Applications of molecular biology in animal husbandry

Reading From Genomes

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Bulls can now be selected for use in breeding by directly evaluating their genotypes, rather their offspring's milk or meat yields! This represents a revolution in animal husbandry

Gene polymorphisms involve variations in the sequence of nucleotides present in the genetic material of living organisms. Such variations occur naturally over the course of evolution as a result of mutations, and they may result from substitutions of single nucleotides (*single nucleotide polymorphism – SNP*), insertions or deletions of one or several nucleotides (*insertion/deletion – InDel*), or a variable number of repetitions of short nucleotide sequences (*short tandem repeats – STR*). Researchers noted early on that variability seen in the sequence of nucleotides in DNA molecules, or the sequence of amino acids in protein molecules, may be an indicator of human or animal characteristics, both external (appearance, build, body size) as well as internal (biochemical changes, metabolic rates, etc.) This discovery led to the concept of genetic markers.

MAS assessment

Genetic markers of yield characteristics in cattle, pigs, and chickens have been studied since the 1950s. The first studies concerned blood type antigens, and blood and milk proteins. It was correctly assumed that it would be possible to predict a given animal's predisposition towards high yield of milk, meat or eggs on the basis of different genetic variants of certain proteins. DNA studies then commenced in the 1980s. By studying DNA polymorphisms it is possible to analyze genotypes in very young animals, to determine genotypes of milk proteins in bulls, and even to genotype embryos. Such methods greatly speed up the selection of bulls qualified for breeding, which can now be evaluated directly based on their own genotypes, rather than on their offspring's milk or meat

Michael MacNeil, USDA

The cattle genome – which has been sequenced for a Hereford cow, L1 Dominette 0144, like that pictured here – is of a similar size to the human genome (around 3 billion DNA base pairs) and includes a similar number of genes
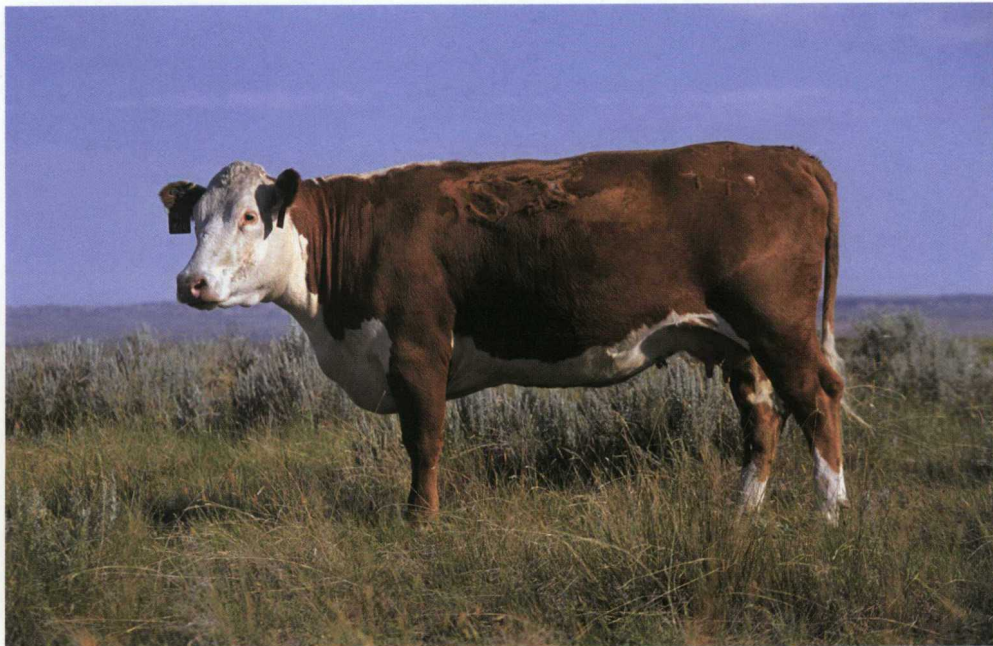
yield. However, MAS (*marker assisted selection*) is not yet in general use for assessing the value of breeding animals.

Quantitative traits

Two methods are used for localization and identification of genes that determine yield characteristics such as milk productivity or meat yield in cattle. The first uses STR polymorphisms to map hypothetical QTLs (*quantitative trait loci*), i.e. the location of genes coding for yield traits in the animal's genome. Based on the analysis of genetic linkages using genetic markers, several hundred QTLs that influence yields of milk, fat and protein, and contents of fat and protein in milk have been identified on various cattle chromosomes. QTLs have also been mapped for other production characteristics, such as the yield and quality of meat in cattle and pigs. By narrowing the position of potential QTLs down to ever shorter chromosomal regions it ultimately becomes possible to clone a given gene, and to identify which mutation of that gene influences the variability in the given characteristic. Such approach has resulted in the identification of a Phe/Tyr mutation in transmembrane domain of growth hormone receptor in cattle as a QTL with a strong influence on the yields of milk and its fat content. The second method of identifying yield markers involves assessing the influence of polymorphisms of candidate genes associated with phenotypic values of the desired yield characteristics. Such studies look at genes with known nucleotide sequences, defined in their role in biochemical and physiological processes that shape a given characteristic.

Double-muscled cattle

One of the most beneficial achievements of cattle genomics has been the discovery of "functional" mutations in the gene coding for myostatin, a protein that inhibits growth and differentiation of muscle tissue. Some of the mutations cause the formation of nonsense "stop" codons or a shift in the reading frame, resulting in a shortened, inactive protein. This leads to augmented muscle growth, with the cattle phenotype known as "double muscled." In sheep the "Texel" mutation in the gene coding for myostatin creates target sites for microRNA (responsible for regulation of protein synthesis), leading to the
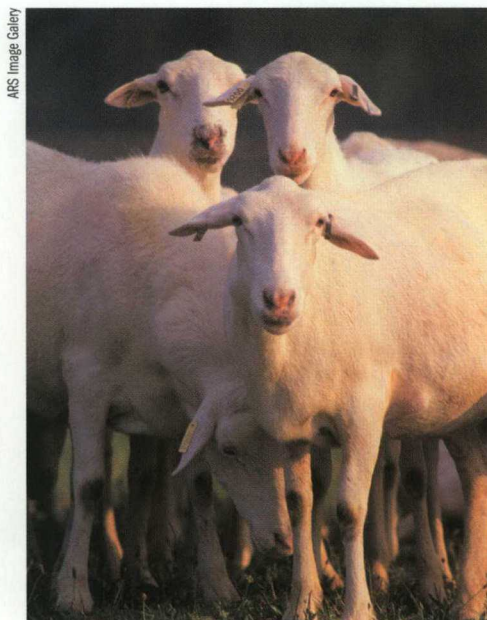
ARS Image Galery

Functional genomics of animals is a rapidly developing discipline. These St. Croix sheep exhibit resistance to parasites and tolerance of high temperatures

inhibition of myostatin secretion. "Callipyge" sheep, in which the mutation in the *DLK-1* gene leads to muscle augmentation in their rumps, derive their name from the Greek for "beautiful buttocks."

Milk protein genes

Genes that code for milk proteins – casein, whey proteins – are recognized markers of composition and quality in milk production. The links between milk protein polymorphisms and cattle yield characteristics were studied in the 1950s, while today we are studying the polymorphisms of genes coding for those proteins. Studies carried out over several years have shown that κ-casein polymorphisms in cattle are connected with the rate of milk coagulation following treatment with rennet. As such they are excellent markers of milk quality as raw material for cheese production. The polymorphism of the β-lactoglobulin gene is connected with bovine milk productivity, and the protein composition of milk. In turn, polymorphisms of the lactoferrin and lysozyme genes are linked with bovine resistance to mastitis (inflammation of the udder), while certain variants of β-casein encode a protein which may be responsible for diabetes and circulatory disorders when consumed by certain people.

In goats the polymorphism in αS1-casein influences the content of α-casein in milk. Several variants of the αS1-casein gene in goats have been discovered – A-F and 0.

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ARS Image Galery

Pork and beef producers have teamed up with scientific research centers to urgently look for genetic markers that will allow for early monitoring of yield and quality of meat in cattle and pigs
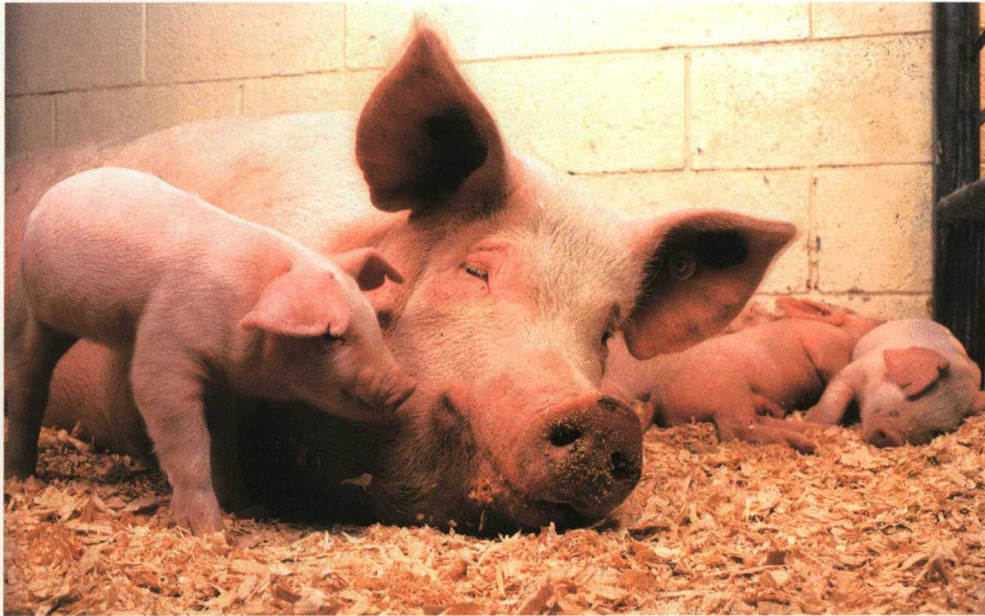
The "strong" variants – A, B, C – determine a high level of α-casein in milk, while the "weak" variants – E, F – determine a low level. Variant 0 results in a complete lack of α-casein in goats' milk. The polymorphism of the αS1-casein gene influences the quality of milk used in cheese production. In France identification of αS1-casein genotype is routinely determined in goats.

Meat quality markers

Overproduction of food means that in developed countries more and more attention is being given to the quality rather than quantity of animal-derived products. Pork and beef producers have teamed up with scientific research centers to urgently search for genetic markers that will allow for early monitoring of yield and quality of meat in cattle and pigs. Several such markers have already been identified. These are mainly genes involved with the growth and differentiation of muscle tissue (synthesis or degradation of muscle proteins). Genes encoding for enzymes of the muscles' proteolytic pathway, such as *CAST*, *CATB* and *CAPN2S*, appear to be promising markers of beef quality, and the polymorphisms of corresponding genes *RYR1*, *PRKAG3*, *PKM2*, *GLUT4* and *MYOG* may be indicators of pork quality. Markers known to be important for the fattening of livestock include the genes for leptin (*LEP*) and its receptor, which play an important role in regulation of the feeding and energy conservation of an organism, as

well as genes encoding for the melanocortin receptor (*MC4R*), a hormone involved in the regulation of feeding, and the family of fatty-acid-binding proteins (*FABP*).

Sequenced cattle genome

To date, the genomes of humans, chimpanzees, mice, rats, chickens, cattle, dogs, horses, cats, and the opossum have all been sequenced. The sequencing of the pig genome is likely to be completed in 2010. Sequencing of the Hereford breed of cattle, L1 Dominette 0144 cow, began in 2003. By 2006 the genome was sequenced 7.1-fold, indicating a high accuracy of the sequencing. The cattle genome is of a similar size to the human genome (around 3 billion DNA base pairs) and includes a similar number of genes.

Scientific studies look more closely at various genome regions – coding sequences of genes, regulatory sequences, non-coding sequences, cDNA and others. They are saved in designated databases, such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html), and are freely available, which makes them invaluable to researchers studying gene polymorphisms.

Learning entire genomic sequences, however, does not fully explain the influences of gene interactions and their variations on phenotype characteristics in fields such as animal husbandry. Scientists are therefore now studying the functioning of genomes. This new scientific discipline – functional

genomics – encompasses various phenomena of gene expression: transcription (synthesis of mRNA – messenger RNA), translation (protein synthesis), and profiles of metabolites related to gene function. Functions of various genomic sequences are being studied using modern molecular techniques of transcriptomics, proteomics, and metabolomics.

Improving meat and milk

The Institute of Genetics and Animal Breeding of the Polish Academy of Sciences (in Jastrzębiec near Warsaw) researches genomics-based methods for the improvement of production and functional characteristics of cattle, and the quality of animal products. Our studies are based on the assumption that gene polymorphisms – substitutions or deletions/insertions of nucleotides – may influence the level of gene expressions or the functions of proteins they code for, and that animals intended for distinct purposes, such of meat or dairy production, differ in terms of profiles of expression of genes specific to different types of tissue. Searching for genes showing different expression in animals of different breeds, varying in genotype or yield potential, may lead to new methods of using genetic markers in the breeding and selection of livestock. We have discovered dozens of new polymorphisms in gene candidates for markers of yield and quality of meat and milk in cattle. We have demonstrated a link between certain polymorphisms in the coding and regulatory regions of bovine genomes with better yields of milk, meat and carcass, as well as improved reproduction and udder health. We are also searching for molecular mechanisms driving the influence of polymorphisms on phenotypic characteristics in animals.

Our most interesting research concerns the cattle transcriptomics. The key question of functional genomics in livestock is as follows: which genes are being expressed, and to what degree does this change in animals with different yield and quality of meat or milk? We have compared levels of expression in tissues and organs of bulls of four cattle breeds that vary greatly in terms of yield and quality of their meat, and their milk productivity – the dairy breed Holstein-Friesian, dairy/beef Polish Red, and typical beef breeds Limousin and Hereford. Using expression microarrays allowed us to simul-

taneously analyze the expression of several thousand of genes in the livers and muscles of bulls. Significant differences in transcriptomic profiles were shown between the breeds and purposes of the cattle. Important differences in levels of gene expression between breeds were shown in tens/hundreds of genes, including many key to shaping the desired characteristics. We have selected several genes whose expression may determine specific production attributes. They are now being tested on large cattle populations.

Tools of tomorrow

It would seem that the huge progress made in recent years in the studies of genetic markers and functional genomics of livestock should quickly find practical applications and that many breeders will start using DNA analysis to select and improve cattle, pigs and poultry. New mutations with potential importance in genetic markers of productivity characteristics are being identified almost every day, and the majority are patented. New tools, such as SNP microarrays, allow for concurrent analysis of hundreds of thousands of mutations in a single animal. However, we are still some way off from seeing animal selection based on marker-assisted selection come into widespread use. DNA analysis remains rather costly, and breeders are not convinced that using MAS will bring them real financial benefits. ∎

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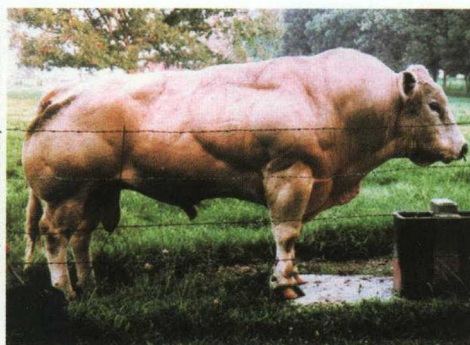
One of the most effective achievements of cattle genomics has been the discovery of "functional" mutations in the myostatin gene. The "double muscled" characteristic of this Blue Belgian bull results from mutation *nt821(del11)* in the myostatin gene

Further reading:

Kadarmideen H.N., Von Rohr P., Janss L.L., (2006). From genetical genomics to systems genetics: potential applications in quantitative genomics and animal breeding. *Mammalian Genome, 17,* 548–564.

Ibeagha-Awemu E.M., Kgwatalala P., (2008). A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. *Mammalian Genome, 19 (9),* 591–617.