 daughters. These sires had a mean of 198 sons per sire and the sons had a mean of 93 daughters per son (Weller *et al.*, 1990). The granddaughter design has an advantage in that the analyses can be carried out by using samples (blood/semen) collected from sires in artificial insemination centres rather than by locating cows on farms (Weller *et al.*, 1990).

CHAPTER 1

The first studies to detect a QTL carried out in dairy cattle used the granddaughter design and focused on milk production (Georges *et al.*, 1995). Several more studies have been conducted in different populations of dairy cattle. See Table 1 for the results and references.

Table 1: QTL screened for traits of interest in dairy cattle breeds

Trait	Experimental designs	Region of QTL	References
Milk production, yield and Protein %	Granddaughter design on *Bos taurus*	BTA1, 6,9.10 7&20	Georges *et al.*, 1995
Milk fat & protein yield	Grand daughter design On Holstein cattle	BTA 6, 14, 20 & 26	Zhang *et al.*, 1998
Fat %	Daughter design of half sib families from Israeli Holstein cattle	BTA 14	Heyen *et al.*, 1999
Milk composition And somatic cell score	Grand daughter design on eight Holstein cattle	BTA 3, 6, 7, 14, 21 &29	Rodriguez-Zas *et al.*, 2002
Fat %	Granddaughter design on Holstein cattle	BTA 3	Plante *et* al., 2001
Milk production	Granddaughter & Daughter design	BTA 3, 6, 9, 14, 20 & 23	Grisart *et* al., 2002
Protein %	Granddaughter design	BTA 14	Looft *et al.*, 2001
Growth hormone receptor		BTA 20	Kim *et al.*, 2002 Blott *et al*., 2003
Fat yield		BTA 26	Gautier *et al.*, 2005

Rodriguez-Zas *et al.* (2002) studied eight Holstein families to implement both interval and composite interval mapping using milk and composite production and somatic cell score indicators in an out bred population. Within-family mapping identified QTL for protein yield that were found at 32 cM on BTA3 in family five and between 26 and 36 cM on BTA6 in family six. Three QTLs were found that affected fat yield: at 74 cM on BTA3 in family eight, at 3 cM on BTA14 in family four, and at 14 cM on BTA29 in family seven. Two QTLs associated with somatic cell score were detected on BTA21, one at 33 cM in family one and another at 84 cM in family three. Two QTLs for milk

yield were also detected at 116 cM on BTA7 in family three and at 0 cM on BTA29 in family seven. According to Rodriguez-Zas *et al.* (2002), these results indicate the possibility of one QTL with pleiotrophic effects or multiple QTLs within a marker interval. Several other studies confirm that BTA6 carries multiple QTLs, at least one of which may affect multiple traits (i.e. a pleiotrophic QTL) including milk, protein, fat, yields and protein percentage and fat percentage. (Figure 3).

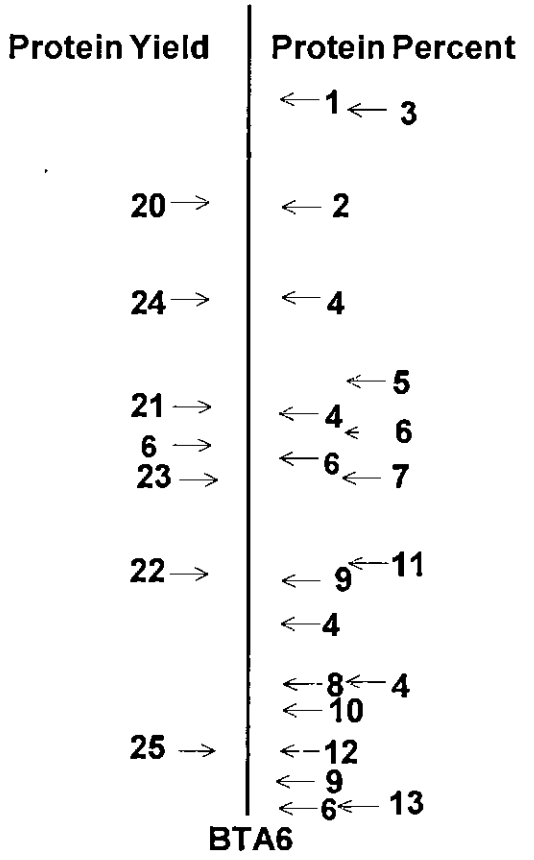

Figure 3: Schematic diagram of bovine chromosome (BTA) 6 showing locations of QTLs affecting milk protein yield and milk protein percentage: 1=Zhang *et al.* (1998), 2 = Nadesalingam *et al.* (2001), 3 = Spelman *et al.* (1996), 4 = Mosig *et al.* (2001), 5 = Freyer *et al.* (2002), 6 = Ron *et al.* (2001), 7 = Ashwell *et al.* (2002), 8 = Ashwell *et al.* (2001), 9 = Velmala *et al.* (1999), 10 = Maki-Tanila *et al.* (1998), 11 = Viitala *et al.* (2003), 12 = Ashwell and VanTassell (1999), 13 = Boichard *et al.* (2003), 20 = Ashwell *et al.* (2004), 21 = Cohen *et al.* (2002), 22 = Freyer *et al.*, 2003, 23 = Kuhn *et al.* (1999), 24 = Rodríguez-Zas *et al.* (2002), and 25 = Weiner *et al.* (2000). The arrows indicate the cM.

Another example is mastitis that has been identified as one of the sources that influence economic losses in the dairy industry by reducing the milk yield and causing deterioration in the milk quality. A QTL that affects the incidence of mastitis was identified on chromosome six in the region of the QTL for milk production. High milk production may influence the increase in susceptibility of mastitis and additional QTL for clinical mastitis were found on BTA 3, 4, 14, and 27 (Klungland *et al.*, 2001; Schulman *et al.*, 2002).

1.3.2 Beef cattle

Crossbreeding between *Bos taurus* and *Bos indicus* has been practiced in subtropical regions due to the benefits of heterosis and breed complementary for reproduction, growth, and carcass traits. Similar crosses have been used in experiments to detect QTL for economic important traits to improve production (Keele *et al.*, 1999; Stone *et al.*, 1999; Casas *et al.*, 2000). Many of these QTL detection studies have used either backcrosses whereby F_1 individuals are interbred (Figure 4) or F_2 designs whereby F_1 individuals are mated to one of the parental populations (Figure 5). The major advantage of the F_2 design over that of the backcross is that three genotypes are present at every QTL in the mapping population. Backcross populations have only two possible genotypes at a QTL. Thus, the F_2 design allows for the estimation of the dominance effect on a QTL where the backcross design does not (Stone *et al.*, 1999). The analysis of either backcross or F_2 families is highly efficient where alternative alleles have been fixed or the allele frequencies are very different in the two breeds/lines. A discussion on recent studies using both backcross and F_2 design in beef production will be discussed.

CHAPTER 1

Kim *et al.* (2003) used both backcross and F_2 design for cattle that descended from Angus and Brahman grandparents to detect the QTL responsible for growth and carcass fatness. Four hundred and seventeen genetic markers, mainly microsatellites, were used to produce a sex-average map of the 29 autosomes spanning 2,642.5 Kosambi cM. A total of 35 QTLs was detected; five QTLs with significant effect that influenced birth and post-weaning growth traits and 30 suggestive QTLs were found on 19 chromosomes under the line-cross and random infinite alleles models. One QTL was found on BTA 1 for yearling weight under the line-cross model and positioned at 68 cM. Four QTLs affecting growth were detected with a significant evidence of linkage under the random infinite alleles model; two QTLs were in the approximate region of BTA 6 and the distal region of BTA 2 for birth weight, a QTL for yearling weight on BTA 5; and a QTL for hot carcass weight on BTA 23 and located at 14 cM. None of these QTLs (except the QTL for yearling weight on BTA 5) were detected under the random infinite alleles model were found through line-cross analyses, suggesting segregation of an alternative allele within one or both of the parental breeds.

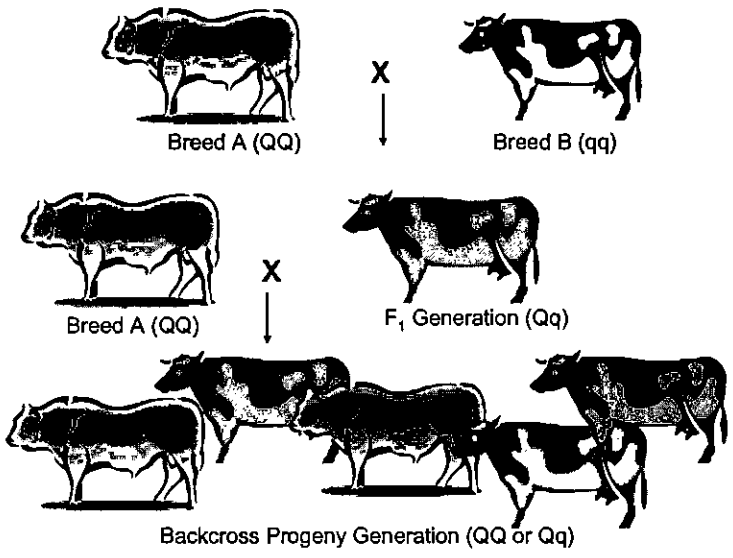

Figure 4: Schematic representation using backcross design.

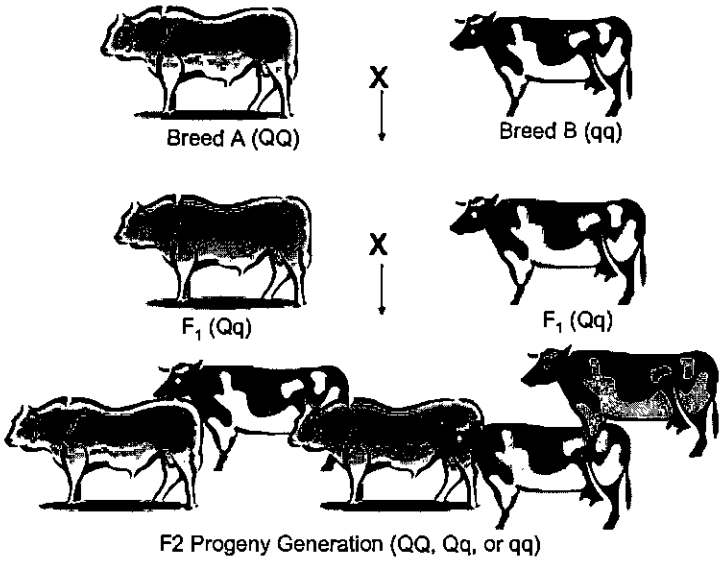

Figure 5: Schematic representation using F_2 design.

CHAPTER 1

Keele *et al.* (1999) used a backcross design of one Brahman x Hereford bull mated to *Bos taurus* cows and 196 microsatellite markers spanning all 29 autosomal bovine chromosomes to identify a QTL for meat tenderness. Tenderness was measured by the Warner-Bratzler Shear force on steaks aged either two or 14 days post-mortem. The QTL peak was located 28 cM from the most centromeric marker on BTA15. The QTL interacted significantly with the slaughter group. The difference in the shear force of steaks aged 14 day post-mortem between progeny with the Brahman paternally inherited allele versus those with the Hereford was 1.19 phenotypic standard deviations for one slaughter group and was not significant for three other slaughter groups.

Stone *et al.* (1999) also identified QTLs that affected carcass and growth traits by genotyping 238 microsatellites on selected backcross progeny from a *Bos indicus* x *Bos taurus* sire mated to *Bos taurus* cows. The genome screens were conducted with markers at 10 to 20 cM intervals on animals selected to represent the extreme values for phenotype as an approach for obtaining the approximate map location of a QTL with a reduced amount of genotyping (Lander & Botstein, 1989). Backcross progeny inheriting the *Bos indicus* allele on BTA5 had a significantly lower dressing percentage and a higher proportion of bone in the wholesale rib cut compared to those inheriting the *Bos taurus* allele. Significant evidence of a QTL for increasing the retail product yield and component traits on BTA2 was mapped at approximately 35 cM on BTA13. The observed QTL effect on BTA2 and BTA13 generally affected the same traits in the same direction. The QTL at approximately 19 cM on BTA14 indicated that the Brahman alleles had a larger longissimus muscle area in comparison to the Hereford alleles. The QTL on BTA1 showed that Brahman alleles increase birth weight in relation to Hereford

alleles. Additional putative QTLs with suggestive effects were detected on BTA 18 and BTA 26. The effect of a Brahman allele on BTA 18 increased rib-fat and decreased the retail product yield. On BTA 26 the Brahman alleles increased rib muscling and decreased rib fat and fat yield.

There are several studies using the genome scan approach with backcross and F_2 designs employing different *Bos taurus* breeds to identify QTLs that have effects on economic traits such as body composition traits, carcass yield and quality, and growth (Casas *et al.*, 2000, 2003, 2004; MacNeil & Grosz, 2002).

Casas *et al.* (2000) conducted a genome scan with 150 markers to identify additional QTL for economically important traits in two half-sib families using the backcross design with Belgian Blue x MARC III (¼ Angus, ¼ Hereford, ¼ Red Poll, ¼ Pinzgauer) and Piedmontese x Angus sires segregating an inactive copy of myostatin. In the family with the Belgian Blue inheritance (n = 246), a significant QTL was identified on BTA6 between 48 and 51 cM for birth and yearling weight and this also suggested a co-located QTL for longissimus muscle area and hot carcass weight. A QTL for marbling was found at 21 cM on BTA17 and at 60 cM on BTA27. In the family with Piedmonts inheritance (n = 209), QTL for fat depth, retail product yield and USDA yield grade were suggested between 62 and 72 cM on BTA5 and between 56 and 65 cM on BTA29 for the Warner-Bratzler shear force at three and 14 days post-mortem.

MacNeil & Grosz (2002) identified a QTL that affect carcass traits by genotyping two paternal half-sib families of backcross progenies produced from a Hereford x composite gene combination (CGC = ½ Red Angus, ¼ Charolais, ¼ Tarentaise) bulls mated with both Hereford and CGC dams. A genome scan was conducted using 229

microsatellite markers spanning 2,413 cM on 29 bovine autosomes. The result showed a significant QTL effect on age constant live weight located at 52 cM on BTA 17 in both families. Similarly in both families, progeny receiving the allele from Line 1 Hereford were approximately 24kg lighter at harvest than their contemporaries that received the allele from CGC. A QTL for marbling was located at 122 cM on BTA 2 and the effect was also similar in both families with progeny that received the allele from Line 1 having approximately 0.6 score units less marbling at harvest than their contemporaries that received the allele from CGC.

Half-sib families of purebred Wagyu were studied using the daughter design to detect QTL (Mizoshita *et al.*, 2004). Eight QTLs for growth and carcass traits were identified using the progeny of a half-sib family of a Japanese black (Wagyu) steer. A genome scan was conducted using 342 microsatellite markers by spanning 2,664 cM of 29 bovine autosomes. The longissimus muscle area and marbling were positively affected by QTLs located on BTA4 at 52 to 67 cM. A QTL for carcass yield was found on BTA5 in the region of 45 to 54 cM. Five QTLs related to growth, including slaughter and carcass weights, were located on BTA14 and were also positively affected by the same region of the haplotype of BTA14 (29-51 cM).

Alexander *et al.* (2007) conducted a genome scan using 328 F_2 progeny in Wagyu x Limousin F_2 progeny derived from eight Wagyu founder bulls and identified QTL regions on five chromosomes involved in lipid metabolism and tenderness. A QTL with multi-faceted effects on conjugated linoleic acid and marbling was observed towards the centromere of BTA2. A QTL that affected the amount of mono-unsaturated fat per 100 grams of dry tissue was located at 125 cM on BTA7. Another QTL affecting the

percentage kidney-pelvic-heart fat was found at 40 cM on BTA7. Also detected were QTLs influencing myofibrils on BTA5, QTLs for fat thickness on BTA1, and QTLs for Warner-Bratzler shear force on BTA 10.

The studies described above have primarily dealt with cattle. However, similar approaches have been used successfully in other farm animal species: swine (e.g., Andersson *et al.*, 1994, Knott *et al.*, 1998; de Koning *et al.*, 1999), sheep (Charlier *et al.*, 2001, Cockett *et al.*, 2001), and poultry (Groenen *et al.*, 1997; Ikeobi *et al.*, 2002; Tuiskula-Haavisto *et al.*, 2004).

1.4 Methods used to analyze genome scan experiments

A variety of statistical methods to analyze or map QTL in outbred populations were developed and implemented to improve livestock production. Hoeschele *et al.* (1997) classified these methods into five groups, group 1: the linear regression, the least squares; group 2: likelihood analysis; group 3: squared difference regression; group 4: residual maximum likelihood and group 5: exact Bayesian linkage analysis.

Group 1 includes linear regression using single or multiple linked markers. The theoretical basis for regression analyses given by Zeng (1993) indicates that the partial regression coefficient of the phenotype on a marker in multiple regressions depends only on those QTLs that are located in the interval bracketed by the two neighboring markers and is independent of QTL located in other intervals. A least squares (LS) analysis for QTLs in half-sib populations was presented by Haley *et al.* (1994), Spelman *et al.* (1996) and Uimari *et al.* (1996b). Haley *et al.* (1994) indicated that the least squares method is suitable for crosses where the lines may be segregating at marker loci but can be assumed

to be fixed for alternative alleles at the major QTL affecting the traits under analysis, for example crosses between divergent selection line or breeds with different selection background. The use of multiple markers in a linkage group simultaneously increases the test statistics and, thus, the detection of the QTL compared to the use of a single marker or markers flanking an interval. The method is relatively simple to apply therefore more complex models can be fitted.

Group 2 includes the maximum likelihood analysis of postulated bi-allelic QTLs using single or multiple linked markers. It has been implemented for half-sib designs in an outcross population (e.g., Weller, 1986). Mackinnon & Weller (1995) derived the likelihood for the single marker and half-sib design, while Georges *et al.* (1995) used multiple linked markers. The assumptions and models for a phenotype given the QTL genotypes are identical to the least squares model. Differences of maximum likelihood compared to least squares are: analyses typically have assumed a bi-allelic QTL and the distribution of phenotype is a mixture of normal distributions with different means corresponding to the QTL genotypes.

Group 3 includes squared difference regression which is based on analyzing the squared difference of the phenotypes of pairs of relatives on the expected proportion of identity-by-descent at a locus, originally proposed by Haseman & Elston, (1972). Gotz & Ollivier, (1994) found that this method was as powerful as least squares for a swine population. The assumptions are random mating and linkage equilibrium and use only pairs of certain types of relatives.

Group 4 includes the residual maximum likelihood based on a mixed linear model incorporating normally distributed QTL allelic effects with a covariance matrix

conditional on the observed marker data. This was developed by Grignola *et al.* (1996a, b, 1997) in half-sib designs for QTL mapping.

Group 5 includes exact Bayesian linkage analysis using single or multiple linked markers and fitting bi-allelic or infinite-alleles QTLs (Uimari *et al.* 1996a). This method takes full account of the uncertainty associated with all unknowns in the QTL mapping problem, including the multi-locus marker-QTL genotypes and the number of QTLs on the chromosome under study (Uimari *et al.* 1996a). It allows for different models of QTL variation and also provides exact small sample posterior variances and co-variances of parameters, exact confidence intervals, posterior distributions of parameters of interest, posterior probabilities of models and it relies on Markov chain Monte Carlo algorithms. Bayesian analysis was implemented via the Markov chain Monte Carlo algorithms for QTL mapping in animal genetics (Thaller & Hoeschele, 1996b for single markers; Uimari *et al.* 1996a for multiple linked markers).

The implementation of any of the above depends on data structure, computational constraints and expertise, and any distributional assumptions an investigator is willing to make. However, the least squares analysis allowing for performing data permutation to determine genome-wide significance thresholds should be a first step in the analysis of each experiment. Moreover, the standard errors parameters and confidence intervals must be obtained via Monte Carlo and bootstrap sampling techniques. QTL Express (Seaton *et al.*, 2002) was developed to make these QTL mapping tools available to the wider scientific community via a user-friendly web-based user interface.[7]

[7] http://qtl.cap.ed.ac.uk

1.5 Examples of gene discovery in QTL studies

Genetic data on gene discovery is available to improve the selection in breeding schemes through the use of QTL information. Information from genetic markers can be implemented in breeding programs through marker assisted selection or marker assisted introgresion. Genes that affect the traits of interest may be discovered in the QTLs and their effects are estimated to refine the selection criteria and to increase the accuracy of the selection to improve animal production (Bovenhuis *et al.*, 1997). Examples of muscular hypertrophy and growth hormone will be discussed,

Muscular hypertrophy was first documented by Culley (1807) and has been the subject of considerable study in cattle populations (Arthur, 1995; Bellinge *et al.*, 2005; Hanset, 1981). This phenotype was mapped by Charlier *et al.* (1995) on BTA2 within 2 cM of the marker loci in a backcross family. Myostatin was identified as the gene responsible for producing double-muscling in cattle (McPherron *et al.*, 1997). Thus myostatin is considered as a major gene because of its great effect in the expression of growth and carcass traits (Arthur, 1995). It is located at the centromeric end of BTA2 (McPherron *et al.*, 1997; Smith *et al.*, 1997; Grobet *et al.*, 1997). Different myostatin mutations had been identified as segregating in different breeds of cattle. Casas *et al.* (2000) in an experiment on Belgian Blue **x** MARC III and Piedmontese **x** Angus cattle, also suggested interactions between myostatin and a QTL on BTA5 affecting the Warner Bratzler shear force at 14 days post-mortem and between myostatin and a QTL on BTA14 affecting fat depth.

Short *et al.* (2002) studied the pleiotropic effects of genes controlling the muscularity in Hereford, Limousin, and Piedmontese F_2 crossbred calves. The results

confirmed that a large increase of muscle through hyperplasia and a decrease in fat was achieved by using the myostatin allele from the Piedmontese. These results also indicated that the effect of this gene is primarily additive in many traits. It also has some form of non-additive gene action. A significant QTL affecting fat and protein percentages as well as milk yield near the centromere of BTA14 were found by several researchers (Coppieters *et al.*, 1998; Riquet *et al.*, 1999; Heyen *et al.*, 1999; Ashwell *et al.*, 2001; Looft *et al.*, 2001). Grisart *et al.* (2002) constructed a corresponding bacterial artificial chromosome contig and identified a nonconservative missense mutation in the positional candidate gene AcylCoA: diacylglycerol acyltransferase (DGAT1). Winter *et al.* (2001) describe the association of a lysine /alanine (Ala) polymorphism (K232A) in DGTAT1 with milk fat content and postulated that this mutation was responsible for a variation in milk fat content. The effect of Lys/Ala polymorphism on milk composition was validated in New Zealand Jersey cattle (Spelman *et al.*, 2002), Holstein-Friesian and Ayrshire, together with Israeli Holstein cattle (Weller *et al.*, 2003) and in German Fleckvieh and Holstein (Thaller *et al.*, 2003a). Grisart *et al.* (2004) presented genetic and functional data that confirmed the causality of the DGAT1 K232A mutation.

Growth hormone (GH) has been used as a functional and positional candidate gene in association studies in several species, including cattle, for its role in growth, lactation, and association with many other traits (Taylor *et al.*, 1998; Vukasinovic *et al.*, 1999; Barendse *et al.*, 2006). Taylor *et al.* (1998) presented a positional candidate gene analysis using GH1 as a model for QTL effects on growth and carcass composition localized to BTA19 by interval analysis in a cross among *Bos taurus* and *Bos indicus*.

The growth hormone receptor gene has also been associated with the QTL for milk yield on bovine chromosome 20. Arranz *et al.* (1998) conducted the fine mapping of QTLs for milk yield and proposed a growth hormone receptor as a positional candidate gene. The QTLs that affected milk yield were also confirmed on BTA 20 (Olsen *et al.*, 2002). The analysis of Blott *et al.* (2003) revealed a substitution of tyrosine (Tyr) for phenylalanine (Phe) in the trans-membrane domain of the bovine GH receptor protein that is associated with a strong effect on milk yield.

1.6 The use of QTL mapping results for marker assisted selection

One reason for conducting a QTL study is to be able to implement a breeding scheme that increases genetic progress through marker assisted selection (MAS). From theoretical and simulation studies Abdel-Azin & Freeman, (2002) confirmed that the application of MAS has the potential to increase the rate of genetic gain especially if the traditional selection is compromised due to important phenotypes being expressed late in life (e.g. fertility and longevity), after slaughter (e.g., carcass yield and meat quality), sex-limited (e.g. milk production and semen traits), or are difficult or expensive to measure (e.g. disease resistance and feed intake). Marker assisted selection may also have considerable value in overcoming antagonisms where unfavourable genetic correlations exist between traits; for example between milk production and fertility (Garnier *et al.*, 2003). Another area where expectations of MAS are high is in the selection for functional traits (Elsen, 2003). In order to use a detected QTL through MAS, an accurate estimation of the QTL location and effect is required (Spelman & Garrick, 1998). Marker assisted selection does not replace the traditional breeding value estimation but

provides additional information to enhance the accuracy of selection. To implement MAS strategies requires genotyping and analysis in a proper breeding population.

The degree to which MAS will be successful relies on the level of precision at which the QTL has been identified (deKoning *et al.*, 2003). These levels include firstly, functional mutations, secondly, MAS can be applied using linkage disequilibrium (LD) markers (loci that are in population-wide linkage disequilibrium with the functional mutation) and, thirdly, linkage equilibrium (LE) markers (loci that are in population-wide linkage equilibrium with the functional mutation) (deKoning *et al.*, 2003). The LE markers can be detected on a genome-wide basis by using breed crosses or the analysis of large half-sib families within the breed, and such a genome scan requires only sparse marker maps (15-50 cM intervals depending on marker in formativeness and genotyping cost) to detect most QTL with moderate to large effects (Darvasi *et al.*, 1993).

The LD markers are close to the functional mutation for sufficient population-wide LD between the marker and the QTL to exist (within 1-5 cM, depending on population structure and background), and they can be identified using candidate genes or through fine mapping approaches (Andersson, 2001; deKoning *et al.*, 2003). The functional mutations are the most difficult to detect because causality is difficult to prove with the results of the limited number of examples available, except for single-gene traits (Andersson, 2001).

The implementation of MAS in dairy cattle has been evaluated and has shown to lead to an increased rate of genetic gain compared with that in beef production (Brascamp *et al.*, 1993). Two categories of MAS schemes have been evaluated (Spelman & Garrick, 1998): Within-family MAS involves selection decisions made on conventional EBV and

QTL information used within-family. Alternatively, BLUP-based selection involves the use of mixed models that incorporate effects for an individual QTL allele and decisions are made depending on EBVs that combine QTL and polygenic components. The MAS schemes that use within-family information from QTLs to pre-select bulls for progeny testing are a practical application of QTL results in the short-term. Spelman & Garrick, (1998) applied two within-family schemes to the top-down and bottom-up MAS schemes. The top-down scheme (Kishi *et al.*, 1990) identifies sires that are heterozygous for the locus based on the granddaughter design with the use of QTL information in the pre-selection of grandsons entering progeny testing. The bottom-up scheme (Mackinnon & Georges, 1997) identifies sires heterozygous for a QTL based on the daughter design and only sons that have the preferred genotype enter progeny testing. Both methods have been shown to increase genetic gain, especially when multiple ovulation embryo transfer technology is used on bull dams (Spelman & Garrick, 1998).

The LD markers near the prolactin gene and segregating in one prominent Holstein sire family have been used for the pre-selection for young bulls (Cowan *et al.*, 1997). In addition, LE markers have been used in several dairy breeding programmes, including the pre-selection of young bulls in the USA based on QTL studies reported by Georges *et al.* (1995) and Zhang *et al.* (1998); in New Zealand (Spelman *et al.*, 2002); and the Netherlands (Spelman *et al.*, 1996; Arranz *et al.*, 1998; Coppieters *et al.*, 1998).

1.7 The Use of QTL mapping results for introgression

Marker assisted introgression is a crossbreeding approach which aims to migrate genes from a donor breed or line into a recipient line through backcrossing (Figure 6).

An F_1 is created followed by a number of back-crossing generations and completed with an intercross to fix the introgressed gene (Soller & Plokin-Hazan, 1977; Koudanda *et al.*, 2000). This introduction of a favourable gene into a commercial population can be effected quickly, precisely and cost-effectively if the desired gene can be identified with markers. Introgression can also be implemented if the targeted gene has been identified. For example, indigenous cattle often have resistance to endemic diseases. If the resistance is at least in part genetic and loci containing the controlling genes can be identified, it is possible to transfer those genes from the resistant indigenous breed to the breed that has been selected for high production by backcrossing (Hospital and Charcosset, 1997). The following studies were conducted through marker assisted introgression for trypanosomosis.

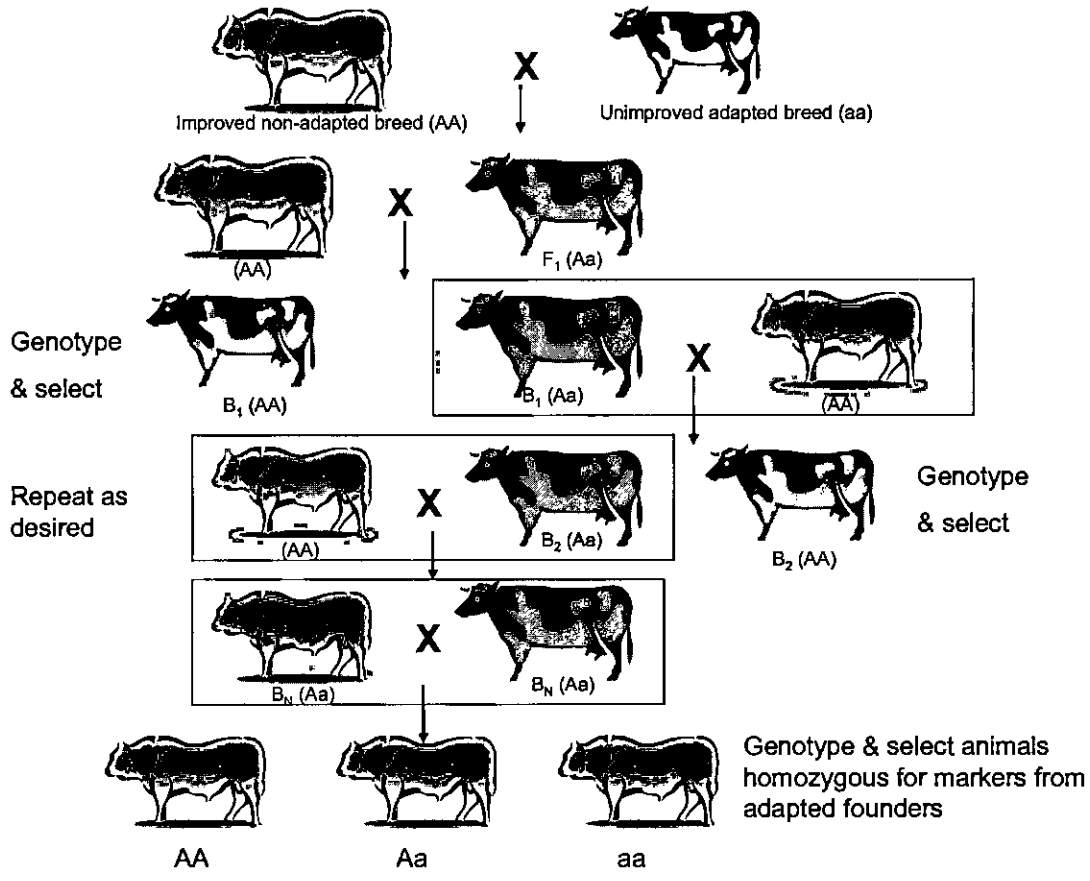

Figure 6: Schematic representation of a marker assisted introgression strategy using a backcrossing design.

Trypanosomosis is regarded as one of the greatest constraints to efficient livestock production in the sub-humid and non-forested portions of the humid zone of Africa. Thus, having genetically resistant and highly productive breeds of livestock would be of great value. Koudande *et al.* (2005) reported the first successful application of marker assisted introgression wherein they introgressed genes for trypanotolerance from resistant mice into susceptible mice. Three trypanotolerant QTLs were identified located on chromosome MMU1, MMU5, and MMU17 in a resistant strain, C57BL/6

(estimated time survival of 68.8 days). These loci were introgressed into a susceptible recipient mouse strain, A/J (estimated time survival 29.7 days). The mice were then subjected to trypanosome congolense environment. The results indicated that the introgression of a trypanotolerant QTL into the susceptible recipient background genotype resulted in greater survival times in the trypanosome congolense environment. Mice with an A/J background that carried the resistant QTL on MMU1, MMU5, and MMU17 survived for an average of 57.9, 49.5, and 46.8 days, respectively. These results indicate that all three donors QTL regions have an impact on survival after parasite infection and demonstrate a potentially valuable application of marker assisted introgression.

N`Dama and West African Shorthorn cattle show the ability to survive trypanosome infection (Trail *et al.,* 1989). Increased trypanotolerance in N`Dama and other Shorthorn cattle from Africa was also confirmed. Thus, Hanotte *et al.* (2003) initiated a search for QTLs affecting trypanotolerance using a F_2 cross between N`Dama (resistant) and Boran (susceptible) cattle in Kenya. Several trypanotolerant QTL regions were identified. Surprisingly some of the resistant alleles came from the susceptible Boran cattle. When validated and carried forward with marker assisted introgression, this QTL identification may provide an opportunity to improve disease resistance and enhance the current status of beef production in the region.

This extensive literature overview indicated the applicability of several techniques for the use of genomics for improving livestock production of importance for a research study

under South African conditions. Therefore the goal and objectives of this study is based on QTLs and the application in two case studies.

1.8 Goal and objectives

Broadly, the goal of the study was to examine ways in which molecular genetics can be used to enhance the performance and sustainability of beef cattle production. To achieve this goal, two case studies were conducted which could be implemented by the South African breeders to improve beef production. In the first case study, we determined quantitative trait loci (QTL) for beef quality as reference points for the genetic control of phenotypic expression. The locality of the QTL that have an effect on beef quality in cattle breeds can be used to improve beef quality in otherwise deficient traits. In the second case study, we investigate the migration of QTLs from one breed to another where a QTL for a particular productive attribute could be introgressed into a locally adapted breed.

Therefore, the specific objectives of the study were: 1) to map QTLs for the fatty acid composition as a measure of beef quality (Chapter 2); 2) the introgression of QTLs of interest using a backcrossing experiment (Chapter 3); 3) to design a QTL detection experiment that will suit South African breeds (Chapter 4).

Chapter 2

Case Study 1

Mapping quantitative trait loci for fatty acid composition in beef cattle

GENETIKA
GENETICS

CHAPTER 2

2.1 Introduction

Meat contains a mixture of saturated, mono-unsaturated and poly-unsaturated fatty acids. Fatty acid composition in beef meat has received considerable interest in view of its implications in human health and meat quality characteristics (Wood *et al.*, 2004). A high level of saturated fatty acid is associated with increased serum low-density lipoprotein cholesterol concentrations and is a risk factor for coronary heart disease (Katan *et al.*, 1994, 2000), while unsaturated fats (mono-unsaturated and poly-unsaturated) are beneficial when consumed in moderation.

In addition to the human health implications of fatty acid composition, beef with the most desirable flavour has a lower percentage of saturated fatty acid and poly-unsaturated fatty acids and a higher percentage of mono-unsaturated fatty acids in the muscle fat (Melton *et al.*, 1982). The Wagyu beef breed is known for its extensive marbling and comparatively less external fat. It has also been found to have a greater absolute level of mono-unsaturated fatty acids and a greater level of mono-unsaturated fatty acids relative to saturated fatty acids compared to other breeds (Sturdivant *et al.*, 1992; May *et al.*, 1993; Boylston *et al.*, 1995; Xie *et al.*, 1996; Yang *et al.*, 1999; Mir *et al.*, 2000).

Experimental comparisons of Limousin (lean) and Wagyu (fat) germplasm indicate breed differences with respect to fat deposition (Mir *et al.*, 2002; Pitchford *et al.*, 2002). These differences provide an opportunity to identify QTLs that have an effect on carcass quality and fatty acid composition, to enhance the palatability of meat and to minimize the human health implications such as coronary heart diseases. Alexander *et al.* (2007) performed a genome scan on 328 F_2 progeny in a Wagyu x Limousin cross and

identified seven new QTL regions on five chromosomes involved in lipid metabolism and tenderness. The objective of this study was to continue on the work of Alexander *et al.* (2007) by searching for QTL affecting the relative amounts of saturated, mono-unsaturated and poly-unsaturated fatty acids.

2.2 Materials and Methods

Wagyu-Limousin bulls and females were purchased from Washington State University in October 1999. Eight Wagyu bulls were mated to 108 Limousin females to produce 121 F_1 females over a three-year period, and three of these bulls sired the six F_1 bulls used. The F_1s were *inter se* mated randomly, except for the fact that mating of known relatives was avoided, to produce 328 F_2 progeny that were born in 2000-2003. All animals were placed in a controlled environment at Fort Keogh Research Institute in Mile City, Montana. Calves were reared by their dams without creep feed until weaning at approximately 175 d of age (SD = 14 d). Each year, before slaughtering and within sex, calves were randomly assigned to a slaughter date in groups of eight to 11 head per day. After weaning, the calves were managed in a two-phase system: a growing phase with diet composition of 50 to 54% DM, 14.4 to 15.6% CP, and 1.06 to 1.18 Mcal/kg NEg and a finishing phase with diet composition 68 to 70% DM, 11.6 to 13.4% CP, and 1.26 to 1.31 Mcal/kg NEg. The finishing diet was fed for a minimum of 113 days until the calves were slaughtered. Within year and sex, groups of calves were slaughtered at two to three week intervals.

Thus, the final group slaughtered each year had been fed the finishing diet for at least 210 days. Calves, aged from 450 to 641 days (average 561 days), were transported

to the abattoir on the afternoon before harvest, held overnight with water and without feed, and slaughtered the next morning using standard industry procedures. Two days postmortem the whole rib [107 North American Meat Processors Association (2002)] was removed from each carcass, vacuum packaged, and aged for 14 days at 2°C. After aging, a three-rib section and four 2.54 cm thick steaks were cut from the posterior end of the wholesale rib, then individually vacuum packaged, frozen at -20°C, and held for further analyses. The data for this study were obtained from Fort Keogh Research laboratory with the help of Dr MacNeil.

2.2.1 Fatty acid analysis

Two steaks were transported to the University of Wyoming by the USDA officials for the determination of fatty acid composition as described by Rule *et al.* (2002). Briefly, the entire core of the longissimus dorsi was sampled (i.e., devoid of trim fat and extraneous muscles) by dicing the muscle into 1.0-cm cubes while the muscle was semi-frozen and weighed into pre-weighed plastic cups with perforated lids. All samples were freeze-dried (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY) and then grounded and homogenized using an electric grinder. Samples were packed into 20-mL plastic vials and sealed to inhibit exposure to air, and then stored at -80° C until analyzed for fatty acids and cholesterol, which occurred within two to four weeks of freeze drying. Approximately 150 mg of dried muscle were weighed in duplicate into 16 mm × 125 mm screw-capped tubes that contained 1.0 mg of tridecanoic acid as the internal standard. The samples were then subjected to direct saponification as described by Rule *et al.* (2002). Samples were reacted with 4.0 mL of 1.18 M KOH in ethanol at 90° C by

vortex-mixing (two to three times per minute for three seconds) until the sample were completely dissolved, except for insoluble collagen that appeared as a white powder in suspension upon mixing. The samples were cooled for 45 minutes, 2.0 mL water were added, and cholesterol extracted with 2.0 mL of hexane that contained 0.1 mg/mL of stigmasterol as the internal standard for the cholesterol assay; the hexane phase was transferred to GLC vials and sealed. One milliliter of concentrated HCl was added to the original tubes and fatty acids extracted in 2.0 mL of hexane for fatty acid methyl ester (FAME) preparation, which was carried out according to Rule *et al.* (2002) using methanolic HCl as a catalyst. The analysis of CLA was hampered by the use of acid catalysts because of the partial geometric isomerization of cis-9, trans-11 CLA to trans-9, trans-11 CLA (Yamasake *et al.*, 1999) and the degradation of CLA to allylic methoxy artifacts (Kramer *et al.*, 1997). However, Murrieta *et al.* (2003) demonstrated that dietary treatment effects on CLA in ovine muscle were maintained when acid catalysts were used for FAME preparation, despite up to 20% loss of cis-9, trans-11 CLA. The preparation of FAME from non-esterified free fatty acids (NEFA) requires the use of the acid catalyst because alkaline catalysts do not react with NEFA to form fatty acid methyl esters (Christie, 1982). For the current study, freeze dried muscle samples were chosen at random from approximately 5% of the samples for FAME preparation using methanolic KOH, which does not affect CLA proportions. No loss of CLA in the samples analyzed was observed (data not shown). Generally, either minimal or no loss of CLA in samples containing low concentrations (about 0.5 mg per 100 mg of total fatty acids) of this fatty acid was observed. The cholesterol concentration was determined using GLC as described by Rule *et al.* (1997), and fatty acids were analyzed by GLC as described by

Murrieta *et al.* (2003). In comparison to Alexander *et al.* (2007) who reported on the absolute quantities of various fatty acids, the study presented here provides results pertaining to the relative amounts of saturated, mono-unsaturated, and poly-unsaturated fatty acids.

2.2.2 Genotyping

DNA was extracted by the Fort Keogh researchers from the semen of sires of the F_1 bulls and from the white blood cells of the F_1 bulls, F1 females, and F_2 calves using standard protocols (Ausubel *et al.*, 1994). Touchdown PCR was performed in MJ Research (Waltham, MA, USA) thermocyclers as described by the United States Department of Africulture.[10] All genotypes were collected on a LiCor 4200 DNA Analysis System (Lincoln, NE, USA). Genotypes were scored by and compared to genotypes obtained by Fort Keogh researchers. PCR was repeated for discrepancies that could not be resolved. All the lab work was done by the USDA-ARS researchers. Anomalous genotypes were detected using GenoProb (Thallman *et al.*, 2001a, b). Genetic linkage maps were constructed using CRI-MAP (Green *et al.*, 1990)[11]. The CRI-MAP program was run by the researcher at the For Keogh laboratory. Initially, 156 markers covering the 29 bovine autosomes were chosen from the Meat Reasearch Centre (MARC)[12] based on marker position, suitability for ease of scoring, and number of alleles (personal communication). The microsatellite markers were optimized by the Fort Keogh researchers following the MARC standards procedure. Fine mapping was performed by

[10] http://biolibrary.licor.com/htdocs/RnP/LabPrim.jsp.
[11] http://compgen.rutgers.edu/multimap/crimap
[12] http://www.marc.usda.gov/genome/cattle/cattle.html

adding an additional 61 markers in areas of the genome exhibiting the presence of a QTL. Selected markers, their order, and mapped positions are given in Table 2.

2.2.3 Data analyses

Quantities of the various fatty acids were summarized by calculating the relative amounts of saturated, mono-unsaturated and poly-unsaturated fatty acids in 100 mg dry tissue. QTLs were identified by least squares regression analysis using the F_2 analysis option of the QTL Express program (Seaton *et al.*, 2002)[13] which generated an F-statistic profile at 1-cM intervals for each chromosome. This QTL program was run and analyzed by the student. For each chromosome, the effect of one additive QTL and one QTL with additive and dominance effects were modeled in separate analyses. The QTL effects were simultaneously adjusted for the classification effects of year and sex and the continuous linear effect of age at slaughter. Thus, given the random assignment of animals to slaughter dates and the statistical model used to analyze the data, the inference here is to an age-constant endpoint. The significance of the dominant QTL effect was determined from the ratio of the effect size to its standard error which is distributed as t ($t^2 = F$). For a newly identified QTL, the observed significance level was adjusted to a genome-wide basis following the procedure described by Cheverud (2001).

[13] http://qtl.cap.ed.ac.uk/

Table 2: Markers used with mapped locations on 29 bovine autosomes.

BTA	Marker	Mapped location	USMARC map location	BTA	Marker	Mapped location	USMARC map location
1	BMS574	0	15.428		BM2901	70.7	54.565
	CA095	7.6	23.94		TGLA345	72.4	56.245
	RM326	43.6	61.551		BM6404	73.3	57.056
	BMS527	45.9	62.454		BMS975	76.8	63.84
	BMS4030	48.7	66.915		BMS585	92.6	83.559
	DIK2121	62.4	69.787		BMS1316	111	101.972
	BMS4001	66.1	73.069		BMS2724	125.3	108978
	BM9019	70.5	75.813	**13**	TGLA23	0	8.993
	BM8246	77.7	83.834		BMS1742	10	22.997
	BMS1170	96	105.153		MNB-69	16.2	18.094
	BM1824	115.2	122.391		DIK4536	31.8	29.494
	BMS4043	137.7	142.244		DIK4317	32.4	30.05
	URB014	151.5	154.672		ILSTS059	48.2	41.728
2	TGLA44	0	3.856		DIK4523	53.3	35.529
	DIK4469	3.5	6.039		DIK1105	56	37.388
	ILSTS026	9.7	10.772		BM720	64	46.63
	TGLA431	12.7	11.912		DIK2089	66.5	49.925
	DIK1172	21.7	18.13		RM327	82.4	73.638
	CSFM050	24.9	20.541		BMS995	114.9	96.022
	TEXAN-2	35.4	25.974	**14**	BMS1678	0	14.011
	TGLA377	44.2	30.736		BL1009	19.2	48.925
	RM356	69.3	56.912		BL1029	30.2	59.439
	ILSTS050	79.5	66.611		BM4513	50	79.785
	BMS1866	96.9	88.761		BM6425	63.9	95.139
	BMS2267	115.2	108.679	**15**	BMS2533	0	13.924
	BM2113	123.4	115.437		INRA50	20.2	41.198
3	UWCA7	0	17.368		Z27076	35.3	67.759
	ILSTS096	7.3	27.411		BMS540	63	87.315
	DIK4196	13.8	31.802		BMS429	85	109.753
	BMS482	18.9	34.038	**16**	TGLA245	0	0.907
	MNB-86	23.8	40.944		DIK4030	11.5	12.022
	BL41	26.2	43.292		BMS1348	15.8	14.768
	TEXAN-9	27.8	45.415		BM311	32.1	32.509
	BMS2790	42.6	62.396		CSSM028	60.3	54.072
	BM4301	72.1	82.675		BM719	79.9	77.572
	BM7225	85.4	101.753	**17**	BMS499	0	5.499
	BMC5227	110.5	124.874		BMS1825	0.9	5.499
4	BMS1788	0	12.544		BMS1101	24.4	38.273
	BMS1237	26.5	34.379		MB008	40.6	54.709
	BM1224	51.6	54.835		TGLA170	62.6	74.83

Table 2: Continued...

BTA	Marker	Mapped location	USMARC map location	BTA	Marker	Mapped location	USMARC map location
	ILSTS062	64.6	72.311		BM1233	87.9	92.066
	IDVGA-51	83.5	89.36	**18**	IDVGA-31	0	0
	CA088	93.7	99.697		INRA121	36.9	30.153
5	BMS1095	0	0		BM7109	52.6	46.976
	BM6026	10.4	6.05		BMS2639	66.2	55.529
	BMS610	16.2	12.01		TGLA227	104	84.087
	BP1	21.2	17.287	**19**	BMS745	0	16.044
	BMS1315	36.7	33.655		BMS2142	25.1	43.319
	BMS1617	58.8	56.303		BM17132	37.9	59.202
	CSSM022	68.5	74.2		IDVGA-44	64.6	86.01
	BM315	104.4	103.169		BMS601	96.5	107.951
	BM49	121.5	117.957	**20**	MB068	0	0.63
	BM8126	128.6	126.144		BMS1282	16.3	19.144
6	ILSTS093	0	0		BMS1128	28.8	37.496
	BM1329	38.5	35.398		DIK4527	55	63.182
	BMS518	58.7	58.967		BM5004	68	71.809
	BMS470	67.4	67.401		UWCA26	79.8	77.091
	BM415	80.5	81.961	**21**	BM8115	0	0
	BP7	95	98.496		BMS1117	10.6	10.969
	BMC4203	114.4	119.048		BM3413	15.2	14.986
	BL1038	131.4	129.985		AGLA233	27.7	21.202
7	BM7160	0	0		IDVGA-45	43.5	30.887
	DIK2870	3	1.554		TGLA337	72.3	52.137
	BL1067	18.3	14.683		IDVGA-39	90.1	69.428
	BMS713	21.1	16.756		BMS2382	99.5	80.276
	BM6105	40.3	36.949	**22**	MB116	0	2.864
	TGLA303	42.2	39.337		BMS742	14	18.313
	DIK2819	48.9	47.908		BM1520	37.9	43.162
	TGLA164	56.3	55.922		BMS875	59.6	64.086
	UWCA20	62	58.552		BMS1932	67.8	75.67
	BMS2258	79.2	77.194	**23**	BM47	0	13.774
	BM9065	99.7	101.117		BM1815	14.7	24.199
	DIK4838	110.5	113.1		BM1258	18.5	28.307
	CST	122	118.12		BM7233	37.8	56.303
	BMS1979	133.4	126.24		BM1443	62.1	73.783
	BMS1247	141.7	133.809	**24**	BMS917	0	6.249
8	Z27077	0	11.342		DIK4200	13.6	18.511
	BM310	27.3	31.429		BMS2270	19.3	23.688
	BMS2072	58	66.033		BMS466	41.3	48.805
	BM711	76.5	92.729		BMS3024	61.6	65.928

Table 2: Continued...

BTA	Marker	Mapped location	USMARC map location	BTA	Marker	Mapped location	USMARC map location
	SRC221	102.9	121.221	**25**	ILSTS102	0	7.199
	BMS836	106.7	122.908		BP28	17.7	23.36
9	BMS2177	0	5.379		BMS1353	34.3	46.438
	ILSTS037	23.3	26.266		BM1864	63.1	68.418
	ILSTS013	53.8	48.736	**26**	BMS651	0	2.862
	BMS1724	78.1	80.265		MB067	21.3	22.862
	BMS2251	88.5	86.58		BM188	40.4	42.48
	BMS1967	119.9	109.287		BMS882	54.6	53.477
10	CSSM038	0	11.027	**27**	BM3507	0	0
	BMS528	11.4	24.014		BMS1001	8	5.389
	BMS861	31.2	42.986		BM6526	10.2	10.061
	BR6027	49.7	69.538		BMS2137	18.1	20.781
	BMS2641	73	87.46		CSSM036	35.9	43.002
	BMS614	83	100.13		INRA027	43.2	57.75
	BMS2614	91.3	109.393	**28**	BMC6020	0	8.046
	BL1134	99.2	111.909		BMS510	23.8	29.158
11	DIK2110	0	12.081		BM7246	33.4	47.844
	BP38	11.6	24.617		MB023	50.6	59.557
	BM7169	42.2	50.312	**29**	TGLA86	0	0.924
	BM8118	74	77.063		ILSTS057	10.6	6.808
	RM150	81.3	70.143		DIK5269	12.2	9.754
	BMS989	101.3	92.179		BMS764	15.3	11.293
	ILSTS028	116.2	112.55		MNB-144	18.8	15.501
12	BMS410	0	0		MNB-97	21.4	18.923
	DIK2916	7.9	5.29		DIK2791	25.3	19.998
	BM6108	20.9	15.119		RM044	29.8	24.481
	BM6116	26.2	20.845		BL1100	53.3	50.408
	ILSTS010	54.4	43.724		ILSTS081	74.3	69.009
	DIK4848	67.4	47.354				

2.3 Results and Discussion

The mapping results from this study are in general agreement with the USDA consensus marker map. Three traits summarized the relative amounts of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA). The results from the interval mapping of QTLs across 29 autosomes are presented in Table 3. Residual variance was relatively less for PUFA than for either SFA or MUFA resulting in more power to detect the effects on PUFA than on SFA and MUFA. The observed significance levels were adjusted to a genome-wide basis following the procedure described by Cheverud (2001). A total of six QTLs were found; on a genome-wide basis two were significant and four were suggestive of potential QTLs. These QTLs were located on five chromosomes.

On BTA2, a QTL was found that had additive effects on SFA (4 cM, $F = 10.07$, $P = 0.04$), MUFA (4 cM, $F = 23.62$, $P < 0.01$) and PUFA (11 cM, $F = 20.74$, $P < 0.01$) (Figure 7). This QTL was situated between markers DIK4469 and ILISTS026. By replacing an allele from Limousin with one from Wagyu the percentages of SFA and PUFA were decreased and the relative amount of MUFA was increased. This QTL, near the centromere of BTA2, was also identified by Alexander *et al.* (2007) as affecting the fatty acid content, marbling and flavour. Wagyu is known for its highly palatable meat and flavour and has been associated with the MUFA oleate (Dryden & Marchello, 1970; Westerling & Hendrick, 1979; Melton *et al.*, 1982).

Table 3: Interval mapping results across 29 bovine autosomes for proportions of saturated, mono-unsaturated, and poly-unsaturated fatty acids summarized by locus of maximum additive and dominance effects an each chromosome, the associated F-statistic and genome-wide level of significance (P).

		Additive effect			Dominance effect		
No.	**BTA trait**	**Locus**	**F**	**P**	**Locus**	**F**	**P**
1	**SFA**	0	1.77	0.99	55	1.35	1.00
	MUFA	0	1.24	1.00	146	3.83	0.70
	PUFA	68	3.02	0.86	72	7.92	0.11
2	**SFA**	3	10.07	0.04	73	3.81	0.70
	MUFA	3	23.62	<0.01	9	2.81	0.89
	PUFA	11	20.74	<0.01	62	11.40	0.02
3	**SFA**	110	1.67	1.00	13	2.02	0.98
	MUFA	110	4.09	0.67	13	1.52	1.00
	PUFA	0	0.88	1.00	39	2.55	0.95
4	**SFA**	27	2.32	0.99	58	0.95	1.00
	MUFA	0	1.78	1.00	83	0.87	1.00
	PUFA	0	0.94	1.00	60	7.23	0.22
5	**SFA**	5	0.88	1.00	61	5.20	0.37
	MUFA	87	2.41	0.98	0	5.76	0.28
	PUFA	29	1.67	1.00	0	4.84	0.43
6	**SFA**	95	1.51	1.00	18	0.66	1.00
	MUFA	95	3.03	0.86	22	1.28	1.00
	PUFA	67	2.30	0.96	128	3.45	0.77
7	**SFA**	99	1.46	1.00	33	2.21	0.96
	MUFA	100	1.78	0.99	0	8.41	0.08
	PUFA	122	13.43	0.01	0	4.70	0.50
8	**SFA**	106	2.41	0.97	5	1.93	0.99
	MUFA	0	1.65	1.00	12	2.32	0.98
	PUFA	0	6.26	0.31	14	2.15	0.99
9	**SFA**	39	0.58	1.00	115	5.44	0.38
	MUFA	0	1.00	1.00	115	8.53	0.09
	PUFA	0	0.87	1.00	57	14.59	<0.01
10	**SFA**	12	0.11	1.00	1	2.90	0.89
	MUFA	71	3.29	0.82	41	1.99	0.98
	PUFA	34	1.72	0.99	39	9.22	0.06
11	**SFA**	111	4.01	0.76	99	3.70	0.82
	MUFA	107	1.72	1.00	110	5.41	0.47
	PUFA	0	1.45	1.00	33	1.07	1.00
12	**SFA**	0	4.59	0.53	125	1.87	0.99
	MUFA	0	1.34	1.00	123	2.59	0.93
	PUFA	0	5.17	0.42	83	0.60	1.00

Table 3: Continued…

		Additive effect			Dominance effect		
No.	**BTA trait**	**Locus**	**F**	**P**	**Locus**	**F**	**P**
13	**SFA**	16	1.84	0.98	3	1.54	0.99
	MUFA	74	0.83	1.00	0	1.46	1.00
	PUFA	84	2.76	0.89	1	0.43	1.00
14	**SFA**	42	2.29	0.99	55	0.66	1.00
	MUFA	8	0.04	1.00	60	0.37	1.00
	PUFA	43	4.43	0.74	51	1.02	1.00
15	**SFA**	20	5.25	0.46	56	0.88	1.00
	MUFA	20	5.69	0.39	60	2.08	0.99
	PUFA	63	5.24	0.47	12	9.67	0.06
16	**SFA**	30	1.77	1.00	79	1.83	1.00
	MUFA	24	0.58	1.00	79	1.78	1.00
	PUFA	39	4.99	0.55	0	2.14	0.99
17	**SFA**	87	0.69	1.00	35	3.71	0.85
	MUFA	1	0.62	1.00	33	4.13	0.77
	PUFA	36	0.89	1.00	8	1.64	1.00
18	**SFA**	47	2.32	0.97	46	1.41	1.00
	MUFA	37	3.53	0.81	100	2.16	0.98
	PUFA	67	4.59	0.58	56	7.14	0.19
19	**SFA**	15	7.62	0.14	78	1.10	1.00
	MUFA	25	4.34	0.61	51	1.84	0.99
	PUFA	0	3.41	0.81	3	7.13	0.18
20	**SFA**	55	6.26	0.30	0	0.51	1.00
	MUFA	43	7.07	0.20	1	2.51	0.96
	PUFA	0	2.47	0.97	44	3.12	0.89
21	**SFA**	47	3.18	0.87	99	6.46	0.27
	MUFA	46	3.21	0.87	99	7.95	0.13
	PUFA	0	0.82	1.00	39	2.39	0.89
22	**SFA**	38	0.63	1.00	49	3.04	0.97
	MUFA	43	0.63	1.00	47	9.62	0.08
	PUFA	59	2.95	0.98	24	0.61	1.00
23	**SFA**	14	3.17	0.96	5	0.29	1.00
	MUFA	0	3.01	0.97	3	0.00	1.00
	PUFA	61	0.05	1.00	40	1.45	1.00
24	**SFA**	61	1.84	1.00	0	10.30	0.07
	MUFA	61	3.65	0.95	0	5.18	0.70
	PUFA	31	3.00	0.99	0	1.00	1.00
25	**SFA**	35	0.07	1.00	58	1.85	1.00
	MUFA	34	0.44	1.00	59	1.69	1.00
	PUFA	63	2.33	0.99	62	0.11	1.00

Table 3: Continued…

		Additive effect			Dominance effect		
No.	**BTA trait**	**Locus**	**F**	**P**	**Locus**	**F**	**P**
26	**SFA**	48	1.37	1.00	37	0.79	1.00
	MUFA	49	1.85	1.00	42	1.04	1.00
	PUFA	1	0.90	1.00	0	0.56	1.00
27	**SFA**	0	0.55	1.00	11	0.89	1.00
	MUFA	0	2.40	1.00	12	0.75	1.00
	PUFA	36	0.60	1.00	22	0.86	1.00
28	**SFA**	0	0.09	1.00	50	0.67	1.00
	MUFA	0	1.02	1.00	50	0.87	1.00
	PUFA	3	4.39	0.83	50	2.07	1.00
29	**SFA**	74	1.96	1.00	74	0.99	1.00
	MUFA	74	0.88	1.00	43	1.56	1.00
	PUFA	0	4.64	0.72	71	2.70	0.98

CHAPTER 2

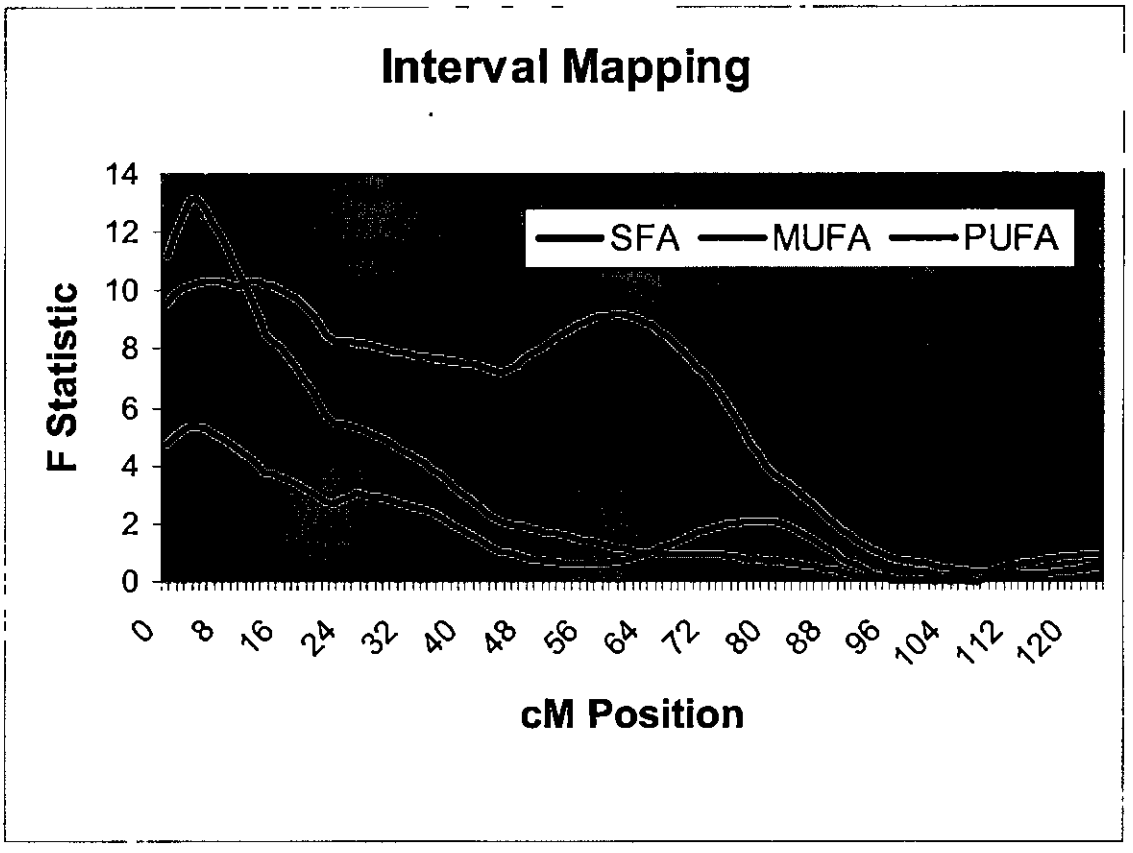

Figure 7: Map of QTL locations far saturated (SFA), mono-unsaturated (MUFA), and poly-unsaturated (PUFA) fatty acids on bovine chromosome 2.

Multiple genes influence marbling or carcass fatness (Barendse *et al.*, 2006). The level of fatness has a major effect on the fatty acid composition of beef. The SFA and MUFA accumulate faster than does the PUFA. One major gene known to affect carcass fatness is the gene responsible for double muscling in cattle, myostatin. It is located at the centromeric end of chromosome 2 (McPherron *et al.*, 1997; Smith *et al.*, 1997). Different myostatin mutations have been identified as segregating in different breeds of cattle. Raes *et al.* (2001) examined the intramuscular fatty acid composition in three myostatin genotypes (double-muscling, mh/mh; heterozygous, mh/+, normal/++) and suggested that

the poly-unsaturated/saturated fatty acids (P/S) ratio is mainly determined by the level of fatness and is increased with low intramuscular fat content.

Two QTLs with dominant effects on MUFA were observed on BTA9 ($P = 0.04$; 2 QTL vs. 1 QTL). The QTL located at 115 cM suggested that the relative amounts of SFA increased (F = 5.44, $P = 0.38$) and MUFA decreased (F = 8.53, $P = 0.09$). Whereas, the QTL located at 57 cM decreased the PUFA content (F = 14.59, $P < 0.01$) and increased the relative amount of MUFA (F = 4.11, $P = 0.64$). Thus, with respect to the MUFA content, the dominance deviations at loci on BTA9 were the reverse of each other suggesting that at one locus the allele from the Limousin was beneficial and, at the other locus, the allele from Wagyu was preferable.

Three additional QTLs suggestive of dominance effects on the relative amounts of fatty acids were also detected. QTL affecting the PUFA content were observed at 31 cM on BTA10 (F = 9.22; $P = 0.06$) and at 12 cM on BTA15 (F = 9.67, $P = 0.06$). Finally, a QTL affecting the MUFA content was found at 47 cM on BTA22 (F = 9.62, $P = 0.08$).

Major fatty acids in beef meat are: SFA (myristic (14:0), palmitic (16:0), stearic (18:0) acids); MUFA (palmitoleic (16:1), oleic (18:1) acids); and PUFA linoleic (18:2), linolenic (18:3), and arachidonic (20:4) acids) (USDA, 2005). Their imbalance may have implications for human health. Consumption of a higher level of MUFA, in conjunction with a reduced level of SFA, is believed to prevent an increase in blood cholesterol levels in the case of oleate and, possibly, to lower blood cholesterol levels.

The fatty acid composition is an important factor for producing high quality beef. The genetic variation in fatty acid synthesis and the deposition in beef are associated with various adipose tissues such as subcutaneous fat, seam and marbling (Webb *et al.,* 1998).

CHAPTER 2

Breed differences are often related to differences in fatness. Perry *et al.* (1998) reported that fatty acid composition is affected by sire breed, independently of a variation in carcass weight. May *et al.* (1993) identified Wagyu beef cattle as one of the breeds with the most marbling or intramuscular fat. Yang *et al.* (1999) characterized Wagyu beef cattle as having soft fat resulting from a high MUFA content. Sturdivant *et al.* (1992) reported that stearoyl-coA de-saturation might be responsible for the elevated MUFA in Wagyu adipose tissue. The growth and fattening of meat animals is associated with increased fat deposition, in subcutaneous and later in intramuscular fat deposits (De Smet *et al.*, 2004). Oak *et al.* (2002) confirmed that genetic differences among sires affected the fatty acid composition of their progeny. Likewise, Rule *et al.* (1997) reported that progeny of high-growth rate potential sires tended to have less SFA and more MUFA in the longissimus muscle than progeny of medium-growth rate potential sires.

Genes, such as leptin (LEP) on BTA4, Thyroglobulin (TG), diacylglycerol O-acryltransferase (Winter *et al.,* 2002; De *et al.*, 2004) fatty acid binding protein (FABP4, Michal *et al.*, 2006) on BTA 14; GH on BTA 19; plus mitochondrial transcription factor A (TFAM, Jiang *et al.*, 2005) have previously been found to have effects on marbling and subcutaneous fat depth. However, these genes do not map to loci coincident with the QTL peaks identified here.

Results of this study indicated that the relative amounts of SFA, MUFA, and PUFA are under some degree of genetic control. Thus it may be possible to improve the quality of the meat by manipulating the fatty acid composition using genetics and appropriate crossbreeding systems.

Chapter 3

Case Study 2

Structural assessment of backcrossing
using microsatellite markers

GENETIKA
GENETICS

CHAPTER 3

3.1 Introduction

Microsatellites are polymorphic genetic markers that have been used in the generation of linkage maps in various livestock species (de Koning *et al.*, 1999). These maps provide the basis for the detection and exploitation of genes segregating at loci that effect on economically important traits; the locations of these genes are referred to as quantitative trait loci (QTL) (Bovenhuis *et al.*, 1997; Devis & Denise, 1998; Grosz, 1998). Microsatellite markers make it possible to identify differences between populations and breeds (D'Surney *et al.*, 2001). One particular breed, namely Hereford, can be genetically manipulated for QTL migration studies.

The Hereford is one of the oldest cattle breeds developed in Herefordshire, England and have been exported to many countries worldwide. These cattle are extremely hardy, have excellent foraging ability and have been farmed for many years for their beef. They are reddish-brown in colour with white on the head, brisket and chest, under-parts of the body, lower legs and tassel. They are susceptible to two conditions, bovine ocular squamous cell carcinoma (cancer of the eye) and sunburned udders. This has been associated with the lack of pigmentation in and around the face and underbelly (Grosz & MacNeil, 2003).[14] Grosz & MacNeil (1999) localized the gene responsible for the Hereford white-face spotting pattern to bovine chromosome 6 within a genomic region displaying conserved synteny with murine chromosome 5. Thus, backcrossing the spotted locus from a non-white faced breed into Hereford was proposed as a potential method of reducing the incidence of bovine ocular squamous cell carcinoma.

[14] http://homepage.usask.ca/~schmutz/index.html

CHAPTER 3

Backcrossing is a well-known and long established breeding method where a characteristic is introgressed from a donor parent into the genomic background of a recurrent parent (Dekkers, 2004). Because backcrossing isolates a gene or chromosomal region in a different genetic background, it aids in dividing the structural design of quantitative traits (Hospital, 2005). It is regarded as one of the few reliable methods to validate the additive effect of a QTL or candidate gene after it is putatively detected. It is generally used when all loci, except for the one being introgressed migrate to the original parental genotype. To decrease the incidence of cancer in Hereford cattle we introduced unlinked loci into Hereford Line 1 breed. Hence the objective of this study was to analyze the genomic structure of Hereford cattle and to study the unlinked loci being introgessed.

3.2 Materials and Methods

Thirteen Line 1 Hereford bulls and 91composite gene combination (CGC) dams were selected for a new breeding program to produce F_1 calves that were then used in a backcrossing experiment that began in 1999 by Dr MacNeil and his staff members. The Line 1 Hereford herd has been maintained as a pure source of the Hereford breed at the Fort Keogh Livestock and Range Research Laboratory (LARRL) in Miles City, Montana, USA since 1934. The CGC herd was started in 1979 at LARRL in order to develop a line of cattle uniquely suited to their environment, specifically the Northern Great Plains. Breed composition of the CGC is ½ Red Angus, ¼ Charolais, and ¼ Tarentaise. The Line 1 and CGC herds have been closed since their founding and no outside sires have been introduced into the respective breeding programmes. All animals subsequent to the founders were selected from within their respective breeding plans. The backcross

experiment produced the initial F_1 progeny and from this point forward, all future generations produced in a separate herd are referred to as the "Redface" herd. From 2002 to 2007, Redface dams were backcrossed to Line 1 Hereford bulls. In 2007 some Redface calves were produced as a result of an intercrossing experiment where a Redface sire born in 2005 was bred to Redface dams. Quantitatively these animals were assumed to be 87.5% Line 1 Hereford. Figure 8 illustrates the design of the experiment and proposed future directions.

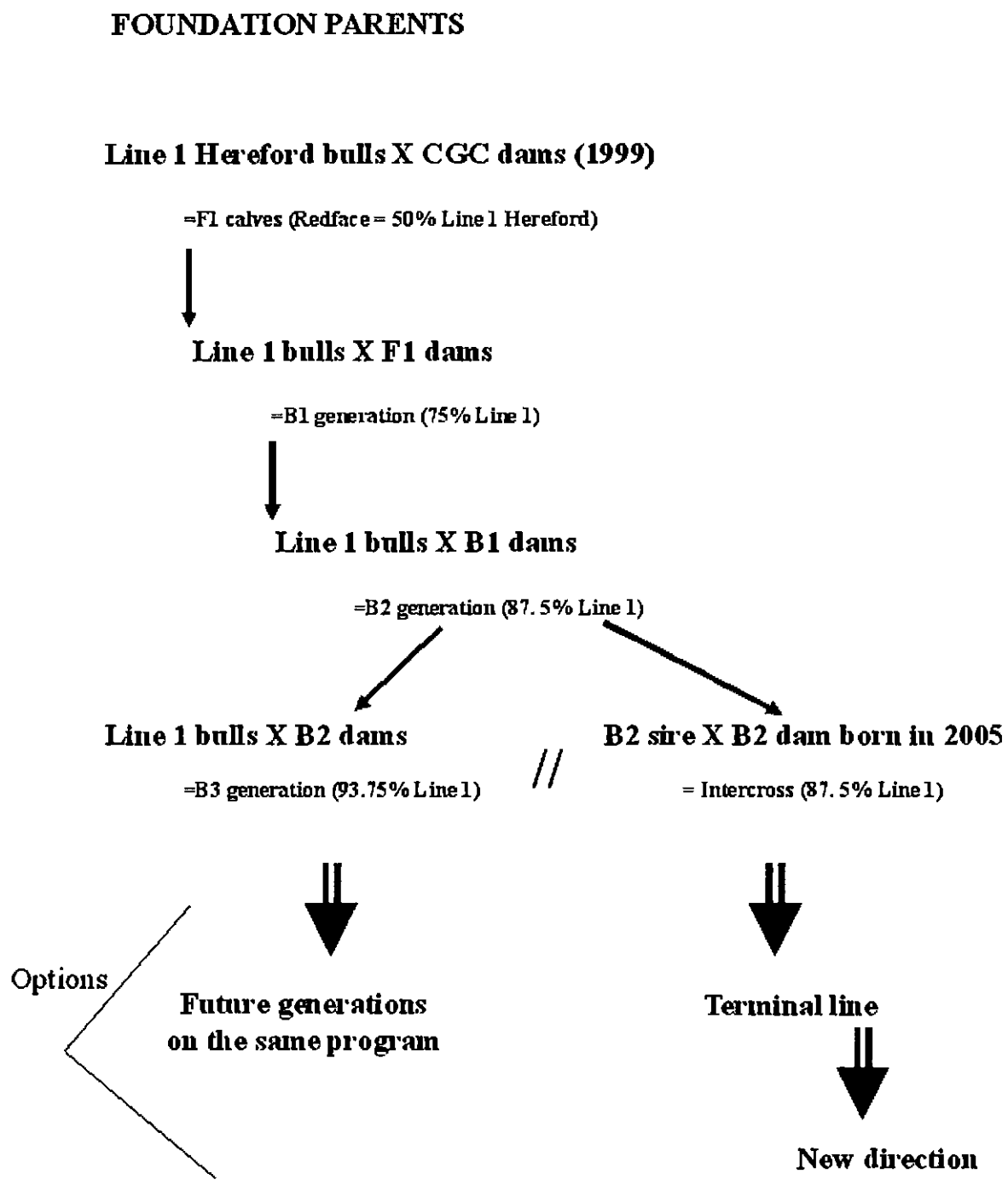

Figure 8: Progression of Redface project.

3.2.1 Genotyping

Blood samples were collected each year for DNA analysis. DNA was extracted from 526 blood samples from Line 1 Hereford bulls, CGC dams, Redface F_1 and Backcross progeny of generations 1, 2, 3 using standard protocols (Ausubel *et al.*, 1994). All samples were extracted by the Fort Keogh technicians including the student for 2007. A total of 34 microsatellite markers encompassing 29 bovine autosomes was selected from the Meat Research Center (MARC)[15] based on marker position, suitability for multiplex reactions and ease of scoring. Touchdown PCR was performed in MJ Research (Waltham, MA, USA) thermocyclers.[16] Genotypes were determined on a LiCor 4200 DNA Analysis System (Lincoln, NE, USA) and were independently scored by two individuals, compared and discrepancies then resolved. PCR was repeated for discrepancies that could not be resolved.

3.2.2 Data analyses

All genotypes were then assessed relative to the purported pedigree using GenoProb (Thallman *et al.*, 2001a, b). GenoProb is a computer program that analyzes genetic marker data in complex pedigrees with missing marker data using an iterative allelic peeling algorithm. Pedigree information for each individual animal as well as marker data was used by the program to determine the genetic model (association of genotype and phenotype marker). Map ID was used to indicate which set marker loci to employ and indicated their properties including chromosomal position (Thallman *et al.*,

[15] http://www.marc.usda.gov/genome/cattle/cattle.html
[16] http://biolibrary.licor.com/htdocs/RnP/LabPrim.jsp

2001a, b). Pedigree errors were resolved based on the genotypes and, when this was not possible, animals with suspect parentage and their descendants were excluded from the study.

A model-based clustering algorithm developed for computer analyses named Structure 2.2 (Pritchard & Wen, 2004) was applied, which identified populations that have distinctive allele frequencies or genetic distances between populations by using genotype data consisting of linked or unlinked markers. Individual animals were placed into population (K) clusters, where K represented the number of populations of the foundation animals, specifically the Line 1 Herefords and the CGC (K=2). Populations were determined in advance but varied across an independent run of algorithms (Pritchard *et al.*, 2000b). Individuals can have a membership in multiple clusters, with membership coefficients summing to 1 across clusters. Input files for running simulations contained parameters such as individual identity, POPDATA (which is the population identity or population origin of each individual animal), POPFLAG column which contained two characters that indicated whether or not to use population information zero represented the population not to be used as part of the learning data, and one represented the learning foundation populations), missing data (-9) and genotype data consisted of 34 loci. The run lengths were selected at a burn-in of 10000-100000. Pritchard *et al.* (2000a) suggested that the longer the run of simulation before collection data minimized the effect of starting configuration and also a longer run of simulation after the burn-in period improved the accuracy of the parameter estimates. In addition, a Q (1, 2, 3, 4, 5, and 6) vector was developed for each population for use with the admixture model for the Redface generation. "Q" refers to the overall number of

populations within the entire dataset to which the animals were individually assigned. This includes learning populations and crosses and backcross generations. The Line 1 Hereford sires were assigned to population number 1; 2 was assigned to the CGC dams and the remaining populations identified the subsequent generations. For example, population 3 represented animals with an expected 50% relationship to Line 1 Hereford, with 4 at 75%, 5 and 6 at 87.5%, and 7 at 93.75%, respectively. Within the STRUCTURE program, the GENSBACK protocol was used with the command USEPOPINFO to verify and display the ancestry population information in the results file to detect whether alleles from the Q populations were related to or inherited specific allele frequencies from either population 1 or 2. Allele frequency was determined when the correlation model was selected (Pritchard *et al.,* 2000a, b). The results were interpreted using the percentage of alleles inherited from either the Line 1 Hereford or the CGC populations. Data results were also presented in the form of bar plots and triangle plots from the Structure program (Pritchard & Wen, 2004).

3.3 Results and Discussion

Results were generated from 526 DNA samples which included animals from the original 13 Line 1 Hereford bulls and 91 CGC dams, their descendents, and the Line 1 Hereford bulls used for subsequent generations of backcrossing pedigrees. Thirty four unlinked microsatellite markers across 29 bovine chromosomes were used in these analyses. In this experiment, the Line 1 Hereford and the CGC were the founding populations (hence, K=2). STRUCTURE provided the probability that genotypes of Redface animals were derived from CGC and Line 1 Hereford. Genotypes from purebred Line 1 Hereford and CGC were included as the learning data to benchmark the founding populations. The estimated proportion of the genotype of animals in each of the Redface generations that originated from Line 1 Hereford and the corresponding expected proportions based on the pedigree are presented in Table 4.

Table 4: Estimates of the proportion of genotypes arising from Line 1 Hereford in each generation.

	Line 1	**CGC**	**F1**	**B1**	**B2**	**Intercross**	**B3**
Expectation	1.000	0.000	0.500	0.750	0.875	0.875	0.938
Mean	0.999	0.002	0.540	0.746	0.819	0.776	0.871
Standard deviation	0.002	0.006	0.060	0.061	0.060	0.080	0.000
Number of animals	64	50	179	153	69	10	1

It was estimated that the 179 F_1 calves actually inherited 54% of the Line 1 Hereford alleles with a standard deviation of 0.06, 4% greater than expected. The 153 B_1 calves were estimated to have inherited 74.6% of their alleles from Line 1 Hereford also with a standard deviation of 0.06. In the B_2 generation, it was estimated that the calves

(N = 69) inherited 81.9% of their alleles from Line 1 Hereford with a standard deviation of 0.06. This is 5.6% less than the quantitative expectation of inherited alleles from a second generation backcross. The results from the B2 generation may be speculatively interpreted to suggest some genome-wide selection for heterozygosity. There were very small numbers of animals in the intercross and B3 generations. Data for the intercross generation is shown in Table 5.

Table 5: Summary of the data from the intercross generation.

Calf	% L1	Dam	Generation	% L1	Sire	% L1
20071413	66.3	20030027	B1	68.9	20051431	83.6
20071420	87.5	20041432	B2	72	20051431	83.6
20071425	82.8	20041413	B1	65	20051431	83.6
20071429	70.8	20041424	B1	70.1	20051431	83.6
20071430	76.2	20030018	B1	65.7	20051431	83.6
20071434	85.6	20030003	B1	80.7	20051431	83.6
20071436	74.4	20030299	B1	71.9	20051431	83.6
20071437	68	20041425	B1	71.7	20051431	83.6
20071439	87.8	20030005	B1	76.5	20051431	83.6
20071441	77.2	20030021	B1	61.8	20051431	83.6

* % L1 = Line 1 Hereford; Dam = Redface (87.5% B2)

Calf that was born in 2006 was expected to inherit 93.75% of its alleles from Line 1 Hereford, based on its pedigree. However, the predicted proportion of alleles inherited from Line 1 Hereford was 87.1%, 6.65% less than expected (Figure 9).

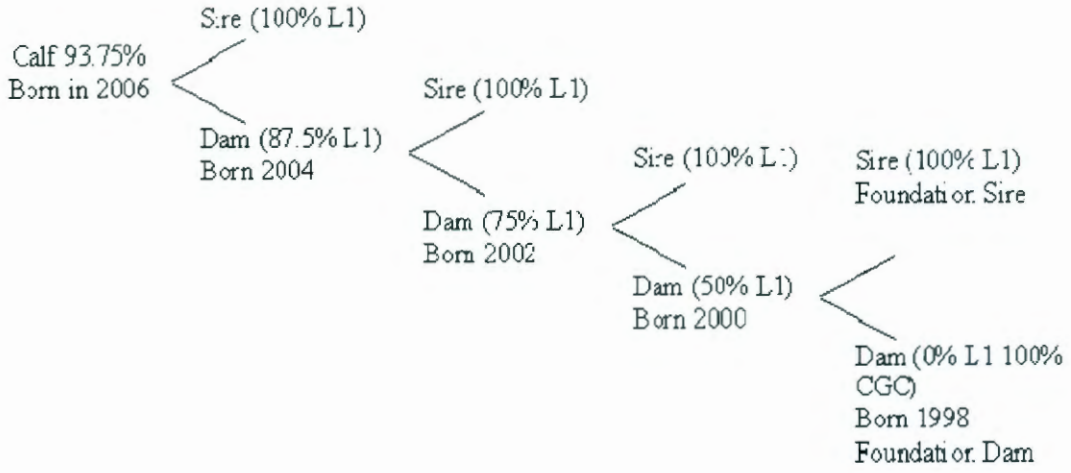

Figure 9: Pedigree for three backcross generations

Presented in Figure 10 is a bar plot of the results for each of the Redface animals sorted by generation. The Line 1 Herefords are coded 1 and assigned the colour red. The CGC animals are coded 2 and assigned the colour green.

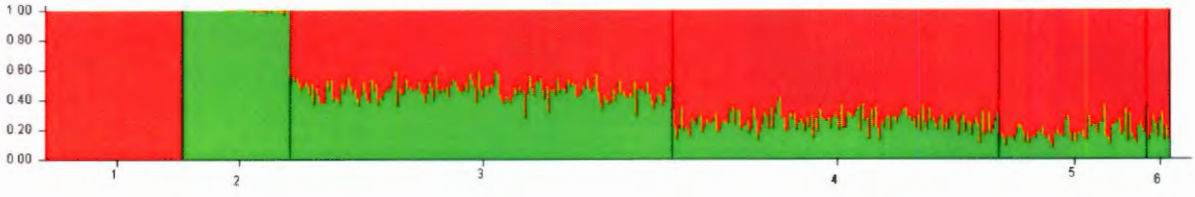

Figure 10: Summary of clustering results for the data of each population (Line 1 Hereford=1, CGC=2, F_1=3, B_1=4, B_2=5 and intercross =6)

The subsequent generations of backcrossing show the variation in alleles predicted to have been inherited from each of these base populations. For example, more red demonstrates the increasing amount of L1 Hereford alleles inherited. The results indicate

animal to animal variation in the predicted genetic composition of the F_1 B_1, B_2 and B_3 animals. These results are amplified in the expanded plot present in Figure 11. Note that the B4 animal is not shown in Figure 10 because it was only one calf.

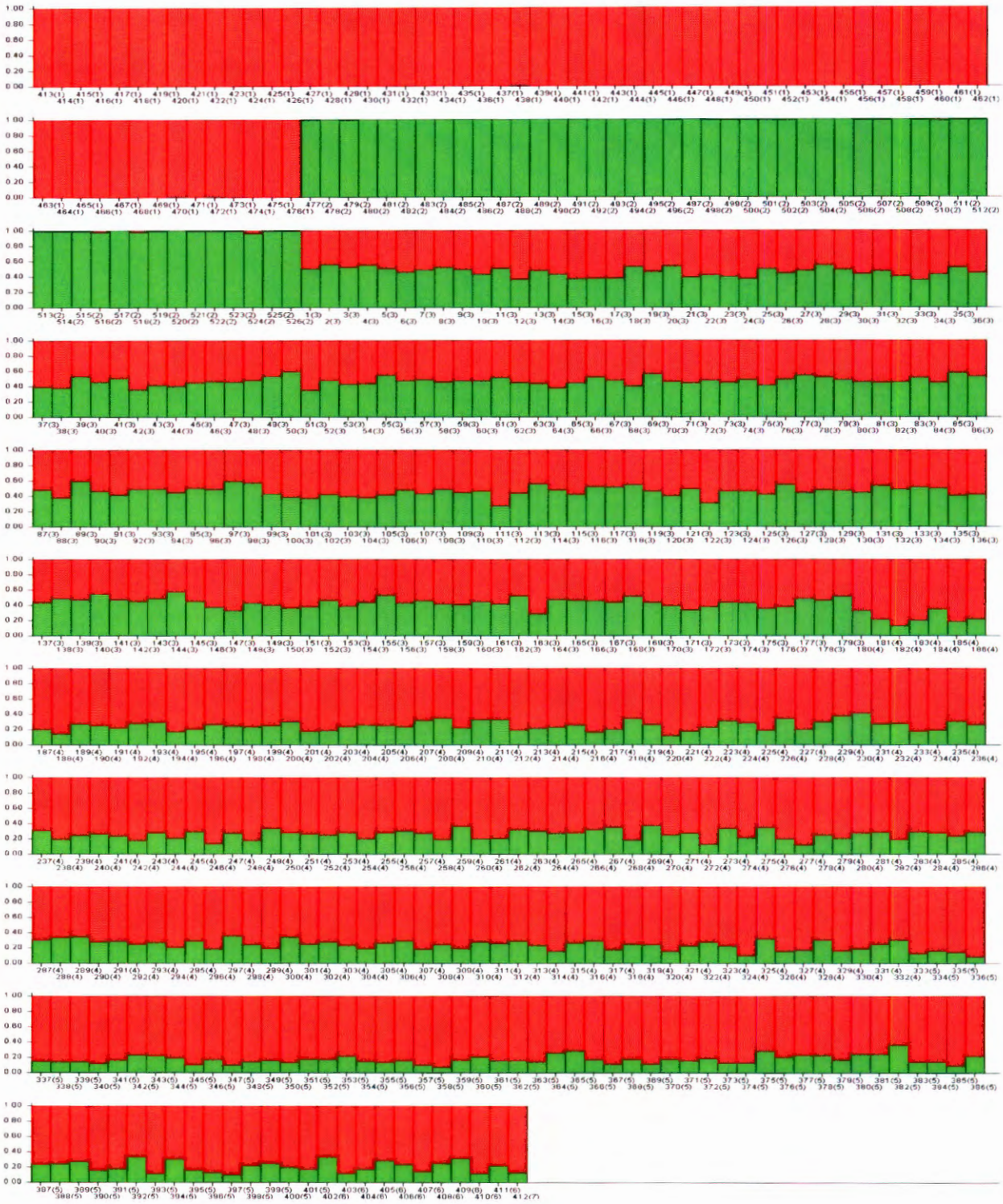

Figure 11: Extended bar plot representing all individual animals.

In a triangular plot (Figure 12) the results from STRUCTURE represented the two foundation populations and the cross and backcross generations. The animals are represented by dots and they migrate towards the Line 1 Hereford population. Again, subsequent generations of backcrossing are shown to migrate toward the Line 1 Hereford, as expected.

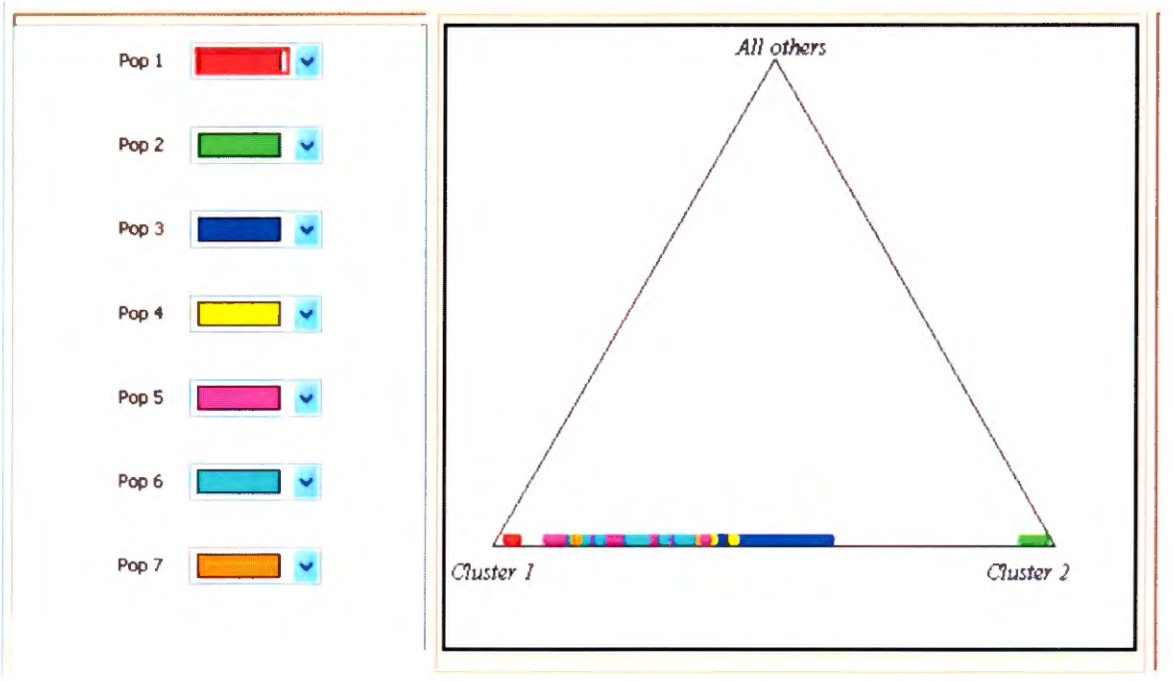

Figure 12: Summary of clustering results for learning populations 1 (cluster 1) and 2 (cluster 2) and their relationships to the cross and backcross generations.

As the number of generations increase, the divergence among the allele frequencies are more highly correlated (Falush *et al.*, 2003). At this point the allelic differences between Redface and Line 1 Hereford become less variable.

As Grosz & MacNeil (1999) localized the gene responsible for the Hereford white-face spotting pattern to BTA 6 within a genomic region displaying conserved synteny with murine chromosome 5, in this study, the five markers this region of BTA 6 most closely linked to whiteface were used for marker assisted selection. Unfortunately, while these markers provided sufficient information to resolve the map position of the spotted locus to within 1 cM Grosz & MacNeil (1999), they did not provide sufficient information to differentially characterize Line 1 Hereford and CGC in Figure 13.

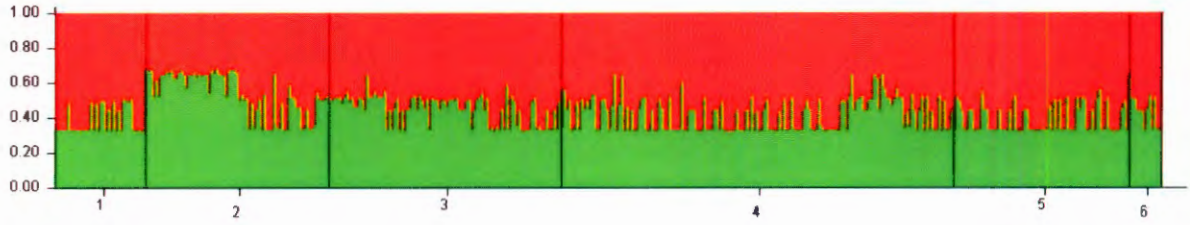

Figure 13: Summary of spotted locus marker of bovine chromosome 6 (Line 1 Hereford=1, CGC=2, F_1=3, B_1=4, B_2=5 and intercross =6).

Thus, these markers have not been particularly useful for this study. In order for this study to be continued, additional research would be required to identify markers in this region that differentiate between Line 1 Hereford and CGC.

Chapter 4

Designing an experiment to Detect and Validate quantitative traits loci in beef cattle

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CHAPTER 4

This chapter will explain the design of an experiment for QTL detection in one indigenous and one British breed from South Africa. With the knowledge gained in the previous two case studies (Chapter 2 and 3) the F_2 a backcrossing experiment was designed, whereby certain genetic characteristics of the South African indigenous cattle breed can be applied to benefit cattle breeding in South Africa. The goals of the experiment should therefore include the following:

- To identify and apply the QTL migration technology into the detection of the QTL that effect growth, tick resistance, stress adaptability and meat quality in the Nguni indigenous and the British Angus breed using a F_2-design.
- To compare / correlate / evaluate the F_2-heifer progeny of the Nguni (South African indigenous breed) and Angus (British breed) under intensive feedlot conditions with regard to growth performance, carcass yield, stress adaptability and meat quality characteristics with associated quantitative trait loci.

Ultimately this design should contribute to the use of marker assisted introgression to increase resistance to ticks, without compromising on meat quality (tenderness, taste, and colour), growth, stress adaptability and carcass quality of F_2 progeny. A cost for such a design will also be included.

Taking the above goals into consideration it is important to have some background information on the two breeds in a South African context.

4.1 Background

The depreciation of livestock production is a concern to South Africa. On the other hand, South Africa has indigenous breeds well adapted with special characteristics such as the Nguni. This cattle breed is mostly farmed by emerging farmers and provide new opportunity for enhancing production. Empowering emerging farmers with new technologies in breeding and developing their entrepreneurship abilities to fight poverty has become a main focus in South Africa. Genetic management using tools such as crossbreeding and artificial selection enhanced with the application of genetic markers for QTL detection can improve beef production and carcass quality of other cattle breeds to meet the market requirements. South African farmers are also faced with the challenge of different environments and marketing systems for optimized production and profitability. This implies that cow herds must remain well adapted to the environment, while their offspring must meet the increasing demand of the beef markets and feedlots. To achieve these challenges farmers will require a basic knowledge of genetic and non-genetic factors which affect adaptation and beef production and quality.

Improving breed genetics can be a major advantage in modern cattle breeding where changes and fluctuations in the market are part of the industry. Environmental adaptation and tick borne diseases are increasingly contributing to the loss of animal production in South Africa. Ticks are responsible for great losses in animal production within the economic system. The indigenous breeds are known for being tick resistant

and identifying genes through QTLs can improve exotic breeds to better adapt to South African conditions.

Research has indicated that the Nguni breed has high tick resistance and should be used to improve other breeds susceptible to ticks (Spickett *et al.*, 1989). For thousands of years, Nguni cattle genetics have been shaped by natural selection in the African environment; however, they are not generally viewed as a breed of commercial value because of their medium to small body frame (Matjuda, 2005). Slaughter weight averages 249 to 342kg at 75-105%, while the carcass weight is approximately 138 to 196kg at 112 days (Strydom *et al.*, 2000a). To date, studies have shown that indigenous cattle such as the Nguni, perform well for economically important traits when used as a dam line (Scholtz, 1988, 2005; Mpofu, 2002; Ramsay *et al.*, 2000).

Angus cattle have the ability to reach heavy weights without becoming overly-fat and are thus suitable for a range of commercial markets. Where a high degree of tropical adaptation is needed, Angus bulls can be crossed with adapted breeds in rotational or terminal crossbreeding programs to maintain a market standard. In Australia there is an increasing trend to combine the desirable attributes of various breeds in crossbred bulls, while maintaining a high degree of tick resistance and environmental adaptation. For example, most tropical breeds have been subjected to little, if any, artificial selection for beef production. The reported results of selection for these attributes within temperate breeds suggest the potential improvement using crossbreeding (Sarmiento & Garcia, 2007). Such selection may not result in a loss of the survival and fitness traits that made these breeds attractive in the first place, if selection is undertaken within the constraints of the actual production system (Kohler-Rollefson, 2004). With the use of Angus

germplasm in South Africa poor adaptation to harsh environments and susceptibility to tick infestation remain a problem. Thus, farming with Angus cattle in South Africa implies that the farmer needs to spend more on chemicals such as dip and other stock remedies which results in less profit.

To achieve the goals as set out above the objective of this experiment will be to use the Nguni and the Angus to identify QTLs that have an effect on tick resistance, growth, stress adaptability and meat quality by using a F_2 design. From the case studies done in Chapter 2 and 3 I now have the know-how on planning such an experiment. The first step will be to identify the breeds and then implement a F_2 design.

4.2 The F_2 design

The F_2 design will explain QTL detection, genetic analysis and evaluation of the phenotypes whereby F_1 individuals are interbred (Figure 14). The power of the F_2 design is that three genotypes are present at every QTL in the mapping population as was demonstrated in Chapter 2. It also enables the estimation of both the additive and dominance effects of a QTL. The analysis of F_2 families is highly efficient where alternative alleles have been fixed or allele frequencies are very different in the two breeds. This step has been used for years in many QTL studies to improve animal production. For example, Gasparin *et al.* (2007) identified QTLs for tick resistance on bovine autosome (BTA) 5, 7, and 14 in 382 F_2 animals derived from Holstein x Gir crosses using 23 microsatellite markers. In this study, Angus bulls, which possess a desirable carcass size but are susceptible to tick infestation, and Nguni cows that are not susceptible to tick infestation, will be cross-bred. Two Angus bulls and 252 Nguni cows

will be introduced for two years expecting to produce 150 F_1 and subsequently 250-300 F_2 progenies as shown in Figure 15. Because the genetic markers will be pre-selected to indicate differences between breeds it is expected that calves in the F_1 generation will be heterozygous for all loci. Only a selected number of F_1 bull calves will be used as sires to produce the next progeny. It is expected that this F_2 generation will then have three genotypes.

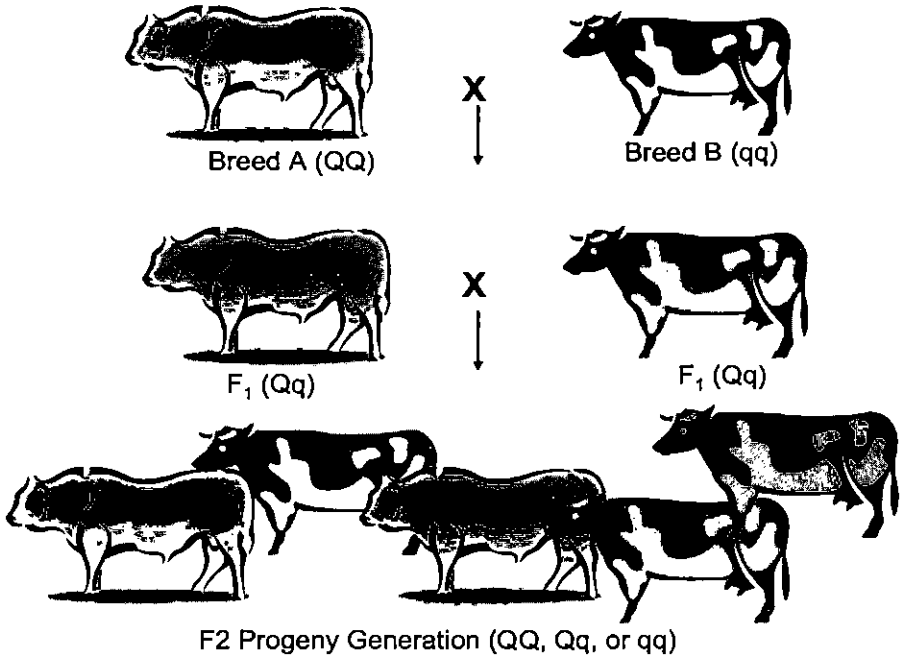

Figure 14: Schematic representation of the F_2 design (A=Angus; B=Nguni; Q & q = QTL).

Hair and blood samples will be collected from all founder (parents) F_1 and F_2 animals. Following DNA extraction, PCR products will be analyzed. Analysis will be carried out using the DNA sequencer model 3130XL. Genotypes will be identified using the specific selected microsatellite markers.

Initially, microsatellite markers evenly spaced at 10 to 25 centiMorgans (cM) will be selected from the bovine genetic map (MARC). The cM is the unit of genetic distance

and is equal to 1% recombination. These markers will cover the whole genome. The initial panel of microsatellites is proposed in Table 6. These markers will be used to genotype 45 Nguni and 45 Angus animals following standard procedures to determine the heterozygosity level at each locus (e.g. Ausubel *et al.*, 1994). Markers that do not segregate between Angus and Nguni will be replaced with other closely linked markers chosen from the MARC microsatellite map until a total of 200 markers are available. After identification of QTLs for growth, carcass traits and tick resistance, additional markers will be added within the region for fine mapping to further narrow the location of interest. Known markers associated with tenderness and marbling such as the CAPN1-316, CAPN1-4751 (micro-molar activated neutral protease), CAST, CAST-Brahman (inhibitor calpastatin) will be determined at MARC or another laboratory accredited to analyze the markers, according to standardized procedures.

Phenotypic data will be collected to include the three genotypes namely tick resistance, growth, and carcass quality obtained from the F_2 progeny and will be linked with the QTLs results that include the 200 markers covering the 29 bovine autosomes. The F_2 progeny will be raised by their dams in an area where ticks are endemic. No pesticides will be used on the calves or their dams. All calves will be weighed at birth and again at weaning. After weaning, the calves will be rounded off to a certain weight in the ARC-feedlot and then slaughtered. The animals will be group-fed and feed efficiency can, therefore, only be determined as a single value for the group (no statistical analysis).

Table 6: Selected microsatellite markers from the Meat Research Center (MARC)

Marker Name	Chr	MARC Position (cM)	Heterozygosity (%)	No. of alleles	Min allele size (bp)	Max allele size (bp)	Annealing temp (PCR)
BMS1928	1	6.9	81	15	138	174	58
BMS4015	1	28.2	75	9	134	158	58
TGLA57	1	46.2	75	7	86	102	58
BM9019	1	67.5	65	5	91	115	58
URB038	1	80.6	87	8	155	173	60
BMS1789	1	100.9	83	7	103	121	58
BMS599	1	125.8	84	12	112	154	58
INRA040	2	7.5	89	24	161	240	58
TEXAN2	2	22.5	66	4	116	122	58
BMS803	2	41	76	6	134	146	58
BMS1126	2	56.3	81	10	122	156	58
TGLA226	2	80	70	4	141	147	58
BMS2519	2	101.5	69	8	101	127	58
IDVGA-2	2	117.8	73	9	119	147	56
INRA006	3	19.5	59	4	106	114	58
BMS482	3	36.2	77	9	137	157	58
BM4129	3	52.3	51	8	78	102	54
ILSTS064	3	77.9	87	13	142	172	54
IDVGA-35	3	102.9	87	12	221	247	58
BMC4214	3	123	73	11	172	196	56
BMS1788	4	8.4	80	11	89	115	58
BMS1237	4	30.6	87	18	140	184	58
BMS2172	4	49.6	68	8	78	96	56
ILSTS062	4	68.3	73	7	188	206	58
BM1500	4	82.8	59	4	135	145	54
AGLA227B	4	101.5	35	3	171	177	58
BMS1095	5	0	81	11	95	117	56
BP1	5	18.8	77	11	302	326	54
BMS1315	5	32.5	61	6	133	145	58
BL4	5	51.2	78	7	149	161	54
BMS1248	5	88.4	74	13	122	162	58
BM315	5	100.1	89	13	107	135	60
BM8126	5	122.1	34	2	127	129	58
ILSTS090	6	11.8	54	2	145	147	56
BM1329	6	35.5	66	6	145	155	58
BMS470	6	63.6	73	6	59	71	58
ILSTS035	6	81	72	17	210	266	56
BM8124	6	94.2	42	9	121	139	58

Table 6: Continued...

Marker Name	Chr	MARC Position (cM)	Heterozygosity (%)	No. of alleles	Min allele size (bp)	Max allele size (bp)	Annealing temp (PCR)
BL1038	6	122.3	68	6	99	113	60
BM7160	7	0	67	6	175	189	58
BMS713	7	15.2	73	7	129	157	58
BM6105	7	35.7	77	8	89	103	58
BMS2840	7	64.3	89	17	217	283	58
INRA192	7	82.4	67	4	124	134	58
RASA	7	103.1	56	4	181	187	52
ILSTS006	7	116	81	10	281	299	54
BL1043	7	134.1	87	14	93	123	58
BMS1864	8	0	68	6	145	159	58
RM372	8	19.1	83	5	126	134	58
BMS678	8	38.4	80	7	97	111	58
BMS2072	8	58.8	81	8	152	168	56
HEL9	8	76.7	80	9	147	165	52
BMS2847	8	112.2	82	10	205	223	58
ETH225	9	8.1	75	7	141	153	58
RM216	9	32	90	10	96	118	54
BMS817	9	38	81	7	114	128	58
BMS1290	9	59	82	8	97	123	58
BMS2251	9	80.8	38	5	94	104	58
BMS1967	9	102.5	90	10	81	101	58
CSSM38	10	7	88	16	160	198	58
BMS2349	10	22.1	79	14	68	110	58
BMS2742	10	38.5	94	13	127	153	58
INRA071	10	59.3	85	11	209	229	58
BMS2641	10	79.1	69	4	173	179	58
SRC323	10	101.4	75	11	101	125	58
BM9067	11	9.5	86	13	99	125	46
RM096	11	31.3	60	7	102	114	56
ILSTS036	11	56.9	74	7	147	159	58
BMS2047	11	73.8	92	18	143	193	58
BL1103	11	90.9	86	9	108	130	58
BMS607	11	105.3	82	10	133	167	58
BMS410	12	0	80	12	82	108	58
BMS2057	12	20.7	80	10	86	108	58
RM094	12	41.7	66	6	146	162	58
BMS975	12	61.2	68	6	86	98	58
BM4028	12	79.7	71	13	102	126	58

Table 6: Continued…

Marker Name	Chr	MARC Position (cM)	Heterozygosity (%)	No. of alleles	Min allele size (bp)	Max allele size (bp)	Annealing temp (PCR)
BMS1316	12	98.7	83	11	96	126	58
TGLA23	13	0	65	9	92	116	58
BMS1742	13	14.8	66	5	157	167	58
ILSTS059	13	34	83	13	162	200	58
BM9248	13	52.8	84	10	115	145	58
AGLA232	13	79.5	90	14	155	183	54
BMS1678	14	6.2	82	7	123	135	56
RM011	14	27.7	86	8	101	121	58
BL1029	14	42.8	70	10	138	174	58
BM4305	14	66.4	86	8	150	168	58
BMS2055	14	84.1	73	8	147	171	58
BMS2533	15	5.2	86	11	128	160	56
INRA050	15	31.1	79	8	132	152	54
IDVGA-10	15	51.6	80	8	161	175	56
BMS812	15	68.8	86	11	111	133	56
BMS429	15	93.4	73	7	125	143	58
TGLA245	16	6.5	86	11	129	161	56
BM121	16	24.4	60	12	118	158	56
BR6504	16	51.2	83	9	121	145	56
INRA048	16	73	73	12	265	303	58
BMS462	16	93.2	51	7	108	130	58
BMS1825	17	3.8	77	14	148	202	58
CSSM9	17	31.4	74	7	147	161	60
BM305	17	51.9	85	14	101	135	58
HUJ223	17	74.8	57	6	150	168	56
BM1233	17	98.6	78	8	166	180	58
BMS1355	18	2.8	75	8	149	165	58
BMS2213	18	26.2	74	9	130	146	58
INRA185	18	42.3	55	3	137	143	58
BMS2639	18	57	90	9	157	185	58
TGLA227	18	84.7	84	11	76	102	56
BM9202	19	0	73	8	85	115	56
X82261	19	19.4	75	8	123	141	58
RM222	19	39.4	62	7	118	136	56
BMS650	19	56.5	87	13	134	174	60
IDVGA-44	19	78.6	78	6	203	215	60
BMS601	19	99.5	65	6	177	187	56

Table 6: Continued...

Marker Name	Chr	MARC Position (cM)	Heterozygosity (%)	No. of alleles	Min allele size (bp)	Max allele size (bp)	Annealing temp (PCR)
HEL12	20	0.7	69	7	145	163	58
TGLA304	20	20	73	4	86	94	56
BMS1128	20	33.9	50	7	91	103	58
BM4107	20	52.4	54	9	157	183	58
BM5004	20	64.3	86	10	120	148	58
BMS521	20	75	68	4	134	142	58
BM8115	21	0	83	9	119	139	52
RM151	21	11.7	74	9	111	133	54
BM103	21	30.5	72	9	146	162	56
BMS868	21	47.9	83	11	124	156	58
TGLA122	21	67.3	86	12	141	181	58
CSSM26	22	0	83	11	238	276	58
INRA194	22	21.8	66	5	145	165	56
BMS2573	22	40.4	75	7	117	131	58
BMS875	22	61.1	53	5	97	105	58
BM4102	22	81.1	71	5	137	173	58
INRA064	23	0	58	7	177	186	58
RM033	23	17.3	64	4	150	156	58
BOLA-DRB1	23	35.4	85	9	208	229	56
BM7233	23	49.1	80	6	104	124	56
BM1443	23	67.1	88	9	145	165	56
BMS2526	24	6	86	9	139	155	58
BMS2270	24	21.2	70	9	78	96	58
BMS66	24	38.2	72	7	132	146	60
BMS1925	24	57.4	75	7	124	146	56
BMC4216	25	0	51	3	163	167	54
BM4005	25	12.3	80	9	102	122	58
BM737	25	27.8	79	10	110	134	52
BMS1353	25	45.3	70	10	100	128	58
BM1864	25	64.9	62	5	125	142	56
BMS651	26	2.5	89	8	117	133	58
HEL11	26	20.7	81	12	181	211	58
RME40	26	41.3	87	14	145	189	58
BM804	26	59.6	67	6	142	152	58
MAF36	26	72.6	51	4	151	157	54
BM3507	27	0	84	9	161	185	58
BMS641	27	14.3	89	10	82	104	58

Table 6 Continued...

Marker Name	Chr	MARC Position (cM)	Heterozygosity (%)	No. of alleles	Min allele size (bp)	Max allele size (bp)	Annealing temp (PCR)
CSSM43	27	34.1	76	9	249	265	58
INRA134	27	45.5	60	7	123	143	58
BM203	27	64.1	71	14	203	231	58
IDVGA-29	28	8.7	73	7	136	152	56
BMS510	28	22.1	86	10	83	107	58
BMS1714	28	42.1	54	7	121	139	58
BMS764	29	9.7	75	7	94	108	58
BMS1600	29	28.4	56	5	139	153	58
BL1100	29	46.6	64	5	77	85	58
ILSTS081	29	65	84	9	88	114	56

Animals selected for slaughter will have reached the best market condition and this will be done by visual assessment at an age of 10 - 12 months, so as to produce carcasses in the A-age class and fatness class 2 or 3 of the current South African Beef Carcass Classification System. All animals will be slaughtered at the ARC-Irene abattoir under controlled conditions. Post-slaughter treatment will involve electrical stimulation for 45 sec (500V) 10 min after exsanguinations and chilling at 4°C within 2 h after slaughter will apply.

Carcass traits will be measured using ultrasound prior to slaughter and in the abattoir cooler after slaughter. The pH and temperature of the carcasses will be measured every hour for the first six hours and again at 24 hours post slaughter. The standard procedure for sampling will be followed.

4.3 Statistical analysis

Least squares statistical methods will be used for analysis of the data as it is more suitable for crosses where the lines may be segregating at marker loci but can be assumed to be fixed for alternative alleles at the major QTLs affecting the traits (Haley *et al.*, 1994). The use of multiple markers in a linkage group simultaneously increases the test statistics and thus the detection of the QTL compared to a single marker or markers flanking an interval. The least squares analysis allows after performing data permutations to determine the genome-wide significance thresholds. In addition, the standard errors parameters and confidence intervals will be obtained using the bootstrap sampling technique. Statistical analysis will also be performed on the F_2 back-crosses to include results for meat tenderness, electrical stimulation and the ageing period of the meat using multiple analyses of variance (MANOVA). ANOVA and regression analyses will be used to statistically evaluate the meat quality results.

4.4 Time frames and costs

The feasibility of any design experiment depends on the time frame and cost of such a project. In this case the time frame for this F_2 design experiment is estimated at a period of seven years (Figure 15). Year one is the first cross between Angus bulls and Nguni cows. The second year will be additional crosses between the two breeds to obtain sufficient F_1 progeny. In the third, forth and fifth years the F_1 progeny will be crossed to obtain sufficient F_2 generation. The sixth and seventh years will be used for statistical analyses and publication writing.

The estimated budget of this F_2 design experiment over a period of seven years is projected see Table 7. This includes animal husbandry (feeding, etc.), artificial insemination (reproduction), experimental phase (ticks, etc.), molecular genetic analyses, quantitative genetic analyses and carcass and meat analyses, human resources and overheads.

4.5 Conclusion

To conclude, if the goals can be reached using this experimental design, the results should lead to the use of marker assisted introgression to increase resistance to ticks, without compromising the meat quality (tenderness, taste, and colour) of the South African Angus and to improve the carcass characteristics, increase stress adaptability and meat quality (tenderness, taste, and colour) of Nguni. To integrate the knowledge gained in the preceding Chapters, especially Chapters 2 and 3 a new search for QTL can be applied using the progenies of the F_2 design.

Figure 15: Time frame of the project

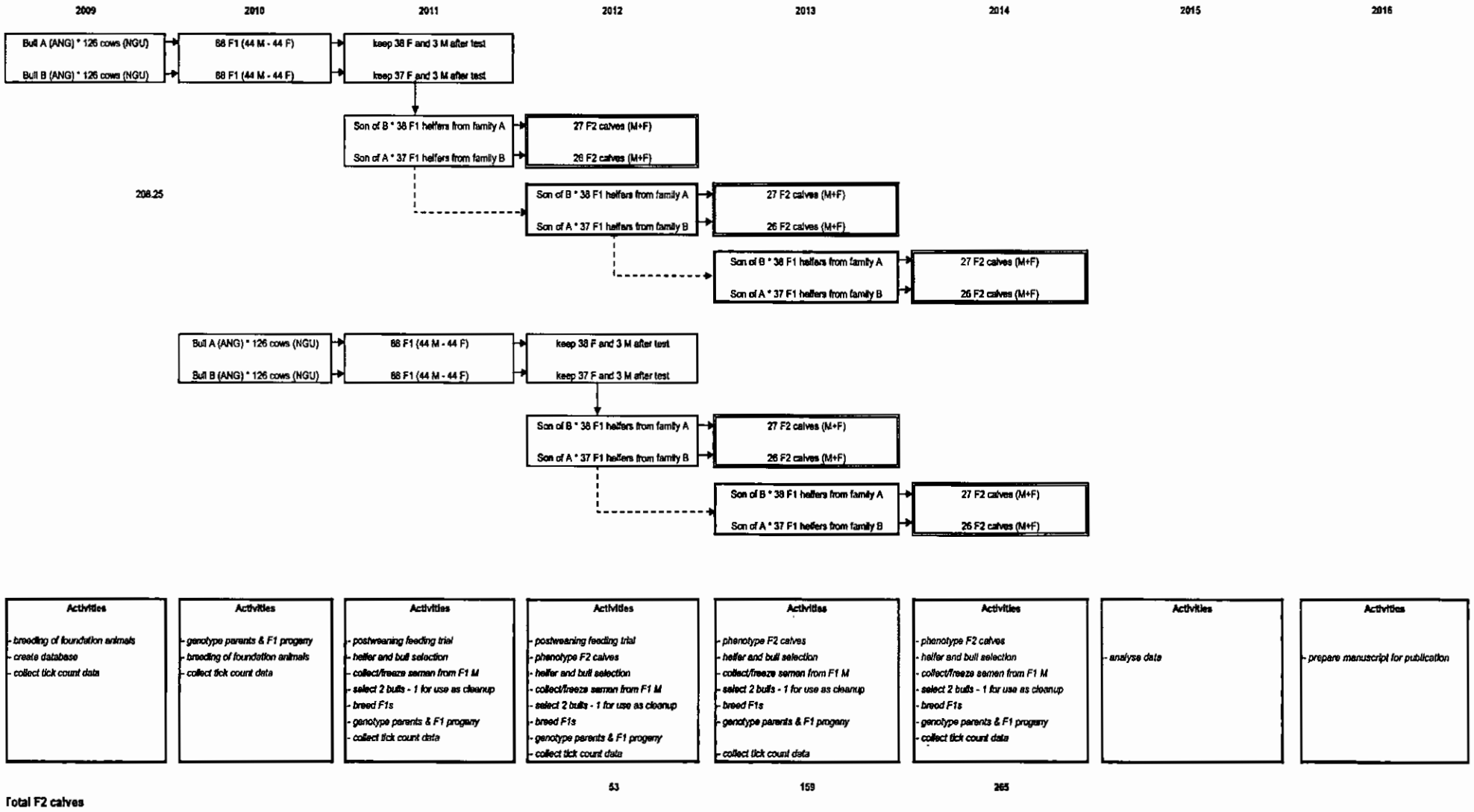

Table 7: The projected budget of the F2 design

Activity	Year						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
1. Collection of Tick Count data - Sept, Oct, Nov & Dec							
accommodation for 2	R1 200	R2 320	R13552	R14907	R6398	R18 038	R19841
food @ R100 per day	R1000	R1100	R1210	R1331	R1464	R1611	R1772
subsistence @ R60 per day	R960	R1056	R1162	R1278	R1406	R1546	R1701
travel @ R2.50 per kilometer	R4100	R4510	R4961	R5457	R6003	R6603	R7263
human resource R200 or R150 per hour	R25600	R27136	R28764	R30490	R32319	R34259	R36314
2. Collection of phenotypes at end of test		R5088	R5393	R5717	R6060	R6423	R6809
3. Collection of weights - monthly weight till weaning							
accommodation				R14400	R15840	R17424	R19166
food				R3520	R3872	R4259	R4685
subsistence @ R80 per day				R640	R704	R774	R852
travel @ R3.50 per kilometer				R11480	R12628	R13891	R15280
human resource				R32000	R33920	R37312	R41043
4. Creating and managing a database	R6000	R1272	R1399	R1539	R1693	R1862	R2049
5. Data analysis & publication							R6800
human resource							R36000
Total	**R53746**	**R57730**	**R62085**	**R99835**	**R108225**	**R117359**	**R295589**
1. sample collection	R1100	R970	R2400	R1500	R1040	R1040	
2.Extractions	R13615	R11350	R13540	R9632	R9803	R9803	
3. PCR	R210500	R700	R200	R10300	R10300	R10300	
4.Sequencing	R5100	R3720	R5100	R3000	R3000	R3000	
5.Capital equipment	R382500						
research tech X2 cost	R5088	R5393	R5717	R6060	R6423	R6423	
Scientist x2	R25600	R27136	R28764	R30490	R32319	R34259	R36314
training and congress	R100000	R60000	R60000	R60000	R60000	R60000	
6. Publication							R20000
Total	**R743505**	**R115869**	**R126021**	**R12982**	**R122885**	**R122885**	**R56314**
Meat science analysis							
metabolic profile, meat quality, biochemical & histology	R10155					R2691075	R194510
research tech X2 cost	R5088	R5393	R5717	R6060	R6423	R6423	
Scientists x2 cost	R25600	R27136	R28764	R30490	R32319	R34259	R36314
training and congress	R100000	R60000	R60000	R60000	R60000	R60000	
6. Publication							R20000
Total	**R140843**	**R92529**	**R94841**	**R96550**	**R98742**	**R2791757**	**R250824**
Animal husbandry	R200000						
Reproduction	R1000000						
Overall budget	**R38094**	**R266128**	**R282947**	**R209367**	**R329852**	**R303200**	**R602727**

Chapter 5

Summary

GENETIKA
GENETICS

CHAPTER 5

Summary

The study presented here was conducted in order to identify ways in which molecular genetics can be used to enhance the performance and sustainability of beef cattle production. The use of QTL mapping results for marker assisted selection and markers assisted introgression has been suggested as a method that can be used to improve livestock production. The study presented here analyzed QTLs as reference points for the genetic control of phenotypic expression by migrating of a specific gene of interest from one population to another.

Chapter 2 focused on determining the health status of beef as determined by fatty acid composition. High levels of saturated fat are associated with increased serum low-density lipoprotein cholesterol concentrations and pose a risk factor for coronary heart disease. Fatty acid composition is believed to be under a degree of genetic control. Thus a search for QTLs that affect relative amounts of saturated, monounsaturated and polyunsaturated fatty acids in beef meat was conducted. A major QTL with additive effects on fatty acid composition near the centromere of chromosome two was observed. In addition, five less significant QTL with dominance effects on fatty acid composition was detected. Results of this study indicate that the relative amounts of SFA, MUFA, and PUFA are under some degree of genetic selection. The fatty acid composition of beef can possibly be improved by locating the relevant QTL through genetic markers and appropriate crossbreeding systems.

In Chapter 3, a backcrossing experiment was described which plays an important role in introducing new genetic material into established breeds or lines for beef production. When coupled with marker or gene assisted selection, it can be used to move

a specific gene or chromosomal region from one population into another. The genomic structure of beef cattle produced by backcrossing was assessed for loci that are unlinked to a specific locus that was being moved from a donor breed to a recipient breed in when the particular genetic variant was not otherwise present. Genotypes of the two parental populations, their F_1 progeny, and two subsequent backcross generations of animals were determined at 34 independent loci. Results indicated that the introgression was compromised because the markers used did not sufficiently segregate between the founder populations.

In conclusion, chapter 4 is F_2 design experiment to integrate the knowledge gained in the preceding chapters with a new search for QTLs using Nguni and Angus cattle. If successful, the results of this study might lead to the use of marker assisted introgression to increase resistance to ticks in South African Angus and (or) add value to the carcass quality and carcass weight to Nguni.

Chapter 6

Opsomming

GENETIKA
GENETICS

CHAPTER 6

Opsomming

Hierdie studie is uitegevoer om maniere te identifiseer waarop molekulêre merkers gebruik kan word om die produktiwiteit van plaasdiere te verbeter, asook om die volhoubaarheid van verhoogde produksie te verseker. Die gebruik van QTL kartering resultate vir merker-gebaseerde seleksie met die hulp van merker-gebaseerde introgressie is voorgestel as 'n metode wat gebruik kan word om lewendehawe se produksie te verbeter. In hierdie studie is QTL as 'n verwysingspunt vir die genetiese beheer van fenotipiese uitdrukking met die migrasie van 'n spesifieke geen van belang vanaf een populasie na 'n ander geanaliseer.

Hoofstuk 2 het gefokus op die analise van vetsuursamestelling wat die gesondheidsstatus van beesvleis kan beinvloed. Hoë vlakke van versadigde vette word geassosieer met 'n verhoogde serum lae-digtheid lipoproteïen cholesterol konsentrasie wat 'n risiko faktor vir koronêre hartsiektes inhou. Daar word geglo dat vetsuursamestelling, in 'n mate, onder genetiese beheer is. Dus, in hierdie studie is 'n soektog vir QTL gedoen wat die relatiewe hoeveelhede versadigde, mono-onversadigde en poli-onversadigde vetsure in beesvleis affekteer. 'n Belangrike QTL met bykomende effekte op vetsuur-samestellings wat naby die sentromeer van chromosoom twee voorkom, is waargeneem. Bykomend is vyf minder belangrike QTL, met dominante effekte of vetsuursamestellings, waargeneem. Die resultate van hierdie studie het aangedui dat relatiewe hoeveelhede van versadigde, mono-onversadigde en poli-onversadigde vetsure in 'n mate onder genetiese beheer is. Dus is daar 'n moontlikheid dat die vetsuur-samestelling van beesvleis verbeter kan word deur die posisie van die

relevante QTL te bepaal deur gebruik te maak van genetiese merkers, en deur gebruik te maak van die regte kruisteling-sisteme.

In Hoofstuk 3 is die terugkruisingseksperiment, wat 'n belangrike rol in die toevoeging van nuwe genetiese materiaal in 'n gevestigde ras of lyne van beesproduksie, beskryf. Wanneer dit gekoppel word met merker of geen teenwoordigende seleksie, kan dit gebruik word om 'n spesifieke geen of chromosoom area vanaf een populasie na 'n ander te skuif. Die genomiese struktuur van vleisbeeste wat geproduseer is met terugkruising is getoets vir loki wat nie gekoppel is tot 'n spesifieke lokus nie en wat geskuif is vanaf 'n skenker ras tot 'n onvanger ras waarvan die spesifieke genetiese variant andersins nie teenwoordig sou wees nie. Genotipes van die twee ouer-bevolkings, hul F_1 nageslag en die twee opeenvolgende terugkruisings generasies van beeste is bepaal by 34 onafhanklike loki. In hierdie studie is daar 'n kompromie aangegaan met die introgressie omdat die merkers wat gebruik is nie genoegsaam gesegregeer het tussen die twee stigters-populasies nie. In opsomming, met die integrasie van die kennis wat verkry is uit die vorige studies, is 'n nuwe soektog vir QTL voorgestel deur gebruik te maak van Nguni en Angus beeste. Indien suksesvol, sal die resultate van hierdie studie dalk lei tot die gebruik van merkergebaseerde introgressie om die weerstand teen bosluise van die Suid Afrikaans Angus te verhoog en die voedingseienskappe en karkaskwaliteit van Ngunis te verbeter.

Chapter 7

References

CHAPTER 7

References

Abdel-Azim, G. & Freeman, A. E. (2002). Superiority of QTL-Assisted Selection in Dairy Cattle Breeding Schemes. J. Dairy Sci.85:1869-1880.

Alexander, L., MacNeil, M.D., Geary, T., Snelling, W.M., Warren, el al. (2007). Quantitative trait loci with additive effect on palatability and fatty acid composition of meat in a Wagyu –Limousin F2 population. Animal Genetics (*in press*).

Anderson, D.E. (1970). Cancer eye in cattle. Mod Vet Pract 51:42-47

Anderson, S.B. (2000). Amplified Fragment Length Polymorphism (http://wwwagsci:.kvl.dk/breed/kortieg1/background/markertypes/aflp-markers.htm, accessed 09/02/01).

Andersson, L. (2001). Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet. 2:130-138

Andersson, L., Haley, C.S., Ellegren, H., Knott, S.A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I., Hakansson, J. & Lundstrom, K. (1994). Genetic mapping of quantitative trait loci for growth and fatness in the pig. Science 263:1771-1774.

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

Arthur, P.F. (1995). Double muscling in cattle: A review. J. Agric. Res. 46: pp 1493-1515.

CHAPTER 7

Ashwell, M.S. & Van Tassell, C.P. (1999). Detection of putative loci affecting milk, health, and types of trait in a US Holstein population using 70 microsatellite markers in a genome scan. J. Dairy Sci. 82:2497-2502.

Ashwell, M.S., Heyen, D.W., Van Tassell, C.P., Sonstegard, T.S., Da, Y., VanRaden, P.M. (2004). Detection of quantitative trait loci affecting milk production, health, and reproductive trait in Holstein cattle. J. Dairy Sci. 87:468-475.

Ashwell, M.S., Schnabel, R.D., Sonstegard, T.S. & Van Tassell, C.P. (2002). Fine mapping of QTL affecting protein percent on BTA6 in a popular US Holstein family, in: Proceeding of the 7th world Congress on Genetics Applied to livestock Production, Montpellier, France, 19-23, ISBN 2-7380-1052-0, paper 09-29.

Ashwell, M.S., Van Tassell, C.P. & Sonstegard, T.S., (2001). A genome scan to identify QTL affecting economically important traits in a U.S. Holstein population. J. Dairy Sci. 84:2535-2542.

Ausubel, F., Brent, M.R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., & Struhl. K. (1994). In Current Protocols in Molecular Biology 1. John Wiley & Sons, Inc. New York.

Barendse, W., Armitage, S.M., Kossark, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, L., Neibergs, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McGraw, F., Ron, M., Teale, A.J., Fries, R., McGraw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E., & Hetzel, J. (1994). A genetic linkage map of the bovine genome. Nat. Genet. 6:227-236.

Barendse, W., Bunch, R.J., Harrison, B.E. & Thomas, M.B. (2006). The growth of hormone 1 GH1:c.457C>G mutation is associated with intramuscular and rump

fat distribution in a large sample of Australian feedlot cattle. Anim. Genet. 37:211-214.

Barendse, W., Vaiman, D., Kemp, S.J., Sugimoto, Y., Armitage, S.M., Williams, J.L., Sun, H.S., Eggen, A., Agaba, M., Aleyasin, S.A., Band, M., Bishop, M.D., Buitkamp, J., Byrne, K., Collins, F., Cooper, L., Coppettiers, W., Denys, B., Drinkwater, R.D., Easterday, K., Elduque, C., Ennis, S., Erhardt, G., Ferretti, L., Flavin, N., Gao, Q., Georges, M., Gurung, R., Harlizius, B., Hawkins, G., Hetzel, J., Hirano, T., Hulme, D., Jorgensen, C., Kessler, M., Kirkpatrick, B.W., Konfortov, B., Kostia, S., Kuhn, C., Lenstra, J.A., Leveziel, H., Lewin, H.A., Leyhe, B., Lil, L., Burriel, I.M., McGraw, R.A., Miller, J.R., Moody, D.E., Moore, S.S., Nakane, S., Nijman, I.J., Olsaker, I., Pomp, D., Rando, A., Ron, M., Shalom, A., Teale, A.J., Thieven, U., Urquhart, B.G.D., Vage, D.I., VandeWeghe, A., Varvio, S., Velmala, R., Vikki, J., Weikard, R., Woodside, C., Womack, J.E., Zanotti, M. & Zaragoza, P . (1997). A medium density genetic linkage map of bovine genome. Mamm Genome 8:21-28.

Bellinge, R.S.H., Liberles, D.A., Iaschi, S.PA., O' Brien, P.A., & Tay, BK. (2005). Myostatin and its implications on animal breeding: A Review. Anim. Genet. 36:1-6

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

Blott, S., Kim, J.J., Moisio, S., Schmidt-Kuntzel, A., Cornet, A., Berzi, P., Cambisano, N., Ford, C., Grisart, B., Johnson, D., Karim, L., Simon, P., Snell, R., Spelman,

R., Wong, J., Vilkki, J., Georges, M. Farnir, F. & Coppieters, W. (2003). Molecular dissection of a QTL: A phenylalanine to tyrosine substitution in transmembrane domain of bovine growth hormone receptor is associated with major effect on milk yield and composition. Genetic, 163:253-266.

Boichard, D., Grohs,F., Bourgeois, F., Cerqueira, F., Faugeras, R., Neau, A., Rupp, R., Amigues, Y., Boscher, M.Y. & Leveziel, H., (2003). Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Selection Evol. 35: 77-101.

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

Boylston, T.D., Morgan, S.A., Johnson, K.A., Busboom, J.R., Wright, R.W., & Reefes, J.J. (1995). Lipid content and composition of Wagyu and domestic breeds of beef. J. Agric. Food Chem. 43:1202-1207.

Brascamp, E.W., van Arendonk, J.A.M. & Groen, A.F. (1993). Economic appraisal of the utilization of genetic marker in dairy cattle breeding. J. Dairy Sci. 76:1204-1214.

Casas, E., Keele, J.E., Shackelford, S.D., Keele, J.W., Koohmaraie, M., & Stone, R.T. (2004). Identification of quantitative traits loci for growth and carcass composition in cattle. Anim Genet. 35(1): pp 2-6.

Casas, E., Shackelford, S.D., Keele, J.W., Koohmaraie, M., Smith, T.P.L., & Stone, R.T. (2003). Detection of quantitative traits loci for growth and carcass composition in cattle. J. Anim. Sci. 81(12): pp 2976-2983.)

CHAPTER 7

Casas, E., Shackelford, S.D., Keele, J.W., Stone, R.T., Kappes, S.M., & Koohmaraie, M., (2000). Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. J. Anim. Sci. 78: 560.

Charlier, C., Coppieters, W., Farnir, F., Grobet, L., Leroy, P.L., Michaux, C., Mni, M., Schwers, A., Vanmanshoven, P., Hanset, R., & Georges, M., (1995). The mh gene causing double-muscling in cattle maps to bovine chromosome 2. Mamm. Genome 6:788-792

Charlier, C., Segers, K., Karim, L., Shay, T., Gyapay, G., Cockett, N. & Goerges, M. (2001). The callipyge mutation enhance the expression of coregulated imprinted genes in cis without affecting their imprinting status. Nature Genetics. 27:367-369.

Cheverud, J.M. (2001) A simple correction for multiple comparisons interval mapping genome scans. Heredity 87:52-58.

Christie, W.W. (1982). The preparation of derivatives of lipids. In Lipid Analysis, 2nd ed. Pergamon Press, New York, N.Y.

Clawson, M.L., Heaton, M.P., Chitko-McKown, C.G., Fox, J.M., Smith, T.P., Snelling, W.M., Keele, J.W. & Laegreid, W.W. (2004). Beta-2-microglobulin haplotypes in U.S. beef cattle and association with failure of passive transfer in newborn calves. Mamm. Genome 15:227-236.

Clutter, A. C. & Schinckel, A. P. 2001. Genetic improvement of sire and dam lines for enhanced performance of terminal crossbreeding systems. Swine Genetics Fact Sheet No. 14. National Swine Improvement Fed., Purdue Univ. Coop. Ext. Serv. West Lafayette, IN.

CHAPTER 7

Cockett, N.E., Shay, T.L., & Smit, M. (2001). Analysis of the sheep genome. Physiol. Genomics, 7:69-78

Cohen, M., Serrousi, E., Ron, M., Reichenstein, M., Plis-Finarov, A., Shani, M., & Weller, J.I. (2002). Population wide linkage disequilibrium between a SNP and a QTL affecting milk production on BTA6 in dairy cattle. Proc. 7th World Cong. Genet.Appl. Livestk. Prod., Montpellier, France, 19-23 August 2002, ISBN 2-7380-1052-0, paper 9-12.

Coppieters, W., Riquet, J., Arranz, J.J., Berzi, P., Cambisano, N., Grisart, B., Karim, L., Marcq, F., Moreau, L., Nezer, C., Simon, P., Vanmanshoven, P., Wagenaar, D., & Georges, M. (1998). A QTL with a major effect on milk yield and composition maps to bovine chromosome 14. Mamm. Genome 9:540-544

Cowan, C.M., Meland, O.M., Funk, D.C., & Erf, D.F. (1997). Realized genetic gain following marker-assisted selection of progeny tested dairy bulls. Abstract P296 in Proc. Plant and Animal Genome V. Available: htt://www.intl-pag.org/pag/5/abstracts/p-5j-296.html.

Culley, G. (1807). Observations on livestock, 4th ed.G. Woodfall, London, UK.

D'Surney, S.J., Shugart, L.R., & Theodorakis, C.W. (2001). Genetic markers and genotyping methodologies: an overview. Ecotoxicology, 10:201-204.

Darvasi, A., Weinreb, A., Minke, V., Weller, J.L., & Soller, M. (1993). Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943-951.

de Koning, D.J., Dekkers, J.C.M. & Haley, C.S., (2003). Designs for QTL detection in livestock and their implications for MAS. Conference Marker-assisted Selection:

A Fast Track to Increase Genetic Gain in Plant and Animal Breeding? Session II. MAS in Animals. In: *Conference of the FAO Biotechnology Forum Molecular marker-assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock and fish in developing countries*. (10°, 17th November - 12th December, 2003, Turin, Italy). http://www.fao.org/biotech/Conf10.htm.

de Koning, D.J., Janss, L.L., Rattink, A.P. Van Oers, P.A., De Vries, B.J., Groenen, M.A., Van der Poel, J.J., De Groot, P.N., Brascamp, E.W., & Van Arendonk. (1999). Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs (Sus scrofa). Genetics 152:1679-1690.

De Smet, S.L., Raes, K., & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. Anim. Res. 53:81-98.

De, S., MacNeil, M.D., Wu, X.L., Michal, J.J., Xiao, Q., Garcia, M.D., Griffin, K.B., Gaskins, C.T., Reeves, J.J., Bussboom, J.R., Wright Jr., R.W., & Jiang, Z. (2004). Detection of quantitative trait loci for marbling and backfat in Wagyu x Limousin F2 crosses using a candidate gene approach. Proceedings, Western Section, American Society of Animal Science 55:95-98.

Dekkers, J.C.M., & Hospital, F. (2002). The use of molecular genetics in the improvement of agricultural populations. Nature Reviews Genetics 3:22-32.

Dekkers, J.C.M. (2004). Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci.82 (no. 13 Electronic Suppl.):E313-E318.

Devis, G.P. & DeNise, S.K. (1998). The impact of genetic marker on selection. J. Anim. Sci. 76 2331-2339

CHAPTER 7

Dryden, F.D., & Marchello, J.A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. J. Anim. Sci., 31(1):36-41.

Eggen, A., (2003). Basics and tools of genomics. Outlook on Agriculture 32:215-217

Elias Calles, J.A., Gaskins, C.T., Busboom, J.R., Duckett, S.K., Cronrath, J.D., & Reeves, J.J. (2000). Sire variation in fatty acid composition of crossbred Wagyu steers and heifers. Meat Science, 56:23-29.

Elsen, J.M. (2003). Utilization of genomic information in livestock improvement. Outlook on Agriculture 32:241-245.

Everts-van der Wind, A., Kata, S.R., Band, M.R., Rebeiz, M., Larkin, D.M., Everts, R.E., Green, C.A., Liu, L., Natarajan, S., Goldammer, T., Lee, J.H., Mckay, S., Womack, J.M., & Lewin, H.A. (2004). A 1463 Gene Cattle-Human Map With Anchor Points Defined by Human Genome Sequence Coordinates. Genome Res. 14:1424-1437.

Fadiel, A., Anidi, I., & Eichenbaum, K. D. (2005). Farm animal genomics and informatics: an update. Nucleic Acids Res. 33:6308-6318.

Falush, D., Stephens, M., & Pritchard, J.K. (2003). Inference of population structure II. Linked loci and correlated allele frequencies. Genetics 164:1567-1587

Freyer, G., Kuhn, C., Weikard, R., Zhang, Q., Mayer, M., & Hoeschele, I. (2002). Multiple QTL on chromosome six in dairy affecting yield and content traits. J. Anim. Breed. Genet. 119:69-82.

CHAPTER 7

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

Gagnoux, D., Daccord, R., Gibon, A., Poffet, J.R., & Sibbald, A.R. (2000). Livestock farming systems: integrating animal science advances in the search for sustainability. European Association of Animal Production, Publication 97.

Galal, S., Boyazoglu, J., & Hammond, K. (Eds). (2000). Developing strategies for lower input animal production environments. ICAR Technical Series, 3.

Gasparin, G., Miyata, M., Coutinho, L.L., Martinez, M.L., Teodora, R.L., Furlong, J., Machado, M.A., Silva, M.V.G.B., Sonstegard, T.S., & Regitano, L.C.A. (2007). Mapping of quantitative trait loci controlling tick [Riphicephalus (Boophilus) microplus] resistance on bovine chromosomes 5, 7, and 14. Animal Genetics, 38:453-459

Garnier, J.P., Klot, R., & Platow, G. (2003). The potential impact of current animal research on the meat industry and consumer attitudes towards meat. Meat Sci. 63:79-88.

Gautier, M., Barcelona, R.R., Fritz, S., Druet, T., Biochard, D., Eggen, A., & Meuwissen, T.H.M. (2005). Fine mapping and physical characterization of two linkage quantitative trait loci affecting milk fat yield in dairy cattle on BTA 26. Genetics 172:425-436.

Georges, M., Nielsen, D., Mackinon, M., Mishra, A., Okimoto, R., Pasquino, A. T., Seargeant, L. S., Sorensen, A, Steele, M. R., Zhao, X., Womack, J. E. &

Hoeschele, I. (1995). Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139: pp 907-920.

Gotz, K.U. & Ollivier, L. (1994). Theoretical aspects of applying sib-pair linkage tests to livestock species. Genet. Sel. Evol. 24:29-42.

Green, P., Falls, K., and Crooke, S., (1990). Documentation for CRI-MAP. Ver2.4 Washington School of Medicine, St. Louis, MO.

Grignola, F.E., Hoeschele, I., & Tier, B. (1996a). Mapping quantitative trait loci via residual maximum likelihood: I. Methodology Genet. Sel. Evol. 28:479-490.

Grignola, F.E., Hoeschele, I., Zhang, Q., & Thaller, G. (1996b). Mapping quantitative trait loci via residual maximum likelihood: II. A simulation study. Genet. Sel. Evol. 28:491-504.

Grignola, F.E., Zhang, Q., & Hoeschele, I. (1997). Mapping linked quantitative trait loci via residual maximum likelihood. Genet. Sel. Evol. (*in press*)

Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Cambisano, N., Mni, M., Reid, S., Simon, P., Spelman, R., Georges, M., & Snell, R. (2002). Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in bovine DGAT1 gene with major effect on milk yield and composition. Genome Res, vol 12: pp 222-231.

Grisart, B., Farnir, F., Karim, L., Cambisano, N., Kim., J.J., Kvasz, A., Mni, M., Simon, P., Frere, J.M., Coppieters, W., & Georges, M. (2004). Genetic and function confirmation of the causality of the DGAT1 K232 quantitative nucleotide in affecting milk yield and composition. Proc. Nat. Acad. Sci. USA101:2398-2403.

Grobet, L., Martin, L.J.R., Poncelet, D., Pirottin, D., Brower, B., Riquet, J., Schoeberlein,A., Dunner, S., Menissier, F., Massabanda, J., Fries, R., Hanset, R., & Georges, M. (1997). A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nature Genet. 17: pp 71-74.

Groenen, M.A.M., Crooijmans, R.P.M.A., Veenendaal, T., Van Kaam, J.B.C.H.M., & Verijken, A.L.J. (1997). QTL mapping in chicken using a three generation full sib family structure of an extreme broiler broiler-cross. Animal Biotech. 8:41-46.

Grosz, M.D. (Bylined by Clint Peck). (1998). How will molecular DNA markers affect the cattle industry? Montana Farmers-Stockman. 8-11.

Grosz, M.D. & MacNeil, M.D. (1999). The spotted locus maps to bovine chromosome 6 in Hereford-cross population. J. Heredity 90: 233-236.

Grosz, M.D. & MacNiel, M.D. (2003).Using markers to introduce novel genes in breeding. Fort Keogh Livestock and Range Research Laboratory, Research Report pp. 58.

Haley, C.S., Knott, S.A., & Elsen, J.M. (1994). Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136: pp 1195-1207

Hanotte, O., Ronin, Y., Agaba, M., Nilsson, P., Gelhaus, A., Horstmann, R., Sugimoto, Y., Kemp, S., Gibson, J., Korol, A., Soller, M., & Teale, A. (2003). Mapping of quantitative trait loci controlling trypanotolerance in a cross of tolerant West African N'Dama and susceptible East African Boran cattle. Proc Natl Acad Sci USA. 100:7443-7448.

Hanset, R. (1981). Double muscling in cattle – its inheritance – its meaning for beef production. Ann. Med.Vet. 125:85-95.

CHAPTER 7

Haseman, J.K. & Elston, R.J. (1972). The investigation of linkage between a quantitative trait and a marker locus. Behav. Genet. 2:3-19

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

Hoeschele, I., Uimari, P., Grignola, F.E., Zhang, Q., & Gage, K.M. (1997). Advances in statistical methods to map quantitative trait loci in outbred populations. Genetics 147:1445-1457.

Hospital, F. & Charcosset, A. (1997). Marker assisted selection introgression of quantitative trait loci. Genetics 147: pp 1469-1485

Hospital, F. (2005). Selection in backcross program. Phil. Trans. R. Soc. B 360, 1503-1511.

Hugo, H. M. (2006). Genetic engineering application in animal breeding. Electronic J. Biotech. 9: No.2.

Ihara, N., Takasuga, A., Mizoshita, K.,Takeda, H., Sugimoto, M., Mizoguchi, Y., Hirano, T., Itoh, T., Watanabe, T., Reed, K.M., Snelling, W.M., Kappes, S.M., Beattie, C.W., Bennett, G.L., & Sugimoto, Y.(2004). A comprehensive genetic map of the cattle genome based on 3802 microsatellites. Genome Res. 14:1987-1998.

Ikeobe, C.O.N., Woolliams, J.A., Morrice, D.R., Law, A., Windsor, D., Burt, D.W., & Hocking, P.M. (2002). Quantitative trait loci affecting fatness in the chicken. Animal Genetics 33:426-435.

Jiang, Z., Kunej, T., Michal, J.J., Gaskins, C.T., Reeves, J.J., Busboom, J.R., Dovc, P. & Wright, Jr. R.W. (2005). Significant associations of the mitochondrial

transcription factor A promoter polymorphisms with marbling and subcutaneous fat depth in Wagyu x with Limousin F2 crosses. Biochemical and Biophysical Research communications 334:516-523.

Kappes, S.M., Keele, J.W., Stone, R.T., Sonstegard, T.S., Smith, T.P.L., McGraw, R.A., LopezCorrales, N.L., & Beattie, C.W. (1997). A second generation linkage map of the bovine genome. Genome Research, 7: pp235-249.

Katan, M.B., Zock, P.L., & Mensink, R.P. (1994). Effects of fatty acids on blood lipids in humans: an overview. American Journal of Clinical Nutrition 60: 1017S-1022S.

Katan, M.D. (2000). Nutritional interventions: the evidence. Proceedings of the Nutrition Society, 59:417-418.

Keele, J.W., Shackelford, S., Kappes, S.M., Koohmaraie. M., & Stone, R.T., (1999). A region of bovine chromosome 15 influences longissimus tenderness in the steer. J. Anim. Sci. 77:1364-1371.

Kim, J.J., Farnir, F., Coppieters, W., Johnson, D., & Georges, M. (2002). Evaluation of a new QTL fine mapping method exploiting linkage disequilibrium on BTA 14 and BTA 20 in dairy cattle, in: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, 19-23 August2002, ISBN 2-7380-1052-0, paper 21-23.

Kim, J.J., Farnir, F., Savell, J., & Taylor, J.F. (2003). Detection of quantitative trait loci for growth and beef carcass fatness traits in a cross between *Bos taurus* (Angus) and *Bos indicus* (Brahman) cattle. J. Anim. Sci. 81: pp 1933-1942.

Kishi, Y., Hallerman, E., & Soller, M., (1990). Marker assisted selection of a candidate bull for progeny testing programmes. Anim. Prod. 51:63-74.

CHAPTER 7

Klungland, H., Sabry, A., Heringstand, B., Olsen, H., Gomez-Raya, G.L., Vage, D.I., Olsaker, I., Odegard, J., Klemetsdal, G., Schulman, N., Vikki, J., Ruane, J., Aasland, M., Ronningen, K., & Lien, S. (2001). Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Mammalian. Genome, 12:837-842.

Knott, S.A., Marklund, L., Haley, C.S., Andersson, K., William, D., Ellegren, H., Fredholm, M., Hansson, I., Hoyheim, B., Lundstrom, K., Moller, M., & Andersson, L. (1998). Multiple marker mapping of Quantitative trait loci in a cross between outbred wild boar and large white pigs. Genetics 149: pp 1069-1080.

Kohler-Rollefson, I. (2004). Farm animal genetic resources-safeguarding national assets for food security and trade. GTZ, FAO, CTA, Exborn, Germany.

Koudande, O.D., Iraqi, F., Thompson, P.C., Teale, A.J., & Van Arendonk, J.A.M. (2000). Strategies to optimize markers assisted introgression of multiple QTL. Mamm Genome 11:145-150.

Koudande, O.D., Van Arendonk, J.A.M., & Iraqi, F. (2005). Marker assisted introgression of trypanotolerance QTL in mice. Mamm Genome, 16:112-119.

Kramer, J.K., Fellner, G.V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.M., & Yurawecz, M.P. (1997). Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total transfatty acids. Lipids 32:1219-1228.

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

Lander, E.S. & Botstein,D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

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

Looft, C., Reinsch,N., Karall-Albrecht, C., Paul, S., Brink, M., Thomsen, H., Brockmann, G., Kuhn, C., Schwerin, M., & Kalm, E. (2001). Mammary gland EST showing linkage disequilibrium to a milk production QTL on bovine chromosome 14. Mamm. Genome 12:646-650.

Mackinnon, M.J. & Weller J.I. (1995). Methodology and accuracy of estimation of quantitative trait loci parameters in a half-sib design using maximum likelihood. Genetics 141:755-770

Mackinnon, M.J. & Georges, M. (1997). A bottom up approach to markers-assisted selection. Livest. Prod. Sci. 54:227-248.

MacNeil, M.D., & Grosz, M.D. (2002). Genome-wide scan for QTL affecting carcass traits in Hereford x composite double backcross population. J. Anim. Sci. 80 (9); pp 2316-2324.

Maki-Tanila, A., De Koning, D.J., Elo, K.T., Moisio, S., Velmala, R., & Vilkki, H.J. (1998). Mapping multiple quantitative trait loci by regression in half sib designs, in: Proceedings of the 6th World Congress on Genetics Applied to Livestock

production, Armidale. 26, 6 WCGALP Congress office, University of New-England, Armidale. 26:269-272.

Matjuda, L.E., (2005). The role of the Angus breed in the emerging beef sector in South Africa. Proc. 9th World Angus Forum Technical Meeting. Cape Town, South Africa Angus Society, 61-65.

May, S.G., Sturdivant, C.A., Lunt, D.K., Miller, R.K., Smith, S.B. (1993). Comparison of sensory characteristics and fatty acid composition between Wagyu crossbred and Angus steers. Meat Science 35:289-98.

McPherron, A.C., Lawler, A.M., & Lee, S.J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-β super family member. Nature,387: pp 83-90.

Melton, S.L., Amiri, M., Davis, GW., & Backus, W.R. (1982). Flavor and chemical characteristics of ground beef from grass-, forage- grain- and grain finished steers. J. Anim. Sci. 55:77-87.

Michal, J.J., Zhang, Z.W., Gaskins, C.T., & Jiang, Z. (2006). The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. Animal Genetics 37:400-402.

Mir, P.S., Mir, Z., Kuber, P.S., Gaskins, C.T., Martin, E.L., Dodson, M.V., Calles, J.A.E., Johnson, K.A., Busboom, J.R., Wood, A.J., Pittenger, G.J., & Reeves, J.J. (2002). Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenges in high percentage Wagyu, Wagyu x Limousin, and Limousin steers fed sunflower oil-containing diets. J. Anim. Sci. 80:2996-3004.

Mir, Z., Paterson, L.J., & Mir, P.S. (2000). Fatty acid composition and conjugated linoleic acid content of intramuscular fat in crossbred cattle with and without Wagyu genetics fed a barley based diet. Can. J. Anim. Sci. 80:195-197.

Mizoshita, K., Watanabe, T., Hayashi, H., Kubota, C., Yamakuchi, H., Todoroki, J., & Sugimoto, Y. (2004). Quantitative trait loci analysis for growth and carcass traits in a half-sib family of purebred Japanese Black (Wagyu) cattle. J. Anim. Sci. 82: pp 3415-3420.

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

Mpofu, N. (2002). Camparison of indigenous and foreign cattle for beef production at Matopos Research Station in Zimbabwe. http://agtr.Cigar.org/Casestudy/case-mpofu-Matopos-51.htm.

Murrieta, C.M., Hess, B.W. & Rule, D.C. (2003). Comparison of acidic & alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Science 65:523-529.

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

Oak, A.., Iwaki, F., Dohgo, T., Ohtagaki, S., Noda, M., Shiozaki, T., Endoh, O., & Ozaki, M. (2002). Genetic effect on fatty acid composition of carcass fat of Japanese Black Wagyu steers. J. Anim. Sci. 80:1005-1011.

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

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E. & Tanksley, S.D. (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721-726

Perry, D., Nicholls, P.J., & Thompson, J.M. (1998). The effect of sire breed on the melting point and fatty acid composition of subcutaneous fat in steers. J. Anim. Sci. 76:87-95.

Pitchford, W.S., Deland, M.P.B., Siebert, B.D., Malau-Aduli, A.E., & Bottenma, C.D.K. (2002). Genetic variation in fatness and fatty acid composition of crossbred cattle. J. Anim. Sci. 80: 2825-2832.

Plante, Y., Gibson, J.P., Nadesalingam, J., Mehrabani-Yeganeh, H., Lefebvre, S., Vandervoort, G., & Jansen, G.B. (2001). Detection of QTL influencing milk production traits on 10 chromosomes in Holstein cattle. J. Dairy Sci. 84:1516-1524.

Pretty, J. (1998). The living land. London,UK: Earthscan Publications.

Pritchard, J.K. & Wen, W. (2004). Documentation for STRUCTURE software: Version 2.2. http://pritch.bsd.uchcago.edu/software/structure22/readme.pdf.

Pritchard, J.K., Stephens, M., & Donnelly, P. (2000a). Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Pritchard, J.K., Stephens, M., Rosenberg, N.A. & Donnelly, P. (2000b). Association mapping in structured populations. Am. J. Hum. Genet. 67: 170-181.

Raes, K., De Smet, S., & Demeyer, D. (2001). Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Anim. Sci. 73:253-260.

Ramsay, K., Smuts, M. & Els, H.C. (2000). Adding value to South African landrace breeds conservation through utilisation. Rare Breeds International Mini Symposium, Zurich, Switzerland 20 August 1999.

Riquet, J., Coppieters, W., Cambisano, N., Arranz, J.J., Berzi, P., Davis, S.K., Grisart, B., Farnir, F., Karim, L., Mni, M., Simon, P., Taylor, J.F., Vanmanshoven, P., Wagenaar, D., Womack, J.E., & Goerges, M. (1999). Fine mapping of quantitative trait loci by identity by descent in outbred populations: Application to milk production in dairy cattle. Pro. Nat. Acad. Sci. USA 96:9252-9257.

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

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

Rule, D.C., Broughton, K.S., MacNeil, M.D., & Short, R.E. (1997). Influence of sire growth potential, time on feed, and growing-finishing strategy on cholesterol and fatty acids of the ground carcass and longissimus of beef steers. J. Anim. Sci. 75:1525-1533.

Rule, D.C., Broughton, K.S., Shellito, S.M., & Maiorano, G. (2002). Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. J. Anim. Sci. 80: 1202-1211.

Sarmiento, R.M. & Garcia, J.P. (2007). Estimation of genetic parameters and variance components for growth traits in Romosinuao cattle in the Colombian humid tropics. Gent. Mol. Res. 6(3):482-491.

Scholtz, M.M. (1988). Selection possibilities of hardy beef breed in Africa: the Nguni example. In Proc. 3rd Wld. Cong. Sheep and Cattle Breeding, Paris.

Scholtz, M.M. (2005). The role of research and livestock industry *in situ* conservation of livestock genetic resource. Proceedings of 4th AACAA and 31st TSAP Annual Meeting, 311-316.

Schulman, N.F., Moisio, S.M., de Koning, D.J., Elo, K., Maki-Tanila, J.A., & Vilkki, J. (2002). QTL for health traits in Finnish Ayshire cattle. Proc. 7th World Cong. Genet. Appl. Livest. Prod. August 19-23, Montpellier, France. Paper 09-9.

Seaton, G., Haley, C.S., Knott, S.A., Kearsey, M. & Visscher, P.M. (2002). QTL Express: mapping quantitative trait loci in simple and complex pedigree. Bioinformatics 18:339-340.

Short, R.E., MacNeil, M.D., Gross, M.D., Gerrard, D.E., & Grings, E.E. (2002). Pleiotrophic effects in Hereford, Limousin, and Piedmontese F2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele. J. Anim. Sci. 80:1-11

Smith, T.P.L., Lopez-Corrales, N.L., Kappes, S.M., & Sonstegard, T.S. (1997). Myostatin maps to the interval containing the bovine mh locus. Mamm. Genome 8: pp 742-744.

Soller, M. & Plotkin-Hazan, J. (1977). The use of marker allele for the introgression of linked quantitative alleles. Theor Appl Genet, 51:133-137.

Spelman, R.J., Coppieters, W., Karim, L., Van Arendok,J.A.M., & Bovenhuis, H., (1996). Quantitative trait loci analysis for five milk production traits on chromosome six in Dutch Holstein Friesian population. Genetics 144: 1799-1808.

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

Spelman, R.J., Ford, C.A., McElhinney, P., Gregory, G.C., & Snell, R.G. (2002). Characterization of DGAT1 gene in the New Zealand dairy population. J. Dairy. Sci. 85:3514-3517.

Spickett, A.M., De Klerk, D., Enslin, C.B., & Scholtz, M.M. (1989). Resistance of Nguni, Bonsmara and Hereford cattle to ticks in bushveld region of South Africa. Onderstepoort Journal of Veterinary Research 56:245-250.

Stone, R.T., Grosse, W.M., Casas, E., Smith, T.P., Keele, J.W., & Bennett, G.L. (2002). Use of bovine EST data and human genomic sequences to map 100 gene-specific bovine markers. Mamm. Genome, 13:.211-215

Stone, R.T., Keele, J.W., Shackelford, S.D., Kappes, S.M., & Koohmaraie, M., (1999). A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits. J. Anim. Sci. 77: 1379.

Strydom, P.E. (1998). The characterisation of indigenous cattle in relation to production and product characteristics. PhD thesis, University of Orange Free State, Bloemfontein, South Africa. Meat Science 55:79-88.

Strydom, P.E, Naudé, R.T, Scholtz, M.M, Van Wyk, J.B (2000*a*) Characterisation of indigenous cattle breeds in relation to carcass characteristics. *Animal Production* 70: 241–252.

Sturdivant, C.A., Lunt, D.K., Smith, G.C., & Smith, S.B. (1992). Fatty acid composition of subcutaneous and intramuscular adipose tissues and M. longissimus dorsi of Wagyu cattle, Meat Science. 32:449-458.

Taylor, J.F., Coutinho, L.L., Herring, K.L., Gallagher Jr, D.S., Brenneman, R.A., Burney, N., Sanders, J.O., & Turner, J.W. (1998). Candidate gene analysis of GH1 for the effects on growth and carcass composition of cattle. Anim. Genet. 29;194-201.

Thaller, G., & Hoeschele, I. (1996b). A Monte Carlo method for Bayesian analysis of linkage between single markers and quantitative trait loci: A simulation study. Theor. Appl. Genet. 93:1167-1174.

Thaller, G., Kramer, W., Winter, A., Kaupe, B., Erhardt, G., & Fries, R. (2003). Effects of DGAT1 variants on milk production traits in German cattle breeds. J. Anim. Sci. 81;1911-1918.

Thallman, R.M., Bennett, G.L., Keele, J.W., & Kappes, S.M. (2001a). Efficient computation of genotype probabilities for loci with alleles. Allelic peeling. J. Anim. Sci. 79:26-33.

Thallman, R.M., Bennett, G.L., Keele, J.W., & Kappes, S.M. (2001b). Efficient computation of genotype probalities for loci with many alleles. II. Iterative method for large, complex pedigrees. J. Anim. Sci. 79:34-44.

Thue, T.D. & Buchanan, F.C. (2004). Linkage mapping of NPY to bovine chromosome 4. Anim. Genet. 35:.265-269.

Trail, J.C.M., D'Ieteren, G.D.M., & Tael, A.J. (1989). Trypanotolerance and the value of conserving livestock genetic resources. Genome 31:805-812.

Tuiskula-Haavisto, M., De Koning, D.J., Honkatukia, M., Schulman, N.F., Maki-Tanila, A., & Vilkki, J. (2004). Quantitative trait loci with parent-of-origin effects in chicken. Genet. Res. Camb. 84:57-66.

Uimari, P., Thaller, G., & Hoeschle, I. (1996a). The use of multiple markers in Bayesian method for mapping quantitative trait loci. Genetics 143:1831-1842.

Uimari, P., Zhang, Q., Grignola, F.E., Hoeschle, I., & Thaller, G. (1996b). Analysis of QTL workshop I granddaughter design data using least squares, residual maximum likelihood and Bayesien methods. J. Quant. Trait Loci 2: (7).

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

Vignal, A., Milan, D., San Cristolbal, M., & Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genet. Sel. Evol. 34:275-305.

Viitala, S.M., Schulman, N.F., De Konning, D.J., Elo, K., Kinos R., Virta, A., Maki-Tanila, A., & Vilkki, J.H. (2003). Quantitative trait loci affecting milk production trait in Finnish Ayrshire dairy cattle. J. Dairy Sci. 86:1828-1836.

Vukasinovic, N., Denise, S.K., & Freeman, A.E. (1999). Association of growth hormone loci with milk yield traits in Holstein bulls. J. Dairy Sci. 82:788-794.

Webb, E.C., De Smet S., Van, N.C., Martens, B., & Dremeyer, D.I., (1998). Effect of anatomical location on the composition of fatty acids in double-muscled Belgian Blue cows. Meat Science 50:45-53.

Webb, J. (2001). New technology for genetic improvement of livestock. London Swine Conference- The Pork Industry and Public Issues, pp 27-41.

Weiner, P., Maclean, I., Williams, J.L., & Woolliams, J.A. (2000). Testing for the presence of previously identified QTL for milk production traits in new population. Anim. Genet. 31:385-395.

Weller, J.I. (1986). Maximum likelihood techniques for the mapping and analysis of quantitative trait loci with the aid of genetic markers. Biometrics 42:627-640.

Weller, J.I., Golik, M., Seroussl, E., Ezra, E., & Ron, M. (2003). Population–wide analysis of a QTL affecting milk fat production in the Israeli Holstein population. J. Dairy. Sci. 86:2219-2227.

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

Westerling, D.B., & Hedrick, H.B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. J. Anim. Sci., 48(6):1343-1348.

Williams, J.L. (2005). The use of molecular marker-assisted selection in animal breeding and biotechnology. Rev. Sci. Tech. Off. Int. Epiz., 24:379-391.

Wimmer, K., Murani, E., Ponsuksili, S., Yerle, M., & Schellander, K. (2002). Detection of quantitative traits loci for carcass trait in the pig by using AFLP. Mamm Genome, 13:206-210.

Winter, A., Kramer, W., Werner, F.A.O., Kollers, S., Kata, S., Durstewitz, G., Buitkamp, J., Womack, J.E., Thaller, G., & Fries, R. (2001). Association of lysine - 232/alanine polymorphism in bovine gene encoding acyl-Coa: diacylglycerol acyltransferase (DGAT1) with the variation at a quantitative trait locus for milk fat content. Proc. Nat. Acad. Sci. 99:9300-9305.

Winter, A., Kramer, W., Werner, F.A.O., Kollers, S., Kata, S., Durstewitz, G., Buitkamp, J., Womack, J.E., Thaller, G., & Fries, R. (2002). Association of lysine-232/alanine polymorphism in bovine gene encoding acyl-CoA: diacyiglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proceedings of the National Academy of Science, USA99:9300-9305

Womack, J.E. (2005). Advances in livestock genomics: opening the barn door. Genome. Res: 15 (12), pp 1699-1705

Wood, J.D., Richard, R.I., Nute, G.R., Fhisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M. (2004). Effect of fatty acids on meat quality: a review. Meat Science, 66 (1):21-32.

Xie, Y.R., Busboom, J.R., Gaskins, C.T., Johnson, K.A., Reeves, J.J., Wright, R.W., & Cronrath, J.D. (1996). Effects of breed and sire on carcass characteristics and fatty acid profiles of crossbred Wagyu and Angus steer. Meat Sci. 43:167-177.

Yamasake, M., Kishihara, K., Ikeda, I., Sugano, M., & Yamada, K. (1999). A recommended esterification method for gas chromatographic measurement of conjugated linoleic acid. Journal of the American Oil Chemists Society 76:933-938.

Yang, A., Larsen, T.W., Powell, V.H., & Tume, R.K. (1999). A comparison of fat composition of Japanese and long-term grain-fed Australian steers. Meat Sci. 51:1-9.

Zeng, Z.B. (1993). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA 90:10972-10976

Zhang, B., Schomoyer, D., Kirov, S., & Snoddy, J. (2004). GOTree Machine (GOTM): a web-based platform for interpreting sets of interesting genes using Gene Ontology hierarchies. BMC Bioinformatics 5:16.

Zhang, Q., Boichard, D., Hoeschele, I., Ernst, C., Eggen, E. Murkve, B., Pfister-Genskow, M., Witte, L.A., Grignola, F.E., Uimari, P., Thaller, G., & Bishop, M.D. (1998). Mapping Quantitative Trait Loci for Milk Production and Health of Dairy Cattle in a Large Outbred Pedigree. Genetics 149:1959-1973.

Zhi-Liang Hu & Reecy, J.M. (2007). Animal QTLdb: beyond a repository A public platform for QTL comparisons and integration with diverse type of structural genomic information. Mamm. Genome. Spring Science + Business Media,10. 1007/s00335-006-0105-8.

Footnotes

[1] www.angus.org.au/Databases/BIRX/omia; www.ncbi.nih.gov/entrez/query.fcgi?db=gene ; http://sol.marc.usda.gov/cattle ; http://pigest.genome.iastate.edu/index.html
[2] www.bovineqtlv2.tamu.edu/index.html and http://www.animalgenome.org/QTLdb
[3] http://www.ncbi.nlm.nih.gov/about/primer/est.html
[4] http://www.ncbi.nlm.nih.gov//mapview/map_search.cgi?taxid=9031
[5] http://pigest.genome.iastate.edu/index.html
[6] http://pigest.genome.iastate.edu/index.html
[7] http://qtl.cap.ed.ac.uk
[8] http://qtl.cap.ed.ac.uk
[9] http://www.marc.usda.gov/genome/cattle/cattle.html
[10] http://biolibrary.licor.com/htdocs/RnP/LabPrim.jsp.
[11] http://compgen.rutgers.edu/multimap/crimap
[12] http://www.marc.usda.gov/genome/cattle/cattle.html
[13] http://qtl.cap.ed.ac.uk/
[14] http://homepage.usask.ca/~schmutz/index.html
[15] http://www.marc.usda.gov/genome/cattle/cattle.html
[16] http://biolibrary.licor.com/htdocs/RnP/LabPrim.jsp