Chapter 2
Genetic Control of Meat Quality Traits

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Introduction

Meat was originally produced from non-specialized animals that were used for a variety of purposes, in addition to being a source of food. However, selective breeding has resulted in "improved" breeds of cattle that are now used to produce either milk or beef, and specialized chicken lines that produce eggs or meat. These improved breeds are very productive under appropriate management systems. The selection methods used to create these specialized breeds were based on easily measured phenotypic variations, such as growth rate or physical size. Improvement in the desired trait was achieved by breeding directly from animals displaying the desired phenotype. However, more recently sophisticated genetic models have been developed using statistical approaches that consider phenotypic information collected, not only from individual animals but also from their parents, sibs, and progeny. This combined information allows the genetic potential of individuals to be the better predicted. The predicted potential for several traits can then be combined into an index which provides a measure of the overall genetic merit of the individual. Using these statistical approaches animals are selected for breeding using the index of their estimated breeding value (EBV), rather than directly on their phenotype.

The results of these phenotype focused selection approaches have been highly successful, with dramatic improvements in the traits under selection. Modern broiler chickens used for meat production are eight times larger and grow much more rapidly than layer types that have been selected for egg production. Specialized beef cattle grow rapidly reaching a mature size in less than a year compared with the 24–36 months required to "finish" traditional breeds. Milk production from specialized dairy cows has also increased dramatically over the past 20 years under selection. However, these past selection choices have resulted in new problems,

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such as a decrease in fertility, which in some breeds now threatens the viability of production. Fertility is currently a major cause for concern for dairy cattle and broiler poultry producers. Dairy farmers are also faced with increasing lameness in their herds while poultry breeders have to cope with birds that have brittle bones. These are major welfare problems as well as threatening productivity. In addition, the inadvertent selection for genetic defects linked to desirable production characteristics is a potential risk, especially when selection programes focus on a limited number of breeding individuals.

The traits that are routinely recorded are, through necessity, simple and focused on the commercially important traits. There has been little opportunity, and in some cases desire, to select other traits, many of which in the past were considered less relevant. However, for the livestock production to remain sustainable, it will be necessary to consider a wide range of traits in selection programs, particularly those that have an impact on the health and welfare of the animals. While there has been an increase in the quantity of production, arguably, until recently there has been little or no attention paid to the quality or composition of the products. With the growing awareness of the consumers with respect to choices, there is now an increasing demand for better quality, as well as lower cost products. Consumers are increasingly more conscious of their own health and also the welfare of animals in agricultural production systems. Over many years, there have been welfare concerns regarding, for example, battery farming for egg production. The frequent focus of the mass media on both human and animal health issues has drawn the public attention to health and welfare problems in, or arising from, agricultural production. This has resulted in increased pressures to change the associated production practices. Following on from the devastating BSE outbreak in the UK in the late 1980s, the public has seen *Salmonella* contamination in egg production, Escherichia coli contamination of beef products, and Foot and Mouth disease in cattle and sheep to name a few examples, all of which have had a major impact on the credibility and financial sustainability of the livestock industry. In addition to the public concerns over the risks from pathogens, there is now the desire to have food with healthy composition, in particular, meat products with lower fat content. In response to these demands the industry has turned its' attention to the quality as well as quantity of production. The major problem to date has been that improvement can only be made in traits where there are reliable measurements recoding variations in the phenotype. This information is required to develop selection strategies. For many health and quality related traits, this data is difficult and expensive to obtain. Additionally, the information can be difficult to apply in breeding programs: e.g., measurements of meat texture, composition or flavor are complicated to carry out, require specific samples, and can only be made after an animal is slaughtered. It is therefore difficult to collect these data in a routine way, and obviously post-slaughter there is no opportunity to breed from animals with superior characteristics. A further complication is that a large part of the variation, particularly in meat quality traits, results from differences in environmental conditions, in particular differences in feed and handling.

Approaches and Tools for Genetic Selection

Although the traits that are important for beef production are influenced by the environment, e.g., management conditions, nutritional status and handling pre- and post-slaughter, the genetically controlled variation (heritability) of the traits important for production, and product quality is relatively high, between 0.15 and 0.35 (e.g., Wheeler, Cundiff, Shackelford, & Koohmaraie, 2004). This suggests that an appreciable proportion of the variation is under genetic control, and hence could be improved by selection. Knowledge of the genes controlling the variation in a trait would open the opportunity to use genomic information in selection programs. By choosing to breed from the animals with the most favorable alleles at important genes, the rate of animal improvement could be significantly increased. Importantly, these gene-based methods have the potential to facilitate the improvement of traits that are difficult to select for by the traditional phenotype based methods. Information on polymorphisms in genes controlling particular traits, and understanding the biological effects of these polymorphisms, will allow genetic information to be used effectively in animal improvement programs. However, progress can be made even before these "trait genes" have been identified. Markers that are genetically linked to the traits genes can potentially be used in marker-assisted selection (MAS) programs (Dekkers, 2004).

As recombination events are relatively rare, large regions of chromosomes are passed intact from one generation to the next. Thus, polymorphisms in the DNA sequence that are close to the trait genes, if used with care, can be used to predict the alleles present at the trait loci. The MAS approach has received considerable attention as the results of numerous genome mapping studies have been published which identify markers linked to the genes controlling important production traits. The majority of traits that are important in livestock production are "complex", that is, they are under the control of several genes, each contributing to a part of the observed phenotypic variation. Therefore, these have been called quantitative traits and hence the genetic loci controlling them quantitative trait loci (QTL). By using a relatively small number or markers, it has been possible to crudely identify the chromosomal locations of QTL containing some of the major the genes controlling a number of important production traits in livestock species. Markers for these QTL could be used for MAS. However, the major drawback is that it is necessary to determine the alleles at the linked markers that are predictive of the favorable allele at the trait locus, which is known as "phase". Phase has to be determined in every population and family in which the MAS will be undertaken, as recombination at the population level means that the association between markers and the trait gene cannot be assumed. In addition, the phase may change from one generation to the next, because of recombination; thus the linked markers can only be used with confidence for a limited number of generations. The likelihood of recombination between the trait gene and the markers is dependent on the genetic distance separating them. Having markers either very close to the trait gene or ideally knowing the functional variation within the gene means that there is a very low probability of recombination

between the markers and the gene. In such cases, the markers can be used directly without first having to determine the phase.

Over the last two decades there has been a considerable effort to develop several different types of maps that cover the whole genomes of many species, including all the major livestock species. Genetic maps (e.g., pig: Archibald et al., 1995; Rohrer et al., 1996; and cattle: Barendse et al., 1997; Kappes et al., 1997) have been produced using recombination to determine the relative position of polymorphic markers to each other based on the frequency of recombination between them. In addition, several types of physical maps have been produced, ranging from large fragment clone maps (Snelling, Chiu, Schein, & The International Bovine BAC Mapping Consortium, 2007) and maps produced by *in situ* hybridization (Hayes et al., 1995; Solinas-Toldo, Lengauer, & Fries, 1995; Chowdhary, Fronicke, Gustavsson, & Scherthan, 1996) to maps produced based on the probability of chromosomal breaks occurring between loci following irradiation (McCarthy, 1996). Together these genome wide maps have led to rapid advances in understanding the structure of genomes and have provided the markers required for genetic mapping studies to localize and identify the trait genes. Following the publication of the human genome sequence (Lander et al., 2001), several projects were initiated to sequence the genomes of a large number of other species. For livestock, the first sequence to be published was that of the chicken (International Chicken Genome Sequencing Consortium, 2004); a draft of the bovine sequence was also made available in 2004 and a more complete draft is soon to be published by the Bovine Genome Consortium (2008). Work is progressing rapidly on sequencing the pig genome, with a full draft sequence expected early in 2009. Work is also underway on the sequence of the sheep genome. Availability of the whole genome sequence provides information on the number and location of genes, and on gene regulation and genetic variations. Availability of the genome sequence also allows tools to be developed which can be used to identify the genes controlling target traits more rapidly.

This chapter will describe the approaches that have been followed to investigate the genetic control of meat production traits and provide examples of the identification of trait genes controlling variation in meat quality related traits.

Definition of Meat Quality

Meat quality can be defined in a number of ways, but the focus is on those factors that affect consumer appreciation of the product. The main sensory factors which influence purchase are color and visible fat, and the primary factors affecting the enjoyment of consuming meat are texture and flavor. However, consumers are increasingly concerned with food safety from the point of view of health implications, e.g., the composition of poly-unsaturated vs saturated fat, and microbiological contamination. The management of animals can influence these factors, for example, feed can affect fat composition and flavor, while the rate of growth and hence age at slaughter can affect the texture. Texture and color of meat are strongly influenced by the way an animal is handed prior to slaughter, and then the treatment and

processing of the carcass post-slaughter. The genetics of the individual may have a smaller influence on many quality characteristics than these management aspects, but nevertheless, genetic variation can make a difference. Indeed, genetic variation may control the way different management practices affect meat quality, such as propensity for and animal convert food into fat vs muscle. Genetics also affects muscle composition and hence texture. In principle, molecular genetic approaches could be applied to defining the response of an individual to environmental factors, and help to establish the correct management conditions to optimize meat composition and physical characteristics. In addition to contributing to the control of microbiological infections, the genetic control of immune response and susceptibility to disease is important for improving animal health and reducing the use of antibiotics and other veterinary products that may contaminate food products. The reduction of pathogens through improved genetic resistance would also reduce the potential for bacterial contamination on carcasses and processed meat.

Traditional Genetic Selection

Genetic improvement of livestock has been achieved by selective breeding, which has been highly successful in improving some traits. However, to establish breeding programs it is necessary to have performance records for the traits that will be selected for. In a commercial context, only simple measurements and recording procedures are possible, without interfering with the management practices and hence adding significantly to the costs of production. Hence the traits routinely recorded are easily and quickly measured. At slaughter, basic information is routinely collected on carcass quality. Some more complex measurements are undertaken by some producers, such as the use of ultra-sound to measure back-fat or muscle depth. However, in general these measurements are not systematically recorded in a centralized way that would allow the data to be used effectively is selection programs. Measurement and routine recording of the more difficult-to-measure traits has not been attempted at a commercial level for obvious reasons: cost or because the measurements are imprecise. Many of the traits associated with, e.g., meat quality, or health, are subjective, dependent on the criteria set and the person carrying out the measurements. Without establishing standardized and detailed protocols for recording traits it is impossible to compare measurements taken in different places and at different times. To standardize trait recording it is often necessary to carry out complex measurements, which are difficult to apply in large populations, and certainly in a commercial setting. There is an increased interest by producers in developing selection criteria that are aimed at improving quality, efficiency, and health traits. In some countries, the recording of more complex traits has been centralized at a national level, e.g., centralized health recording was pioneered by the Scandinavian countries and more recently, Ireland has introduced a national animal health recording system. However, to be effective, the recording protocols should be simple and standardized, preferably at an international level. To achieve this, the

international organization responsible for animal recording, ICAR, can play an important role.

For some species, especially cattle, artificial insemination (AI) has contributed significantly to breed improvement by allowing individual elite sires to produce large numbers of progeny. When improvement is required in a trait that is sex limited, such as milk-related traits, or that which can only be determined post-slaughter, such as meat quality or composition, testing of the progeny of a sire allows his genetic quality for target traits to be estimated. The genetic index of an animal calculated from the performance of daughters or sons can be used to select the highest merit sires for breeding. Sophisticated statistical methods have been developed to analyze this "progeny test" data and identify sires that are above average for the desired trait. Commercial progeny test schemes have maximized the genetic gain in traits such as milk yield and composition, which are easy to measure in a commercial setting compared with meat quality traits. Even with easily measured traits, the progeny test schemes are very expensive; especially considering that a large number of the sires tested will not be used for breeding. To recover the costs of testing, a large number of semen doses have to be sold for each elite bull. Unless carefully managed, this breeding strategy can risk high levels of inbreeding and the associated loss of vigor, and the concentration of deleterious recessive alleles. Progeny testing and artificial reproductive approaches have been used extensively in dairy cattle breeding, where AI is now used ubiquitously, and as a result milk yields of the Holstein breed have more than trebled over the past 25 years. However, this highly focused selection strategy has lead to an alarming reduction in the effective population size of the breed. The occurrence of bovine leukocyte adhesion deficiency (BLAD) was a dramatic example of the potential problems associated with using a limited pool of elite sires. BLAD is a result of a mutation in one gene (CD18) that originated in a single bull, probably about 60 years ago. The effects of the mutation are recessive; therefore while the mutated allele remained at low frequency the adverse affects were not observed. However, a carrier bull turned out to be highly productive and large numbers of his sons were used extensively for breeding as elite AI sires, and in their turn their sons were used to breed further elite sires. By 1990 the frequency of the mutated allele had reached 15% in some countries, and animals homozygous for the mutations started appearing in the Holstein population. The effects were observed as a disease of young Holstein calves characterized by pneumonia, delayed healing of wounds and death (Gilbert et al., 1993). The mutated allele has now been effectively removed from the Holstein population by genetic testing. This example illustrates why selection programs should be coupled with good breeding management to maintain the effective population size and hence the genetic diversity present in the population: to avoid inbreeding and the accumulation of recessive defects.

For progeny selection to be effective, a large number of sires have to be tested, which is very expensive. Therefore, approaches to identify potentially superior animals at an early age would help to reduce cost and would mean that a higher proportion of bulls selected for testing were of high quality. For slow-growing or late-maturing species, juvenile predictors of adult performance can be used to speed

up selection and reduce costs (Meuwissen, 1998). Juvenile predictors would also allow animals with high potential to be selected before many of the rearing costs had been incurred. However, up to now few reliable juvenile predictors have been identified. DNA markers offer the potential to select breeding animals at a very early age, indeed as embryos, and to enhance the reliability in predicting the mature phenotype of the individual.

New Opportunities

There are undoubtedly genetic factors that affect meat quality, such as fatty acid composition, fat distribution, muscle fiber type, etc. Up to 35% of the variation seen in muscle composition is under genetic control (Wheeler et al., 2004). In addition, significant differences have been found in the organo-leptic properties and composition of meat produced from different breeds of cattle and pigs, which also suggests that genetics plays an important role in controlling variations in meat quality. Analysis of the genetic make-up of breeds has shown that, although there is genetic variation *within* breeds, this is small compared with the variation found between breeds (e.g., Blott, Williams, & Haley, 1999). Thus, genetic selection could be used to improve meat quality, and would be most effectively achieved using DNA markers. However, up to now few of the genes controlling variability in meat quality and composition have been identified, and specifically, few functional variations within the genes that control the phenotypic differences are known. With recent technological advances this situation may be about to change.

The application of simple phenotype-guided selection will inevitably be influenced by conflicting choices when considering the diverse range of traits that are important at different levels of the production chain. Some traits appear to be obligatorily in conflict: i.e., when alleles of a particular gene are beneficial for one trait but have negative effects on another. Molecular genetic approaches can be used to aid breeding decisions and may allow selection for a wide variety of traits. Another problem occurs when the genes controlling different traits are close together on a chromosome. In this case, it may appear that there is only one locus having an effect on both traits, as alleles at the closely linked genetic loci will generally be inherited together. However, even for very closely linked genes, by examining sufficient individuals (meiosis), some chromosomes will be identified where recombination has occurred, even between very closely linked loci. Knowledge of the alleles at particular genetic loci and their genetic effects will allow direct selection choices to identify individuals with the most beneficial combination of alleles. Therefore, in theory at least, a strategy to simultaneously select for improved performance in a number of traits could be devised using genetic markers, even when at the phenotypic level the traits may seem to be in conflict. If applied with care, the use of molecular information in selection programs has the potential to increase productivity, enhance environmental adaptation, and maintain genetic diversity.

Advances in Knowledge

Over the past 20 years there have been rapid advances in the development of molecular biological techniques, which have been applied to understanding the regulation of gene expression and function. The application of these techniques to the field of genetics has advanced the knowledge of the structure of the genome and the identification of sequence variations between individuals, some of which have known effects on gene function and phenotypic variation. The most significant advance in the past few years has been the completion of the human genome sequencing project (Lander et al., 2001). This project spurred the development of new technologies that are allowing biological problems to be addressed on a large scale. With these new technologies, it is now possible to analyze many thousands of DNA sequences in a single day. And instead of studying the expression of individual genes, it is now possible to examine the expression of all the genes in the genome simultaneously and to address the interactions between genes. The resources developed to sequence the human genome were subsequently used to sequence the genomes of many other species. The first draft of the bovine sequence was released in October 2004, and a more complete sequence including the annotation of the genes was made available in 2007 (http://www.hgsc.bcm.tmc.edu/projects/bovine/ and http://www.ensembl.org). A genome-sequencing project for pigs is currently underway, and the entire pig sequence is likely to be available in 2009. The sequences of these genomes, together with the information on genetic variations, gene structure, expression and regulation, together with the new technologies for rapidly sequencing the genomes of individuals, will facilitate the identification of the genes controlling variations in commercially relevant traits. Information on polymorphisms, within these genes, could then be used to enhance selection programs, or to develop improved management strategies. Information on large numbers of genetic polymorphisms together with the highthroughput methods to genotype them opens the possibility of genome-wide selection, rather than focusing on a limited number of loci.

For genetic studies, the most important developments arising from the genome sequencing projects has been the identification of large numbers of differences (polymorphisms) in the DNA sequence between individuals. Some of these polymorphisms may be functional, in so far as they alter levels of gene expression or the activity of the protein encoded by the sequence, e.g., changing the affinity of a receptor for its ligand, or the activity of an enzyme. Other variations may be neutral if they occur in inter-genic regions outwith regulatory regions, or within coding regions of genes but are conservative, i.e., do not change the amino acid in the protein. The functional polymorphisms may be involved in controlling variations in phenotypes including those relevant to meat quality, such as muscle composition or structure. The number of DNA polymorphisms known, and the way they are detected, is rapidly changing the way the identification of the genes controlling particular traits is carried out. Before discussing these advances, some examples of different genetic markers will be described.

Genetic Markers

The earliest form of DNA marker used to construct the first true genomic maps was the Restriction Fragment Length Polymorphism (RFLP). Bacterial "restriction" enzymes bind and cut DNA molecules at highly specific recognition sequences. Variation in the target restriction enzyme binding site results in differences in the size of the fragments generated, following digestion with a restriction enzyme. Initially, RFLPs at specific positions in the genome had to be identified individually. This was a laborious process that could only investigate one gene at a time. A special form of RFLP allowed variations in loci that are present in multiple copies throughout the genome to be investigated at the same time. These "Variable Number Tandem Repeat" VNTR markers were successfully used to identify familial relationships between individuals in wild populations by creating "genetic fingerprints" and were also used in genetic mapping studies (e.g., Jeffreys, Wilson, & Thein, 1985; Georges et al., 1990). A major breakthrough came with the identification of microsatellite sequences. These are loci in the genome that contain typically 5–20 copies of a short sequence motif 2 and 4 bp in length, repeated in tandem (e.g., CGCGCGCG). These sequences have a relatively high mutation rate, resulting from DNA replication errors, and so at a population level the number of repeat units at a locus can be highly variable, providing a large number of alleles that can be used as markers in genetic analyses. The number of alleles at these "microsatellite sequences" is approximately proportional to the number of repeat units present.

This high allele number and amenability of microsatellite loci to polymerase chain reaction (PCR) amplification make them excellent markers for use in genetic studies, and indeed most of the gene mapping studies carried out in the past 10 years have used this type of marker. Genotyping the microsatellite locus was initially achieved by PCR using primers that flanked the microsatellite repeat region. The PCR products were labeled by the incorporation of radioactive nucleotides in the reaction, and alleles identified by determining the sizes of PCR product by gel electrophoresis. More recently, the use of fluorescent dyes and automated DNA analyzers allowed the simultaneous analysis of, typically, 5–10 different microsatellite loci simultaneously (multiplexing). Nevertheless, the gel electrophoresis-based methods required to genotype this type of marker mean that it is difficult to automate the procedures and the cost of genotyping remains high.

Genetic variations fall into two classes: insertions or deletions of DNA sequence (indels), of which the microsatellite loci are a special type, or changes to the nucleotide sequence, often at individual bases. These single nucleotide polymorphisms (SNPs) are much more frequent than indels in the genome and occur in both coding and non-coding regions. Estimates from genome sequencing projects in different species suggest that SNPs occur at a frequency of one in every 200 bp, on average. Thus, there are potentially many millions of SNPs in the genome. SNPs within coding regions may have no effect on the protein coded by the gene (silent polymorphisms) or may result in a change in an amino acid. The latter

are more likely to be the functional polymorphisms responsible for variations in traits, although in some cases the functional polymorphism may occur in intergenic regions (see, e.g., IGF2 below). There have been considerable technological advances to facilitate the high throughput genotyping of SNP variations, and current and future whole genome genetic studies are likely to use this type of marker.

Identifying the Genes Controlling Phenotypic Variations

There are several approaches that can be used to identify the genes controlling phenotypic variations. For simple monogenic traits, knowledge of the physiology of the trait may allow the biochemical pathways involved to be analyzed to identify the gene(s) likely to be responsible for the observed differences in the phenotype. If the pattern of expression of these genes among tissues is known, this information can be used to clone the gene(s). Once cloned, the genes from individuals displaying different phenotypes can be sequenced and compared to reveal if there are polymorphisms present that are responsible for the phenotypic variation. Some of the first genes controlling variations in monogenic traits were identified in this way. However, to be successful, a good *a priori* knowledge of the trait and the underlying physiology is clearly required. Another approach is to use comparative information between species; in some cases knowledge of the gene that controls a phenotype in one species may suggest a candidate gene that could be tested in another. Finding polymorphisms within the candidate genes that co-segregate with the phenotypic variation in the trait will indicate if the gene is responsible for the variation. This comparative approach requires a good knowledge of the phenotype, as even subtle differences may be the result of the action of different genes. Even with a good knowledge of the physiology of the trait, other genes, that are not obviously part of the biochemical pathways involved, may contribute to the variation and so would not be considered as candidates. Therefore for complex traits, it may be better to start with no prior assumptions regarding the physiology and to use a genetic, or linkage, mapping approach.

There are two requirements for identifying genes controlling particular traits by linkage mapping. The first are the genetic markers that are used to track inheritance of chromosomal regions in families segregating for the trait. The second are the families that are segregating for the target traits, for which both phenotype and pedigree information is available. Using statistical methods to correlate inheritance of the phenotype with inheritance of the genetic markers in the families, it is possible to localize the gene(s) controlling the trait to broad regions of chromosomes. The chromosomal location is then used as the starting point to identify the trait genes themselves. In practice, the identification of the "trait genes" starting from a chromosomal location is not easy, and the successful identification of trait genes has usually been achieved using a combination of genetic mapping, to localize the QTL region on a chromosome, followed by a candidate gene or positional cloning approaches to identify the trait gene within the QTL region.

Following an initial low resolution linkage mapping study, it is usual that several putative QTLs will be identified. The likely position of these QTL may span quite

large chromosomal regions, typically a quarter or possibly half of a chromosome, which may contain between 200 and 400 genes. It is therefore necessary to refine the QTL localization by fine mapping before assessing putative candidate genes in the region. The precision of the localization of the QTL is dependent on the number of recombination events in the region, which will reduce the amount of flanking chromosomal region that is inherited with the QTL. To increase the possibility of recombination in the target region, additional individuals in the segregating families have to be studied, and additional markers will be required within the region to detect the recombination events. The fine mapping of a trait gene can be achieved most efficiently by examining the target chromosomal region inherited by different branches of a family arising from a common ancestor. The more generations that have separated the different branches of a family from the common ancestor, the greater the reduction in the ancestral genome inherited with the trait gene: i.e., the amount of the genome in linkage disequilibrium (LD). The genomic regions coming from the common ancestor are *identical by descent* (IBD) and can refine the location of a QTL to small chromosomal intervals (see Anderson and Georges, 2004). Once the QTL region has been fine mapped, the next step is to examine the region carefully for any genes, which from their known function, may be involved in controlling the trait (a candidate gene). Likely "candidate" genes will then be examined for variations that may alter their function and hence may be responsible for the phenotypic variation observed. In the absence of any good candidate genes it will be necessary to clone and sequence the refined QTL region from individuals carrying different QTL alleles in order to identify specific genetic polymorphisms that are associated with the variation in the trait.

Analysis of gene expression patterns may also indicate specific genes or biochemical pathways that are involved in the regulation of phenotypic variation: genes that have an increased or decreased level of expression associated with a specific phenotype may be directly responsible for the observed variation, or may be involved in the regulation of other genes that are. For many years, the assay for gene expression has been cumbersome and has focused on individual genes. The development of methods to create arrays of thousands of different DNA probes has facilitated the examination of changes in the expression of large numbers of genes simultaneously. The explosion in the amount of genomic information available and the development of these microarrays have resulted in large amounts of data on gene expression from different tissues and individuals with different genotypes to be gathered very rapidly. Analysis of such information may suggest those genes responsible for variations in phenotypes.

Application of the Techniques to Locating the Genes Controlling Meat Quality Traits

In this section, the different approaches to identify genes controlling meat quality traits are described and examples given for the successful application in each approach.

Candidate Genes

The most straightforward approach for identifying the genes controlling a particular trait is to use knowledge of the physiology of the trait to identify the biochemical pathways involved. This will then suggest genes that may be important for controlling key processing in the development of the phenotype. These genes are "*candidate*" genes that can be tested by identifying polymorphisms within the genes and observing whether the occurrence of the polymorphisms can account for some or all of the variation observed in the trait. In order to select the candidate genes, it is necessary to have a good a priori knowledge of the trait and the underlying physiology. However, even with a good knowledge of the trait, not all of the important genes will be identified and important candidates may be missed if they are not obviously involved in the known physiology. An additional drawback of testing candidate genes is that most traits that are important for livestock production, such as feed efficiency, disease resistance, growth rate, or muscle composition, are not under the control of a single gene, but are controlled by several genes that have an additive effect. For most of these "complex" traits, a very large number of genes could be considered as potential candidates. Testing all of these potential candidate genes is not practical; thus the candidate gene approach is better used in conjunction with other approaches that can refine the choice. A two-step approach, in which the chromosomal location of the gene is identified using a conventional linkage mapping approach, then this information used as the starting point to select *positional candidate genes* within the chromosomal location has proved successful. The application of these approaches is discussed below with reference to examples.

Examples of Genes and QTL Mapping in Livestock

Meat quality

In man, some individuals are know to have an adverse response to halothane anesthetic, which results in muscle spasm and a dangerous drop in muscle temperature. This condition is called malignant hypothermia and is a genetic disease controlled by a single major gene (HAL). A similar condition has been described in pigs. In addition to the adverse reaction to anesthetic, pigs carrying the halothane sensitivity gene (HAL) also respond badly to stressful situations, such as handling and transport. The physiological response can result in sudden death and is referred to as the porcine stress syndrome (PSS). Meat from pigs that carry the HAL gene is characterized by a pale color and a soft texture with a very high drip loss, which is referred to as pale, soft, exudative meat (PSE). The appearance of PSE meat is unacceptable to the consumer, and is associated with low yields of cooked and dry-cured ham. However, carcass traits of halothane sensitive pigs are heavier, shorter, and leaner than halothane negative pigs. HAL sensitive carcasses have better lean content, but are worse for pH, color, drip loss, intramuscular fat tenderness, and juiciness. Response to halothane anesthetic was used to identify pigs carrying the HAL

mutation, and breeding programs have used this information to select boars and sows with different HAL carrier status. By crossing between the carriers and non-carriers, heterozygous production animals could be produced, thus taking advantage of the improved lean growth, without the problems of PSE associated with homozygosity. The gene responsible for the halothane response was initially identified in man as the muscle ryanodine receptor, which is a calcium release channel of the sarcoplasmic reticulum in the skeletal muscle (MacLennan et al., 1989). The HAL sensitivity locus was first mapped chromosome 6 in pigs (Fujii et al., 1991), and later the gene responsible was also shown to be porcine ryanodine receptor (RYR1), which in addition to the halothane sensitivity, causes PSS. After initial breeding programs to produce heterozygous animals, pig breeders decided that the detrimental effects of the HAL gene outweighed the positive effects and therefore the marker information has been used to eradicate the HAL-sensitive allele.

Fat, Obesity, and Feed Intake

The obese strain of mouse (*Ob*) has been used for many years as a model for human obesity. The obesity of the Ob strain was known to be the result if a single genetic mutation, and that the obesity of these mice was the result of a very high feed in take. The gene responsible was identified as leptin, using a positional cloning approach (Zhang et al., 1994). The LEP gene encodes a protein composed of 146 amino acids which is expressed in adipose tissue, but is released into the blood, and thus has a general endocrine effect. The protein acts as a hormone that induces satiety and hence regulates food intake and energy balance (Barb, Hausman, & Hoseknechtm, 2001). Studies in livestock have investigated leptin as a candidate gene that may affect carcass fat. Several polymorphisms have been reported in the bovine LEP gene, some of which have been associated to carcass fat levels and, feed intake in cattle (Buchanan et al., 2002; Lagonigro, Wiener, Pilla, Woolliams, & Williams, 2003; Barendse, Bunch, & Harrison, 2005; Liefers et al., 2005). The LEP gene has also been investigated in pigs and seven polymorphisms have been reported in the porcine leptin gene although none of these have been conclusively associated with fat levels in the carcass (Jiang & Gibson, 1999; Kennes, Murphy, Pothier, & Palin, 2001).

Some forms of genetically associated obesity in humans have been associated with non-functional (frame-shift) mutations in the melanocyte receptor hormone (MC4R). This gene is associated with pigmentation, and more than 30 amino acid variations have been found in different ethnic groups, some of which have been associated obesity and impaired melanocyte cell functions (Yeo et al., 2003; Kim, Reecy, Hsu, Anderson, & Rothschild, 2004). In pigs, a mutation in the MCR4 gene results in an amino acid substitution in the coded protein (Asp298Asn) that has been associated with growth, fatness, and feed intake traits (Kim, Larsen, Short, Plastow, & Rothschild, 2000; Huston, Cameron, & Rance, 2004). Different expression levels of MC4R and LEP genes have been reported in two pig breeds with significantly different levels of intra muscular fat (D'Andrea, Fidotti & Pilla, 2005). In cattle, several mutations have been reported in the MC4R gene and also in the

pro-opiomelanocortin (POMC) gene that is the precursor of alpha-melanocyte stimulating hormone (αMSH), which is an agonist of MC4R (Buchanan, Thue, Yu, & Winkelman-Sim, 2005). But up to now these have not been associated with variation in fat traits. Thus, candidate genes selected because they show a major effect in one species may have less obvious effects in another species.

Meat Tenderization

The overriding factor that affects the liking of meat when consumed is the texture, or specifically tenderness. The structure of muscle has a major effect on meat tenderness, but post-slaughter aging and maturation of meat plays a major part in the quality achieved in the final meat product. This aging process includes the proteolytic degradation of proteins in the meat by calcium dependent proteases. Hence, the tenderization process is affected by the activity of the proteases and the availability of calcium. A family of photolytic enzymes, called calpains, is involved in the proteolysis of muscular proteins during meat aging; the Calpain 1 or μ-Calpain enzyme is encoded by the *CAPN1* gene (Smith, Casas, Rexroad III, Kappes, & Keele, 2000). As its name implies, this protease in activated in the presence of micromolar amounts of calcium and has been associated with degradation of myofibrillar protein in living muscle. A large number of polymorphisms have been described in the bovine *CAPN1* gene (e.g., Page et al., 2002), and two polymorphisms in particular, within exons 9 and 14 of this gene, result in amino -acid substitutions (A316G and I530V), and have been associated with variations in tenderness determined by the Warner Brazler shear force test.

The level of calpain proteolytic activity is regulated by the availability of calcium, and calpains are inhibited by calpastatin (CAST). The *CAST* gene codes for calpastatin, and several polymorphisms, have been found in the bovine *CAST* gene, some of which have been associated beef tenderness measured by the shear force test (Barendse, 2002a; Koohmaraie et al., 1995). There is a strong correlation between calpastatin activity and tenderness measured by Warner-Bratzler shear force (Shackelford et al., 1994). Two SNPs, located in the 3'UTR region of the gene, have been associated with variations in tenderness in several studies (e.g., Nonneman, Kappes, & Koohmaraie, 1999). However, among the different combinations of these polymorphisms (haplotypes), only two have been associated with improved tenderness. Therefore, specific polymorphisms do not have a consistent effect, which suggests that the known polymorphisms are not the functional variations but are linked to it. The use of *CAST* polymorphisms for assessing animals that may produce tender meat has been patent protected, but it should be noted that no QTL for tenderness have been reported on chromosome 7 in cattle, in the genomic region containing the *CAST* gene.

A QTL associated with pork tenderness has been reported on pig chromosome 2, close to the CAST locus (Malek, Dekkers, Lee, Baas, & Prusa, 2001). Several polymorphisms have been reported in the porcine *CAST* gene (Rothschild, Ciobanu, & Daniel, 2004), some of which affected the protein structure and had a large effect on tenderness. Lysyl oxidase, an enzyme involved in cross-linking collagen fibers,

is encoded by a gene (*LOX)*, which is located close to *CAST.* Two alleles at the *LOX* locus have been associated with variation in meat tenderness in cattle. It is therefore difficult to separate the activities of these two neighboring genes to determine which is responsible for the observed variation in meat texture.

Intramuscular Fat and Marbling

The second major factor involved in the appreciation of meat quality, defined by sensory panel testing, is intramuscular fat, which affects both flavor and juiciness. The level of intramuscular fat may vary because of the number of fat cells, or because of variations in fat synthesis. Two genes located on chromosome 14 potentially affect both of these factors. The first Thyroglobulin (*TG*) is proteolytically cleaved to thyroid hormones involved in the regulation of adipocyte development. This gene was initially implicated in fat associated traits as it is close to a microsatellite marker, *CSSM66*, which was associated with carcass fat in QTL mapping studies. Subsequently, polymorphisms within the *TG* gene were directly associated with variation in intramuscular fat (Barendse, 2002b). A second gene located close to *TG* encodes diacylglycerol-O-acyltransferase (*DGAT1*), which is involved in the last stages of fat synthesis and has been identified as the gene underlying a QTL for milk fat synthesis (see below). The polymorphism of the *DGAT1* gene, responsible for the Ala232Lys variation in the protein, is associated with variations in milk fat and also seems to affect intramuscular fat (Thaller et al., 2003). A QTL for beef marbling has been reported on bovine chromosome 14, where both the *TG* gene and the *DGAT1* gene are located, but both the genes, *TG* and *DGAT1*, seem to have independent effects, because the alleles of the two genes are not in linkage disequilibrium.

QTL Mapping

Most of the traits associated with variations in meat quality are likely to be complex in-so-far as several genes will to contribute to the observed variation. Each of these genes will be responsible for a different proportion if the variation, some being responsible for a large amount of the variation, some with only a minor effect. For some traits, it may be possible to postulate candidate genes that contribute to the variation in these *quantitative traits*, but even with a good knowledge of the physiology of the trait, other genes not obviously part of relevant biochemical pathways may contribute to the variation and so would not be considered as candidates. Conversely, many apparently obvious candidate genes will not contribute significantly to the observed variation, e.g., because there are no functional or regulatory variations within the gene. Therefore, for complex quantitative traits, it may be better to start with no prior assumptions regarding candidate genes, and to use a genetic mapping approach. This approach first identifies the chromosomal location of the quantitative trait locus (QTL) and then uses this information as the starting point for identifying the gene and the functional variation within that gene – the *QTN*. The genetic, or linkage, mapping approach has two requirements: families which are segregating

for the trait of interest and DNA markers to track the inheritance of chromosomal regions segregating in the families. The DNA marker information is then correlated with measurements characterizing the variations in the trait, and statistical methods are used to localize the trait genes to broad chromosomal regions.

Families and Data for QTL Mapping

Families segregating traits of interest, in which the traits have been recorded, are a primary requirement for locating the genes controlling the traits. Unfortunately, the range of traits that are routinely recorded in commercial populations is very limited, and through necessity focused on simple traits, such as growth rates and milk yields. An additional consideration is that traits are often sex specific and while selection is applied most strongly in the males, because of artificial insemination (AI), the trait is often expressed in the females, e.g., fertility. In the case of meat quality, accurate measurements of the traits can only be measured post-slaughter and thus cannot be made in the animals intended for breeding.

The structure of the commercial dairy population includes many large half-sib cows produced by AI from a limited number of elite bulls. This population structure is particularly appropriate for mapping QTL. The genetic contribution of bulls to milk production traits can be determined with high accuracy by measuring the phenotypes of their daughters. Bulls with high breeding value are then used extensively through AI to improve the dairy cow population. The sons of bulls with high breeding values are in turn used as AI sires to produce a large number of daughters. Georges et al. (1995) used the US Holstein population to map several QTL involved in milk yield. However, the way bulls are selected in the beef industry is somewhat different. Beef is produced from a large number of breeds, which have not been under the same intense selection as the dairy breeds, and therefore the beef population structure is different. In some countries, there is some systematic recording of beef production related traits in the live animal, such as growth, fat, and conformation traits in order to select the better bulls for breeding. This data provides some opportunities for mapping QTL for simple beef production traits. However, up to now, there has been little direct recording of meat quality traits. Thus, the majority of information published on QTL controlling beef quality traits comes from specifically bred "resource" herds in which the animals used and mating schemes are carefully controlled. These resource herds are usually managed under standardized protocols, which provide the opportunity to record the more difficult traits, which would be impossible using commercial herds (MacNeil, Miller, & Grosz, 2002). Several of the studies investigating meat quality have used resource herds created by cross-breeding between Bos taurus x Bos. indicus types of cattle. There is known to be very large differences in meat quality traits, particularly toughness between these two cattle types. QTL for several beef associated traits have been localized using these extreme cross-bred herds, e.g., for intra-muscular fat or marbling, muscle mass, meat texture, etc. (e.g. Stone, Keele, Shackelford, Kappes, & Koohmaraie, 1999; Casas et al., 2003). However, the value of these QTL in pure-bred populations has yet to be demonstrated.

Genetic Maps

To carry out QTL studies the second requirement is genetic markers to track the inheritance of chromosomal regions. Thus, to be useful, the location of the markers in the genome and their relationship to each other (genetic distances) must be known. The location, or map position, allows markers to be selected that covered the whole genome, or for fine mapping to be concentrated in targeted regions. The chromosomal location of the genetic markers is also used to place the QTL on chromosomes. Over the past decade, genetic and physical maps have been developed for the genomes of all the major domestic species. Two types of genome maps exist: genetic and physical maps. Genetic maps are created by determining the linkage between markers, estimated from their inheritance in families (Snelling et al., 2005; Barendse et al., 1997; Georges et al., 1995; Bishop et al., 1994; Archibald et al., 1995; Rohrer et al., 1996). Recombination will occur between markers that are far apart on chromosomes more frequently than those that are close together. Thus, the recombination frequency between markers can be used to calculate the genetic distance between them. The genetic maps of the livestock species were initially composed predominantly of "anonymous" markers such as microsatellite loci, but more recently genes and expressed sequence tags (ESTs) have been added to the maps. Genetic maps of the cow (Ihara et al., 2004) and other species now contain around 4000 markers. Genetic maps, however, have limited utility for identifying positional candidate genes near QTL; as in general, there are a few markers within genes included within the linkage maps. The resolution of the available genetic maps is also fairly low, as the order and genetic distance between markers can only be determined when there are recombination events between them. For closely linked markers, the probability of a recombination between them is low, and so to determine their order, a large number of individuals would have to be genotyped.

To overcome the deficiencies of the genetic maps three types of physical maps have been constructed. The first examines the chromosomes directly, by *in situ* hybridization of gene probes to chromosomes and direct visualization of the location of the probe by microscopy (e.g., Hayes et al., 1995; Solinas-Toldo et al., 1995). The second type of physical map is created by observing the retention of chromosome fragments in hybrid cells created by fusing together cells of the target species and immortalized cells of another species, typically hamster. Such somatic hybrid cells retain only a fraction of the chromosomes coming from the target species, and therefore the frequency of co-retention of two marker loci can be used to estimate how close together they are on the genome. A refinement of somatic cell hybrid mapping is the radiation hybrid map. These maps are constructed based on the probability of a radiation induced break between the loci in the genome of the target species. To create a radiation hybrid map, cells from the target species are irradiated before fusion to the immortalized cells. As with somatic hybrid cells, the presence or absence of markers in a series of the hybrid cells can then used to determine how close they are in the genome (Itoh et al., 2005; Jann et al., 2006; Everts-van der Wind et al., 2005). High resolution radiation hybrid maps are achieved by increasing the radiation dose, and hence causing a greater fragmentation of the target genome. The third type

of physical map is composed of an ordered set of DNA clones built from large fragment DNA libraries. Currently, the most widely used large fragment cloning vector is the bacterial artificial chromosome (BAC, Zhu et al., 1999). The recovery of DNA from particular genomic regions can be achieved rapidly by selecting the appropriate BAC clone. Using sequence information obtained from clones such as BAC end sequences, clone-based maps can also be used to enhance connections between annotated genome sequences of different species (Gregory et al., 2002). The drawback of physical maps compared with genetic maps is that they lack the polymorphic markers that are needed to refine the locations of the QTL. Therefore, to locate the QTL on a chromosomal region, then fine map that region and ultimately identify the gene(s) controlling the trait, it is often necessary to use the information from both physical and genetic maps.

QTL Discovery by Linkage Mapping

The basic principle underpinning genetic mapping studies and to identify QTL is linkage mapping. This concept is very simple, and is that the likelihood of a recombination event between locations on a chromosome is proportional to the distance they are apart. Therefore, alleles at loci that are on different chromosomes or which are far apart on the same chromosome will be randomly assorted in the successive generations, whereas the closer together genes are, on a chromosome, the more likely they are to be inherited together. By examining the patterns of inheritance of markers and phenotypes, the markers close to the genes controlling the trait are identified as those that show the same, or a very similar, pattern of inheritance. Hence, from knowing the position of the markers in relation to each other in the genetic map, the position of the genes controlling the trait being studied can be deduced. However, the first problem is that each QTL explains only a small fraction of the phenotypic variation, and hence the correlation between the phenotype and the genotype is low. The second problem is that not all of the variation seen in the phenotype is genetic, and environmental factors can have a large impact on the observed variation. The ease with which a particular QTL can be detected is proportional to the amount of the variation in the trait that it explains. A QTL that explains a large proportion of the phenotypic variance, in the extreme case a Mendelian gene which explains all the genetically controlled phenotypic variation, will be easier to detect than one that explains only a small proportion of the variation. The amount of variation in a trait, and the accuracy with which the trait can be measured, is also very important. For traits that show a large variation in relation to the accuracy of measurement, detection of QTL will be easier than a QTL with a smaller effect. Meat quality traits are in general poorly defined, and hence not measured with great precision. Sensory panels, for example, are subjective, and so the definition of a trait by different people will vary. The use of objective measures as surrogates for human taste panels, such as mechanical shear force to define toughness, are poorly correlated with the appreciation of toughness while eating. With such poorly defined traits, only QTL with major effect, i.e., accounting for a large proportion

of the phenotypic variation, will be identified with confidence. To identify a QTL with a reasonable certainty, a large number of individuals will be required to make statistically robust associations between the markers and the traits. As the size of a study increases, and in particular by increasing the number of loci (or number of traits) tested, the likelihood of detecting a chance association between marker and phenotypes increases. It is then necessary to set high thresholds for accepting the associations detected, which risks rejecting some real associations.

The methods suggested to detect QTLs in livestock populations were initially developed to use either two or three generation pedigrees to analyze linkage between a single marker and a QTL (Neimann-Sorensen & Robertson, 1961; Weller, Kashi, & Soller, 1990). The drawback of these methods is that they use information from a single marker at a time. If information from multiple markers is used simultaneously, the confidence with which QTL can be detected is increased. Interval mapping methods, which use information from all markers within a defined genetic interval, have been developed and successfully used to identify QTL using half-sib families (Georges et al., 1995; Knott, Elsen, & Haley, 1996). A web-based software package "QTL Express" (Seaton, Haley, Knott, Kearsey, & Visscher, 2002) implements a multi-marker linear regression method (Knott et al., 1996). This software is publicly available and has been used to analyze the data from many QTL studies in livestock. Alleles at different, independent QTL potentially interact and may influence the same trait, with positive or negative effects, depending on the alleles present at each locus. By considering the interactions of multiple chromosomal regions simultaneously in the analyses, it is possible to increase the power and the precision of QTL mapping in out-bred populations (de Koning et al., 2001).

The results of QTL studies should only be taken as preliminary evidence for the role of a particular genomic region in the control of a trait. Before using QTL information, either in selection programs, or as the starting point to identify the trait gene, it is wise to obtain independent data that supports the existence of the QTL and the size of its' effect. Supporting evidence may include the identification of the QTL in independent studies. Examples of QTL studies, their confirmation, and identifying trait genes from the QTL are discussed below.

Intramuscular Fat

Intramuscular fat, seen as marbling fat in meat, is important for eating quality, as it affects flavor, juiciness, and possibly toughness. The accumulation of subcutaneous fat and intramuscular fat are affected by management, and selection for one is highly correlated with the other. While intramuscular fat is desirable, subcutaneous fat is largely unwanted and is trimmed and discarded when meat is dressed. It would be advantageous for producers if animals could be selected that maintained the accumulation of intramuscular but had a reduced accumulation subcutaneous fat.

A QTL for marbling as an indicator of the amount of intramuscular fat was identified by Casas et al. (1998) on bovine chromosome 2. The QTL effects were initially attributed to myostatin as a candidate gene in the region. Four further

studies also identified a marbling QTL on chromosome 2 (Stone et al., 1999; Casas, Keele, Shackelford, Koohmaraie, & Stone, 2004; Schimpf et al., 2000; MacNeil & Grosz, 2002), which provided some evidence to confirm the first study; however, the QTL peak in each study seemed to be at a different chromosomal position. This uncertainty is because each study used different sets of markers, and genetic maps, in addition to different breeds and measurements of the trait. Thus, it is not certain if the same or different QTL is being detected in each case. QTL with an effect on muscle marbling have be reported on other chromosomes, with evidence for the QTL provided by more than one study; fat associated QTL have been detected on chromosome 3 in three independent studies (Casas et al., 2004; Stone et al., 1999; Casas et al., 2003) and on chromosome 27 in two separate studies (Casas et al., 2000; Casas et al., 2003), although again the positions of the QTL differed between each study. Other QTL affecting fat traits have been reported on chromosome 5 (Stone et al., 1999), 8, 10 (Casas et al., 2001), 9, 14, 23 (Casas et al., 2003) 16 (Casas et al., 2004), 17 (Casas et al., 2000), and 29 (MacNeil & Grosz, 2002) have not yet been detected in other studies.

Tenderness

Tenderness is a primary factor influencing the consumers' reaction to meat. However, tenderness is difficult to define objectively. The appreciation of tenderness when eating is not explained by the force required to cut through a piece of meat, but is affected by the way the muscle fibers breakdown and the release of juice and flavor while chewing. Tenderness has been measured experimentally by three different approaches: either as shear force required for artificial jaws to pass through a piece of meat, the most common mechanical shear method for defining tenderness is Warner-Bratzler Shear Force (WBSF), by taste panel analysis, and by determining the myofibriler fragmentation index (MFI), which is related to the way that meat fibers are broken down. Several QTLs have been reported for WBSF including QTL regions on chromosomes 5, 9, 15, 20 (Keele, Shackelford, Kappes, Koohmaraie, & Stone, 1999, Casas et al., 2001, Casas et al., 2003, Casas et al., 2004), but none of these have been confirmed in independent studies of the same measurement. Gutiérrez-Gil et al. (2007), identified a suggestive QTL for myofibriler fragmentation index (MFI) on chromosome 15, but did not identify a shear force QTL at the same position. Several independent studies have identified a locus on bovine chromosome 29 with effect on tenderness (Casas et al., 2003; Casas et al., 2001; Casas et al., 2000). The calpain 1 (*CAPN1*) gene that codes for a calcium dependent protease involved in meat tenderization post-mortem (see above) is located within this QTL region and is a strong candidate gene for this QTL for meat tenderness.

Unexpected Discoveries

Globally, consumer requirements and trends in preferences of meat quality vary. Currently, the European market demands meat that is low in fat, while the Asian

market requires meat with a high fat content, particularly intramuscular fat. To address local markets selection has resulted in breeds with very different meat characteristics in different global regions. In Europe, cattle breeds, such as the Belgian Blue, Charolais, Limousin, etc., have been selected for rapid, lean growth and good feed conversion efficiency. Traditional European breeds that tended to produce meat with high levels of fat, such as the Hereford and Angus have recently undergone selection to change the breed characteristics and reduce the levels of fat in their meat. In contrast, e.g., in Japan, selection of the Wagu cattle has been strongly focused on developing cattle that produce exceptionally high levels of intra-muscular fat, which is sold at high value in the home market. Similar divergent selection criteria have been applied in pig production. In response to consumer demand, pig breeds used extensively in Europe have been selected for lean growth, particularly in the last 20 years, whereas Meishan pigs from China, have large quantities of fat.

Studies designed to identify QTL controlling fertility and carcass composition in pigs have been carried out using specifically designed cross-bred resource populations from founder breeds with different phenotypes, these include crosses between wild boar, commercial landraces, and local breeds. QTL for carcass and fertility traits have been identified in studies on such extreme breed crosses (e.g., Rattin et al., 2000; Nagamine, Haley, Sewalem, & Visscher, 2003). A QTL that is of particular interest was identified on pig chromosome 7 in a cross between the lean Large White and the fat Meishan pig breeds. This QTL had a particularly large effect, explaining about 30% of the difference in back fat thickness between the two breeds (de Koning et al., 1999). The surprising finding, however, was that the allele associated with lean growth originated from the phenotypically fat Meishan breed.

This example demonstrates that the most beneficial allele for a particular trait may not be found in population showing a desirable phenotype for the trait. This may be because of founder effects, i.e., that the most favorable allele was not present in the individuals initially used to create the population, or breed, or that the most favorable gene may have been lost over time, e.g., because of genetic drift. Alternatively, the favorable allele may have a deleterious effect on another trait and so the benefit is either not seen or the association with undesirable characteristics has placed the gene under negative selection.

Finding the Trait Genes Starting from a QTL

Using a linkage mapping approach for localizing a QTL will define the chromosomal location through flanking DNA markers. These *linked* markers can be used to enhance selection programs by identifying animals that carry the favorable allele at the QTL, which can then be used for breeding. This process is called marker-assisted selection (MAS, Kashi, Hallerman, & Soller, 1990). However, these markers are likely to be at a significant genetic distance from the gene controlling the trait; therefore recombination is likely to occur between the marker and the trait gene. As

a result, at a population level, the alleles present at the marker loci will not predict the alleles present at the trait locus. Therefore, before the linked markers can be use for MAS, it is first necessary to determine the *phase* of the markers, i.e., which alleles at each of the marker loci are linked to the favorable or unfavorable alleles at the trait gene. Determining the phase of markers has to be done within a family by recording the phenotype of individuals in the family and relating this information to the genotype at the linked markers. The phase of allele at the flanking markers and the trait gene may not be the same in different families. Furthermore, recombination can occur at each generation and so the phase of allele at the marker loci and the trait gene has to be frequently reconfirmed. For these reasons, marker assisted selection is not particularly efficient if implemented simply using flanking markers. In contrast, identifying the trait gene, or better, knowing the functional polymorphism that is responsible for the variation in the trait provides markers that can be used directly in the population, without first having to determine their phase. Using information on the gene in selection programs is called gene assisted selection (GAS) and is more effective for enhancing selection than linked markers. Nevertheless, the first step for identifying the trait gene is currently a linkage mapping approach.

There are now a large number of QTLs identified for production traits in livestock (e.g., see http://bovineqtl.tamu.edu/ and http://www.animalgenome.org/QTLdb/). However, so far, few trait genes, and specifically the functional mutations within these genes, have been identified. Identification of the trait genes starting from the chromosomal location is not an easy task. Genetic mapping studies generally localize QTL at low-resolution. Typically studies using microsatellite markers have placed the QTL within a 20–40 centi-Morgan (cM) interval, which roughly equates to 20–40 Mb DNA or a quarter of a chromosome. A QTL region of this size could contain 200 or more genes. Thus, it is necessary to either refine the map position before trying to identify the specific gene that controls the trait, or use other information to select genes within the region for which there is good evidence that they have an effect on the trait.

Linkage-mapping relies on recombination to determine the order of the markers in relation to the trait gene(s) on the chromosome. The unrecombined region of a chromosome that is inherited together with the trait gene is in *linkage disequilibrium* with it. In order to fine map a QTL, it is necessary to examine additional meioses which provide the opportunity for addition recombination events within the QTL region closer to trait gene, and hence reduce the region flanking the gene that is in linkage disequilibrium (LD). In practice, a large number of individuals are required to find those with recombination occurring within the region in LD flanking a QTL. Therefore, fine mapping a QTL region requires a large number of individuals that have been recorded for a trait, within families that are segregating for the QTL. Once the QTL region has been fine mapped, two approaches can then be adopted for identifying the trait gene. The most popular and successful approach so far has been a refinement of the candidate gene approach described earlier, using both the information on a biological function of the gene and its' chromosomal position to select a "positional candidate" gene. The identification of positional candidate genes is assisted using comparative genomics to examine the equivalent chromosomal

regions across species, together with information on known functions of the genes in controlling phenotypes, e.g., from comparative studies in mice. In the absence of a candidate gene, or when the candidate genes identified prove not to include the trait gene, it is then necessary to adopt alternative strategies. To date this has required the cloning of the QTL region followed by sequencing in order to expand information available on the genes and variations present within the QTL region. Sequences of individuals showing differences in their phenotype is then compared to identify animals in which recombination has occurred within the QTL region to reveal specific genetic variations that are associated with differences observed in the trait. Depending on the positions of recombinations, the position of the causative mutation may be refined, or possibly a specific functional polymorphism identified that is responsible for the observed phenotypic variation.

Identification of the trait genes and functional variations

There are now several examples of studies that have identified trait genes using both position candidate gene and positional cloning approaches. Illustrative examples of both approaches and a combination of these approaches are discussed below.

Myostatin

Double muscling is a distinctive phenotype that has been recognized by breeders of several European beef breeds. It is characterized by pronounced muscular development, resulting from both muscular hypertrophy and hyperplasia, and is associated with reduced intra-muscular fat (Ménissier, 1982). Hence, double muscled animals produce carcasses that have an increased yield of the expensive cuts of meat, and are exceptionally lean. The most extreme form of double muscling is found in the Belgian Blue breed where the trait behaves as if it is controlled by a single major gene. To localize the gene responsible, a research population of cattle was created by crossing double muscled Belgian Blue cattle to a non-carrier breed. Using this population and a conventional linkage mapping approach, the double muscling gene was localized to a region on bovine chromosome 2 (Charlier et al., 1995). Examination of the genes within this region identified no strong candidate genes for the trait. A collagen gene that was a possible candidate was ruled out, and so a fine mapping and positional cloning strategy was started. However, at the same time work in mice on the transforming growth factor (TGFβ) family of genes identified a new member of the gene family, GDF8, that had an effect on muscle development. Transgenic mice in which expression of GFF8 gene was "knocked-out" developed hyper-muscularity similar to the double muscling phenotype in cattle (McPherron, Lawler, & Lee, 1997). The GDF-8 gene product was found to be a negative regulator of muscle growth and was therefore called myostatin. Myostatin acts in the developmental pathway which regulates the differentiation of progenitor cells into muscle and fat tissues. In the absence of myostatin, the cells preferentially develop

into muscle instead of fat. Belgian Blue cattle that showed the double muscling phenotype were found to have an 11 bp deletion within the coding region of the GDF8 gene (Grobet et al., 1997; Kambadur, Sharma, Smith, & Bass, 1997). Cattle of other breeds with the double muscled phenotype were subsequently found to have mutations within the coding region of their myostatin gene, e.g., the Piedmontase breed has a single base mutation within the coding region of the myostatin gene (McPherron et al., 1997). Individuals of the Marchigiana beef breed that carry a single nucleotide polymorphism in the 5' promoter region of the gene have a muscularity index 25% higher than individuals without the variation (Crisà, Marchitelli, Savarese, & Valentini, 2003).

Interestingly, the same variation as found in the Marchigiana had also been identified in pigs, where it is associated with higher average daily gain, as well as improved muscling (Jiang, Li, Du, & Wu, 2002). Muscular hypertrophy in Texel sheep is also associated with a variation in the GDF8 gene. In this case, a polymorphism in the 3'UTR in some individuals in this breed creates a target site for microRNAs that destabilize the myostatin gene product. The result is a reduction in myostatin and hence the muscular hypertrophy seen in some Texel sheep (Clop et al., 2006).

Calypyge

In sheep excessive muscular development was observed in the progeny of a single ram, Solid Gold, in the USA in the early 1990s. The trait showed an unusual mode of inheritance, with the expression of the phenotype dependent on whether the "Calypyge" allele was inherited from the sire or the dam, a phenomenon known as imprinting. Linkage mapping studies localized the gene controlling the *CLPG* to sheep chromosome 18 (Fahrenkrug et al., 2000; Berghmans et al., 2001). The region containing the QTL was then sequenced and the polymorphisms identified used to refine the locations of recombination events and reduce the candidate interval to approximately 400 Kb. This interval contained a good candidate gene, *DLK1*. The region of the human chromosome 14 and mouse chromosome 12 that correspond to this region of sheep chromosome 18 have been intensively studied because they show parental imprinting, i.e., allelic expression is dependent on the parent of origin (Schmidt, Matteson, Jones, Xiao-Juan, & Tilghman, 2000). In fact, two genes within this chromosomal region both in mouse and human are imprinted, with paternal allele of *DLK1,* but the maternal allele of *MEG3 is* expressed. This region was fully sequenced from sheep carrying the *CLPG* mutation, and an A to G polymorphism was identified in an inter-genic regulatory region between *MEG3* and *DLK1* that segregated with the trait. The mutation seems to affect a site that regulates the epigenetic modifications involved in the imprinting of the *DLK1-GTL2* region (Freking et al., 2002). While the increase in muscularity associated with *CLPG* is a positive feature which was initially selected for, subsequently the trait was associated with poor meat quality.

Insulin-Like Growth Factor (IGF2)

Resource populations, in which genetically divergent founders with distinct phenotypes are crossed, have been used extensively as a tool to identify QTL, particularly QTL involved in variations in carcass traits. A QTL on chromosome 2 in pigs controlling variation in muscle traits and fat depth was mapped, initially in several resource populations, some created by crossing European with Asian pig breeds. Using the combined data from several different studies, in a meta-analysis, the position of this QTL was refined to a small region including the insulin-like growth factor 2 (*IGF2*) gene (Nezer et al., 1999). Sequencing across the *IGF2* locus revealed 258 polymorphisms in and around the gene (van Laere et al., 2003). These polymorphisms could be assembled into two haplotypes, one defining a chromosomal region of European origin and one from the Chinese breeds. Examination of these haplotype blocks within individual animals in the crossbred resource populations identified several individuals with recombinations between the two haplotypes that further refined the chromosomal location of the causative variation and identified a single SNP, a G to A transition within intron 3 that appeared to be the causative mutation, or "quantitative trait nucleotide" (QTN). Interestingly, intron 3, is not within the region of the gene coding for the *IGF2* protein. One mechanism for the regulation of gene expression through imprinting involves the methylation of cytosine bases to inactivate the gene. The IGF2 QTN occurs in a region of the DNA that is imprinted as a result of cytosine methylation. The expression pattern of *IGF2* is known to show imprinting, and the specific SNP seems to be within a binding site for a protein that regulates gene expression.

Both the *IGF2* and the Callipyge example discussed above (Freking et al., 2002), are traits associated with muscle composition that are controlled by genetic variations outside the protein coding region of the genes involved, and within regions that show imprinting.

Hints from, Fat in Bovine Milk (DGAT1)

Many studies performed on dairy cattle using commercially collected data have identified QTL for milk yield and composition (e.g., Georges et al., 1995). One of these QTL, associated with variations in milk fat content, was mapped to chromosome 14 then fine mapped using new markers produced from a sequencing contiguous set of BAC clones spanning the QTL region (Coppieters et al., 1998). This region of bovine chromosome 14 is equivalent to a region of human chromosome 8 which contain the same set of genes, i.e., shares conserved synteny. Studies in mice have shown that a gene acyiCoA:diacylglycerol acyltransferase 1 (*DGAT1*) with this region on human chromosome 8 affects milk synthesis and mice in which there is no expression of the gene fail to establish lactation (Casas et al., 1998). This *DGAT1* gene was found within a contig of BAC clones spanning the QTL milk region on bovine chromosome 14 which made it a strong candidate gene for the milk fat QTL. Sequencing the *DGAT1* gene from bulls carrying different alleles at the QTL revealed a number of polymorphisms within the gene. These polymorphisms included one in exon 12 of the gene that resulted in an amino acid change (alanine

to lysine K232A) in the mature protein (Grisart, Farnir, & Karim, 2002). Extensive studies of Holstein cattle have shown that cows with the lysine allele have consistently higher milk fat than those with the alanine allele and that the lysine allele is associated with decreased protein content and milk yield.

Knowledge that *DGAT1* is involved in the biochemistry of fat synthesis, and the discovery that variations in this gene present in cattle had an effect on milk fat, prompted studies to investigate the possible effect of this gene on muscle fat. A study of the K232A substitution polymorphism within *DGAT 1* in a crossbred Holstein vs Charolais experimental herd suggested that the lysine allele was associated with increased enzyme activity and higher lipid content of different tissues, including muscle (Thaller et al., 2003).

Applications

The information now available on the association between specific variation in genes and traits such as the examples given here can, and in some cases, are being implemented in selection programs to improve quality, quantity, and efficiency of meat production. The advances in genomic information and the technology available for genomic studies in livestock species should make the discovery of the trait genes and causative variations within the genes more rapid in the future. In addition, the new technologies that are now available for large volume genomic studies, open the possibility to apply novel strategies to enhancing selection programs, using whole genome assisted selection, rather than applying information locus by locus. However, to achieve this it is necessary to have markers at sufficiently high density to identify linkage disequilibrium block in the population.

New Opportunities

Initially, studies to identify the genes controlling commercially important traits used either a candidate gene or gene mapping approach, as discussed above. These strategies were appropriate to the knowledge and technology available at that time. However, information on the role of a gene could also come from other sources, e.g., the level of expression of a gene, or by extension the variation in expression of groups of genes involved in particular metabolic pathways. The study of gene expression has traditionally been carried out gene by gene. The first methods used gel electrophoresis and hybridization of radioactive probes to reveal the expression and crudely quantify the levels of particular RNAs (Northern blotting). More recently, techniques using the polymerase chain reaction (reverse transcribed PCR: RT PCR), and derivatives of this technique (e.g., TaqMan. Applied Biosystems Inc), have facilitated a more accurate quantification of the amounts of a particular RNA molecule present in a sample. Nevertheless, the use of these techniques follows from the identification of a specific gene, or at least a small number of genes that are believed to play an important role in controlling variations in the target trait. To identify candidate genes, when little prior information is available, it would be

necessary to examine many genes, preferably simultaneously, instead of individuals genes.

Several approaches have been developed to examine the expression profiles of several genes, starting from samples obtained from appropriate tissues taken from individuals with divergent phenotypes in the trait of interest. These approaches begin with no prior hypothesis regarding the genes or gene products that play a role in controlling the observed variation. Differential Display-Reverse Transcribed-PCR (DDRT-PCR) uses a "quasi-random" set of oligonucleotide primers and reverse transcriptase to create cDNA fragments representing a large proportion of the expressed genes in a sample. These are then compared with cDNA fragments created from other samples. The fragments are separated by polyacrylamide gel electrophoresis and side-by-side comparisons allow the identification of transcripts that are differentially expressed between the two samples (Liang & Pardee, 1992). This approach has been applied to examine the variations expression associated with muscle fat by comparing the expression in muscle from Charolais vs Holstien breeds which are phenotypically very different for muscle properties (Dorroch et al., 2001). The DDRT-PCR approach identified 277 differentially genes between the breeds. Sequencing followed by mapping of these differentially transcribed genes to assign them to chromosomal regions (Goldammer et al., 2002) provided a number of candidate genes for muscle related traits. Comparing the genome location with the QTL mapping data for intramuscular fat identified one cluster of differentially expressed genes located on bovine chromosome 3 within a QTL for marbling traits. Other studies have used this technique to ask more focused questions, e.g., to associate variation of gene expression with variations in hormone levels (Bellmann, Wegner, Teuscher, Schneider, & Ender, 2004).

The DDRT-PCR allows differences in expression patterns to be observed from PCR fragments produced from expressed genes. However, identifying the gene from which the fragments originate then requires cloning and sequencing of the DNA fragment. A major technological advance that has opened the way for more extensive studies of gene expression patterns, e.g., between cells from animals with different phenotypes or following particular treatments, has been the development of expression-arrays. By creating cDNA copies of RNA coding for particular genes and binding this cDNA to a solid matrix in an organized array, so that the location of each RNA type is known, the expression of that RNA species, e.g., from different cell types can be investigated by hybridizing RNA from cells to the array. The first arrays, macroarrays, were created by spotting the target cDNA onto a nylon matrix and hybridizing with RNA labeled with radioactive probes. Such arrays could investigate the expression of several hundreds to thousands of genes of simultaneously, but quantifying small difference in the expression from the radioactive signal was not very accurate. More recently, microarrays have been developed by printing at high density many thousands of either cDNAs coding for specific genes, or oligonucleotide probes representing fragments of the cDNA sequences, onto glass slides or other matrices. These arrays are then probed using fluorescently labeled probes which can more precisely measure the radioactively labeled probes. In addition, the labeling of the samples to be compared with two different colored fluors, allows

the samples to be hybridized simultaneously to the same array and differences in expression directly compared.

Initial studies used cross species array probes, from humans and mice, as insufficient information was available from the livestock species. A dual color fluorescence approach, using a human array, for example, was used to compare expression patterns in muscle samples from cattle selected for high and low growth potential. From the 1300 human gene probes on the array, 34 genes were identified with different levels of expression between the genetic types (Casser-Malek et al., 2003). Many of the genes identified with differential expression were associated with muscle structure (e.g., titin) or cell regulation (e.g., thyroid hormone receptor). Data from studies using cross species probes can indicate genes with differential expression patterns, but should be treated with caution as the genes identified may not be the equivalents between species, especially when the genes are members of gene families.

Currently, sophisticated technologies are being used to create arrays with hundreds of thousands of "gene features" which can be used to investigate the relative expression of genes with high precision. Using this array technology, the expression of a very large number of genes can be compared between samples, e.g., of tissues from animals with different phenotypes or in different physiological states. With the increase in genomic information available for livestock in recent years, arrays are now available with probes from the target species. Many arrays have been developed for specific tissues, or biochemical pathways, and arrays are now available for cattle and pigs that contain probes for a major part of the expressed genome. Using a microarray approach to investigate fat deposition in Japanese Black (JB) vs Holstein cattle many genes expressed at a higher level in the JB were found that are associated with unsaturated fatty acid synthesis, fat deposition, and the thyroid hormone pathway (Wang et al., 2005). These array data are consistent with the increased monounsaturated fatty acids observed in beef from Japanese Black Cattle.

The microarray approach has also been used to compare gene transcription profiles between pig breeds with different muscle characteristics (Lin & Hsu, 2005). This study identified several genes involved in, e.g., muscle structure (myosin light and heavy chains, and troponin) and energy metabolic enzymes (electron-transferring flavo-protein dehydrogenase, NADH dehydrogenase, malate dehydrogenase, and ATP synthases) associated with lean growth. However, the selection of the tissue for gene profiling studies requires careful consideration. Liver is an organ involved in energy metabolism; therefore, variations in liver function may influence carcass size and quality. Studies carried out on liver samples comparing expression in German Landrace, which has a relatively high fat carcass compared with the Pietrain that is very lean (Ponsuksili, Murani, Walz, Schwerin, & Wimmers, 2007), showed that animals with fatter carcasses have higher expression of genes associated with lipid metabolism pathways (FASN, ACSS2, ACACA) while lean growth was associated with expression of genes for cell growth and/or maintenance, protein syntheses, and cell proliferation pathways (PPARD, POU1F1, IGF2R).

eQTL

The information obtained from mapping and expression studies is different but complimentary. Genes containing polymorphisms identified from mapping studies are not necessarily those that will be differentially expressed, and conversely genes with differential expression will not necessarily contain genetic variations. The polymorphic gene may, e.g., be a receptor that regulates another gene which is involved in the development of the phenotype. Specific polymorphisms in the receptor may not affect the expression of the receptor gene itself, but may be within the ligand binding site, and hence genes which are regulated by the receptor may show differences in expression. Such a case is referred to as trans-acting effects: i.e., when the location of genetic polymorphism and gene with affected expression are at different locations. Alternatively, a variation within a gene may directly affect the expression of that gene, e.g., variation is in a promoter region necessary for expression. Such variations act in "cis", i.e., the differentially expressed gene, and the gene with the genetic variation are at the same location.

In order to locate the gene regulating the variations in expression, an "expression QTL" (eQTL) approach is used. If the expression data is considered as a phenotype, the location of the genes responsible for the variation can be mapped using a conventional genetic mapping approach. If the eQTL(s) maps to the location of the gene(s) with differential expression, i.e., are in *cis*, it is likely that the differential expression is the result of a polymorphism within that gene. If not, a trans-eQTL, implies that the gene with differential expression is being regulated by another gene at a different location on the genome. If the expression eQTL and QTL data for the trait under investigation are combined, together they may suggest the gene(s) and regulatory pathways important controlling variations in the trait. To date, there have been no eQTL studies completed for livestock species, partly because obtaining the expression data is expensive, and partly because the designs of such experiments are not simple. For meat associated traits suitable tissues should be obtained from animals within families that differ in the target trait. However, the choice of tissue is not always obvious, as a trait displayed in one tissue may be the result of a variation in the activity in another. As discussed above, variation in muscle composition may arise from the muscle, liver, or adipose tissues, or indeed, from another tissue, e.g., pituitary.

Proteomic Analysis

The study of gene regulation at the level of the transcript (RNA) may be misleading as amount of RNA coding for a specific protein may not correlate very accurately with the concentration of that protein in the cell, or e.g. in the case of a hormone in the serum. There are many mechanisms in the cell that can alter the rate of translation of an RNA molecule into a protein. Therefore, it may be better to examine the level of a protein directly, instead of inferring the level of a protein from the amount of RNA that codes for it. Additionally, the protein may be post-translationally modified, either

by processing of a precursor, or chemically e.g., by addition of sialic-acid or phosphate, before the protein is functional. By taking a proteomic approach it is possible to consider the levels of a protein and also post-translational modifications, and so relate more accurately the level of active protein with phenotypic effect.

The basic technique for proteomic analysis has not changed significantly over the past 20 years and involves analysis of proteins by two-dimensional gel electrophoresis, where the proteins are separated using two different physical properties, typically electrical charge, using iso-electric focusing electrophoresis, and then by size, on denaturing SDS gel electrophoresis. The patterns produced from the different samples are then compared and where differences observed, the protein extracted from the gel for analysis. The protein is then identified by analyzing the fragments produced following digestion, typically, by trypsin. The resent advances to improve proteomic analysis have not been in the basic technique, but with improvements in the reproducibility of the electrophoresis and the image analysis to compare the patterns. Analysis of tryptic digests is now carried out by mass spectroscopy and large libraries with information on the tryptic digests of many proteins are available to interpret the data (http://www.expasy.ch/tools/). To date, there have been few reports of proteomic studies in livestock associated with meat quality traits. One example investigated the proteins expressed in muscle from double muscled and normal cattle. The data identified that muscles from animals with the 11 bp deletion in the myostatin gene have a protein pattern consistent with an increase in fast-twitch glycolytic fiber number, which is inhibited when myostatin is expressed normally (Bouley et al., 2005).

Genome Sequence

The ultimate map of the genome of any species is the genome sequence. Following the publication of human genome sequence (Lander et al., 2001), the sequencing capacity that had been assembled was deployed to sequence the genomes of other species. A project to sequence the bovine genome started in 2003 and the first draft sequence with a threefold coverage of the genome was made publicly available in November 2004. Continued work has now produced a full draft sequence constructed from a sixfold genome shotgun (random) sequencing of DNA from an inbred Hereford cow combined with a 1.1 fold sequence coverage from a minimum tiling path of BAC clones from a of a Hereford bull. The assembly of the shotgun sequence was assisted using a composite genome map of the bovine genome that incorporated all the available genome mapping data (Snelling et al., 2007). A project to sequence the pig genome is currently underway and is using a well ordered genome-wide set of ordered BAC clones to create the whole genome sequence.

One of the important outputs of the human genome sequencing project has been a large number of SNP markers, that are being used in genetic studies. Currently, more than 4 million human SNPs have been validated (International HapMap Consortium, 2007, http://www.genome.gov/10001688). The cattle genome sequencing project identified over 2 million putative SNPs, but many of these are artefacts from

sequencing errors. Additional genome sequencing from genetically divergent breeds in the bovine Hapmap project identified 30K SNPs, that have been tested and confirmed across many breeds. A similar approach to SNP discovery has been adopted for pig.

Genotyping Technologies

SNP polymorphisms have advantages over other marker types, in-so-far as they can be detected by methods other than electrophoresis, which is slow and difficult to automate. Following the discovery of many hundreds of thousands of SNPs from the human sequencing project, automated assays have been developed using, e.g., fluorescence or Mass-spectroscopy, to genotype SNPs. It is now possible to rapidly genotype hundreds to thousands of individuals for tens of thousands of SNP markers in a few hours.

A panel of about 50,000 SNPs was recently created for the bovine genome by a consortium of researchers in the USA. The power of this panel of markers was recently demonstrated using *DGAT1* (see above) as an example. Using this marker panel with Holstein cattle families, a QTL with an exceptionally high LOD score and a 3 Kb 90% confidence interval was identified centred on the *DGAT1* gene (Schnabel et al., 2008). This panel is now commercially available (Illumina Inc, San Diego USA). This high density of SNP markers will allow genes to be identified directly by the association of regions of DNA that are inherited in *linkage disequilibrium* with the trait gene. To date, the relatively low density of markers (typically around 150–200) used in QTL mapping studies has required using families.

Application of the Data in Breeding

As discussed above, breed improvement, up to now, has been achieved through phenotypic selection focused on easily measured traits. Over the last four decades, the approaches to selection have been refined and trait measurements, made on the individual, have been replaced by calculated "breeding values" that make use of all the available information on the genetic merit of the individual, including information from relatives, parents, progeny, and siblings. However, many of the economically important traits, and certainly those involved in variation in meat quality are difficult to measure and are "quantitative" in nature. The phenotypic variations in these traits were originally thought to result from the interactions between the many genes, each having a small effect on the phenotype - the infinitesimal model (see review by Flint & Mott, 2001). If this were the case, it was thought that identifying the genes controlling a quantitative trait would be impossible. Fortunately, as demonstrated by the QTL examples given above, for at least some economically important traits it seems that, although there may be many genes involved in controlling the variation in the trait, there are usually a small number of *major* genes that control a reasonable proportion of the observed variation. Information on a few genes can be readily incorporated in to selection programs, by genotyping

individuals and adding the information into the calculation of the breeding value currently estimated from phenotypic measurements.

The use of genetic markers to improve estimated breeding values was suggested over 15 years ago (Fernando & Grossman, 1989), but in general the use of marker to increase the accuracy of selection has been restricted by a lack of knowledge of QTLs with large effects. There are now several examples where the genes controlling important meat production traits have been identified, as discussed above, and a few pilot programs are including this information among the selection criteria. However, for most traits the information available is a QTL position based on loosely linked markers, and not the gene, or variation within the gene. The QTL information is specific to the study population in so far as different alleles at the marker loci can be associated with favorable or unfavorable alleles in different populations, thus linkage phase between a marker and QTL had to be established for every family in which the markers are used. The inclusion of neighboring marker information, to create haplotypes containing the QTL, increases the confidence for correctly identifying genomic regions containing favorable alleles compared with using markers individually. Using a high density of markers to identify haplotypes spanning relatively short genetic distance regions that are identical by descent (IBD) and which are conserved at the population level allows recombination events close to the trait gene to be recognized. This information can identify possible errors in correctly assigning alleles at the trait locus. And, as discussed before, knowing the trait gene and variations in that gene allows the information to be used with certainly.

The rate of genetic improvement that can be achieved in selection for meat quality traits, is dependent on the amount of the variation that is genetically controlled. The improvement that is possible using markers in the selection program is proportional to the amount of variation that is explained by the genes included in the selection criteria. For both beef and pork, meat quality traits between 10 and 30% of the variation is genetically controlled (e.g., Burrow, Moore, Johnston, Barendse, & Bindon, 2001), but individual genes may explain only a few percent of this variation. However, some traits that have a well defined biological basis, and which affect specific aspects of meat quality, have a much higher genetic contribution, e.g., the size and number of fibers in particular muscles, which will affect lean muscle development (Rehfeldt, Fiedler, Dietl, & Ender, 2000). Indeed the myostatin gene, which is associated with double muscling in several breeds of cattle, has been shown in mice to have a major influence in regulating muscle fiber size, type, and number (Rehfeldt et al., 2006). However, although the mutation in the myostatin gene has been shown to control a major part of the double muscling phenotype in the Belgian Blue breed, in other breeds, e.g., the South Devon, the same mutation has a more limited effect (Wiener, Smith, Lewis, Woolliams, & Williams, 2002). Therefore, even when the trait gene is known and the effect has been characterized in one population, care should be exercised in extrapolating information for use in another. It is likely that even for a gene that is responsible for a large proportion of the genetic variation the affect on the phenotype may be dependent on interactions with other genes (epistasis).

Eventually, sufficient information will be accumulated to define the biochemical pathways that control particular traits and phenotypes. It will then be possible to select for improvement on several criteria and multiple genetic loci, each of which are involved in the development of the desired phenotype. To identify these pathways, QTL mapping and individual trait gene identification is just the first step. Several approaches will be required to improve the factors involved in regulating meat quality parameters. One route to identifying particular biochemical or developmental pathways that are involved in the meat quality traits, will be to examine the expression patterns of genes and identify those that are co-regulated during particular developmental processes and are associated with, e.g., specific nutritional status or with particular phenotypes. This information along with the genetic information can be used to optimize the selection strategies.

Genome Selection

The use of genome wide marker information has been proposed by several authors (Meuwissen, Hayes, & Goddar, 2001; Gianola et al., 2004), to select the best animals based on their whole genome, rather than on one, or a few markers. The genome selection approach is used to estimate the genetic value of an individual based on all the available genetic marker information, rather than detection of quantitative trait loci. The main challenge with this approach, not withstanding the collection of suitable data, is to develop a functional statistical model that simultaneously relates phenotypes to SNP genotypes taking into account additive genetic effects between different loci and other nuisance effects, such as sex or age of an individual, environment etc. Standard quantitative genetics theory gives a mechanistic basis to the mixed-effects linear model, treated either from classical (Sorensen & Kennedy, 1983; Henderson, 1984) or from Bayesian (Gianola & Fernando, 1986) perspectives. With the availability of a 50,000 SNP genotyping panel for cattle, it has been possible to assess the feasibility of the genome selection approach. Using the data from Holstein cattle, van Tassel et al. (2008) demonstrated the value of a hybrid approach in which genome-wide marker data combined with the phenotype data to calculate a genome assisted breeding value (GAEBV). GAEBV calculated using data from bulls in selection programs between 1995 and 2000 was used to test the accuracy of predictions compared with the standard EBV by examining the actual performance estimated for bulls progeny tested in 2000–2003. For milk yield, protein, fat content and SCC, the GAEBV were considerably more accurate than the standard EBVs.

Conclusions

Use of genetic markers will facilitate more effective genetic selection for improvement in production associated traits in livestock. In the first instance, before the genes controlling the traits under selection have been identified and characterized,

marker-assisted selection based on markers that are linked to the gene controlling variation in the traits can be applied. However, this has to be done within families where the allelic associations between markers and traits (phase) had been determined. Once identified, the functional allelic variation within the trait gene can be used to select more effectively for the best alleles at particular loci. With the discovery of tens of thousands of markers genome-wide and the development of techniques to genotype large numbers of markers efficiently, it has become possible to use genomic selection methods. Using a sufficiently high density of markers, it is possible to use those in markers in strong disequilibrium with trait genes to select for chromosomal regions with the best alleles for a trait, even before the trait gene is identified. This is similar to marker assisted selection, but instead of selecting for individual loci, the technique is applied across the whole genome.

There are several advantages of using markers in selection programs, rather than relying on phenotype-based selection. These include a more rapid and accurate prediction of the phenotype and hence earlier selection of breeding stock. Using the genome selection approach, the estimates of breeding values are increased allowing for a more accurate selection and increased confidence in the choice of the best animals. Such selection methods will also help to optimize improvements in traits controlled by loci with pleiotropic effects, where current selection suggests that progress in one trait may have a negative impact on another important trait. Knowledge of the genetic effects of each region of the genome may suggest ways of improving apparently confounded traits simultaneously.

Although there has been rapid advances in knowledge of the genome sequence, in techniques for assaying variations and in the statistical methods for using this information effectively in selection programs, the major barrier to implementing the techniques remains the lack of populations in which phenotypic traits are systematically recorded. Such information is essential to estimate the proportion of the phenotypic variation that is under environmental and genetic control. Phenotypic information is also necessary to calculate the breeding value of each chromosomal segment for a given trait. Genes with a major effect on a phenotype in one population may be associated with little phenotypic variation in another, depending on the genetic background. It is therefore important to collect phenotypic information from different genetic types, and in different environments. Further information on gene interactions may come from gene expression studies. Gene expression microarrays have now been produced for the majority of livestock species, allowing the expression patterns of many thousands of genes to be assayed simultaneously. Building up information on patterns of gene expression in different tissues and species could reveal co-regulated physiological pathways that are currently unknown. This data will add to the information derived from the analysis of the genome and contribute to our understanding of variations in meat quality and other traits.

The increasing availability of genomic sequence from a wide range of species will allow comparison of coding and non-coding regions across species, to identify functional domains and regulatory sequences. When this information is combined with gene expression studies it will be possible to identify those regions of the genome that regulate expression of metabolic pathways involved responsible for

complex traits. In some, possibly many, cases the genetic polymorphism responsible for phenotypic variation in traits may not be obvious if it lies in a regulatory region rather than within the coding part of a gene, as found for fat deposition controlled by *IGF2* in pigs or the *Callipyge* muscling phenotype in sheep. And, as with these examples, the expression of these genes may not be simple, with the expression of specific functional allele dependent on the parent of origin (imprinting). Improved knowledge of genetic and epigenetic effects and interactions between genes (epistasis and pleioptropy) and genes with the environment will allow information to be effectively incorporated into selection and management strategies. These ever-increasing refinements will provide breeders with better tools to rapidly respond to changing market demands for meat products with different qualities.

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