Wool Technology and Sheep Breeding

Volume 50, *Issue* 3 2002 *Article* 31

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ISSN 0044 - 7875/02 Wool Tech. Sheep Breed., 2002, 50 (3), 401-409

A Review of Markers for Wool and Sheep Carcase Quality Traits

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Summary

The use of DNA and protein markers for selecting wool and carcase traits is reviewed. The molecular technologies are unlikely to completely replace traditional genetic evaluations for these product-quality traits for a number of reasons. For sheep selection, using protein markers detected by the use of two-dimensional polyacrylamide gel electrophoresis technology is probably too expensive. However, discovering which specific proteins are expressed in superior animals enables the use of high-throughput, immunochemistry laboratory or in-field assays to detect those protein markers. Alternatively, the process of gene detection and location of single nucleotide polymorphisms (SNPs) within the genes that can be used as DNA markers will be greatly assisted by protein expression studies. DNA or protein chip arrays to detect the presence of DNA or proteins markers will make the process less expensive. Relative abundance of structural proteins is probably more important than their presence or absence in determining quality-trait phenotypes.

Keywords: protein markers, proteomics, DNA markers, wool quality, carcase quality

Introduction

DNA or protein marker technologies are more likely to be used by breeders for traits that are difficult to measure and have a high economic value. Markers for disease-resistance traits are more likely to meet these criteria than are most wool- and meat-quality traits. To be widely used by breeders, markers need to be sufficiently accurate and cost-effective compared to traditional non-marker selection methods. Molecular technologies are unlikely to completely replace traditional genetic evaluations (Burrow and Johnston 2000). Quantitative trait loci (QTL) breeding values need to be balanced with polygenic breeding values, or the long-term average merit of the population may reduce due to lower selection intensity for polygenic effects (Garrick 1997).

DNA markers

The human genome contains about 3,165 million DNA base pairs, with the 30,000 to 40,000 genes occupying only about 2% to 10% of the genome (www.ornl.gov/hgmis). The remaining DNA consists of repetitive (25% to 30%) and non-repetitive, non-coding (60% to 65%) DNA. Knowledge of the genetic map of sheep by cytogenetic or linkage assignments has been increasing rapidly (Broad *et al.* 1997; Maddox *et al.* 2001). Prior to 1994, only 17 DNA markers were known, whereas a web-based sheep database (www.thearkdb.org), as of August 2002, listed 1,722 loci, of which 370 were designated as genes and 1,352 were non-coding polymorphic DNA segments. Most markers are from studies of non-coding microsatellites (Ms), restriction fragment length polymorphisms (RFLP), (fluorescent) *in situ* hybridisation ((F)ISH) or single-stranded conformation of polymorphisms (SSCP). The cattle database listed 2,621 loci, of which 668 were designated genes. There are many DNA sequences that are common to both sheep and cattle, as they only diverged phylogenetically about 17 million years ago (Pepin *et al.* 1995).

The chromosome positions and names of the 370 sheep genes coding for various enzymes, hormones, growth factors, receptors and structural proteins are given on the ark website. Wool- and carcase-quality traits are presumably controlled by genes affecting the expression of various structural proteins that make up wool fibres and carcase, e.g., keratins, collagens, actin and myosin, and/or genes affecting fluxes across metabolic pathways that control cell growth and differentiation, timing or tissue specificity of gene expression and/or ligand-receptor systems that control cell function, e.g., fibroblast and epidermal growth factors, bone morphogenetic proteins, Janus family tyrosine kinases (Jaks) and signal transducers and activators of transcription (Stats). Bost *et al.* (1999) have modelled how QTL may control the activity or quantity of enzymes in metabolic pathways.

Research on marker assisted selection (MAS) using DNA markers linked to production or disease traits and QTL has focused on the candidate gene approach or the genome scan approach, which uses Ms spread over the genome at about 10 to 20cM intervals (Dekkers 1999). Initially, markers flanking QTL are identified, then more closely linked markers are found where the haplotypes (different numbers of repeats in Ms) associated with QTL hold across the whole population, not just within families. The final aim is to find a polymorphic marker within the gene controlling the phenotype (Georges 2001).

The candidate gene approach has focused on short expressed sequence tags (EST), usually 100 to 200 bases long, that can act as an identifier of a gene hypothesised to play a role in the trait of interest. EST sequence data are usually processed into contigs to reduce redundancy and generate longer contiguous sequences from overlapping ESTs. Messenger RNA from tissues of interest is copied to cDNA and inserted into bacterial plasmids to produce gene libraries. DNA fragments from these inserts can be sequenced and then screened against known genes from other species to help establish gene function (Franklin *et al.* 2000). The EST expression patterns are characterised

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using (F)ISH, and flocks can be screened for associations between genetic variation at these loci and phenotypic performance. The EST work aims at distinguishing genetically superior animals from the patterns of expression of thousands of genes measured simultaneously on DNA microarrays or chips. If their costs reduce, the microarray methods may move beyond being only used for research.

Genes with major effects on halo hairs, medullation, lustre and crimp are listed by Lauvergne *et al.* (1996). Wool- and carcase-quality traits with reported DNA markers are given in Tables 1 and 2, respectively. None of these markers are being used commercially. Parsons *et al.* (1994) reported evidence for linkage between high-glycine-tyrosine keratin gene loci (KAP 6 and 8) and wool fibre diameter in medium-wool Merinos, as have Beh *et al.* (2001) and Henry *et al.* (1998) in a Merino x Romney backcross flock using Ms. The size of the QTL effect reported in these studies has been up to 3μm. It is difficult to postulate the possible morphological basis of this effect, as KAP 6 and 8 only make up about 5% of the volume of cortical proteins. However, they may well be located in the orthocortex (Powell and Rogers 1994; Powell 1996), which increases as a percentage of the fibre volume as fibre diameter increases (Orwin and Woods 1980).

Table 1 DNA markers or genes for wool-quality traits

Trait	DNA microsatellites	Gene/protein	Reference
Fibre diameter (μm)	OARDB6, RM65	KAP 6 and KAP 8	Parsons *et al.* (1994); Henry *et al.* (1998); Beh *et al.* (2001)
Staple strength (N/ktex)	ETH3	KRT 1.2, KAP 1.1 and KAP 1.3	Rogers (1994)
Clean colour (Y-Z)	TGLA77	transmembrane signaling (K^+ transport)	Benavides *et al.* (2000); McKenzie *et al.* (2001)
Recessive dark fibre (/100g)	HUJ616, Ms	Agouti	Parsons *et al.* (1997); Allain *et al.* (1998)
FDCV(%), length (mm), crimp	Ms		Allain *et al.* (1998); Ponz *et al.* (2001); Bray *et al.* (2002)

Table 2 DNA markers or genes for sheep-carcase-quality traits

Trait	DNA marker	Gene/protein	QTL size effect (%)	Reference
Rib eye muscling	Ms	Carwell	-	Nicoll *et al.* 1997
Muscling, fat depth	Ms	myostatin	-	Broad *et al.* 2000
Dressing percentage	Ms	callipyge	6	Leymaster and Freking 1998
Lean percentage	Ms	callipyge	12	Leymaster and Freking 1998
Muscling	Ms, OAR18	callipyge	-	Lien *et al.* 1999; Fahrenkrug *et al.* 2000
Meat tenderness	SSCP, SNPs, RFLP	calpastatin	16	Palmer *et al.* 2000
Shear force	Ms	callipyge	-	Leymaster and Freking 1998

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There are a number of sheep marker studies in progress that have not yet produced publicly reported results of commercial significance (Crawford 2001). These include a Merino * Awassi flock (Raadsma *et al.* 1999), a DNRE strong-wool/fine-wool Merino flock (N. Robinson), a CSIRO resource flock (I. Franklin), an AgResearch Romney/ Superfine Merino flock (K. Dodds), an AgResearch Texel/Coopworth flock (J. McEwan), an AgResearch Coopworth flock (T. Broad), an INRA flock (D. Vaiman), a USDA Romanov/Suffolk flock (K. Leymaster) and the Roslin Institute Scottish Blackface flocks (S. Bishop). Only heterozygous sheep inheriting the gene from their sire (polar overdominance) express muscular hypertrophy (Georges *et al.* 1996), so the callipyge gene would be difficult to use commercially. There has been more research on beef-carcase-quality DNA markers (Hetzel *et al.* 1997; Morris *et al.* 2001) than on sheep markers. Some of the beef-carcase-quality DNA markers may be relevant to sheep, due to DNA conservation across livestock species.

SNPs can be discovered from public EST databases (Buetow *et al.* 1999) and found on websites, e.g., www.ncbi.nlm.nih.gov/SNP/. SNPs are single nucleotide substitutions that follow Mendelian inheritance and are the most frequent type of DNA polymorphism. SNPs usually give rise to biallelic sequence variations that occur every 500 to 1,000 nucleotides in both coding and non-coding regions of the genome (Brookes 1999). SNPs account for the majority of an individual's genotypic "individuality". Unfortunately some regions, especially those coding for functional proteins, are more highly conserved and therefore are less likely to contain SNPs when compared to the regions outside of genes. SNP discovery can also be targeted to introns of some genes.

SNPs are expected to take the place of Ms polymorphisms as markers, especially for disease mapping, just as Ms rapidly replaced RFLPs in genome scans to detect QTL (Landegren *et al.* 1998). Genome scans could be undertaken using SNPs with a 1cM marker density, requiring in the order of 1,500 to 3,000 SNPs (Kruglyak 1997). It seems reasonable to propose that, shortly, SNPs will be used to test candidate genes or well-defined candidate loci from QTL studies for association between haplotypes where regions of interest have been narrowed considerably. The cost of determining parentage by measuring 8 Ms has reduced to about $A25 /sheep. The cost-effectiveness of this would be improved if the Ms were also closely linked to a QTL or if SNPs could be screened more cheaply. Many SNP discovery techniques are impeded by the manipulations required for gel-electrophoresis and PCR amplification of fragments. MALDI-time-of-flight (MALDI-TOF) mass spectrometry is a technique capable of fast and efficient molecular weight determination of DNA fragments without the need of PCR amplification (Lewis *et al.* 1998). Mass spectrometry can also be used to study proteins separated by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) or by 2-D liquid chromatography (Hancock *et al.* 2002).

Protein markers

There has been far less emphasis placed on the ultimate outcome of multiple gene expression, i.e., proteins. The genetic map codes for proteins that may be expressed within a cell at various levels of abundance, depending on other promoters and cofactors.

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Major genes can lie in control regions and not affect the form of a protein but affect the rate of transcription and relative abundance. A desired gene may be present in a selected animal, but it may not always express the protein that leads to the desired metabolic and phenotypic effects. Each gene codes for a unique protein, which may be further modified in various ways within the cell. Thus, there is often more than one gene product present for each gene. Multiple protein forms found following 2-D PAGE may be due to alternative mRNA splicing, translational frame shifts, and co- and post-translational modification (phosphorylation, glycosylation, deamination, methylation, acetylation etc.) and differing enzymatic cleavage patterns (Lisacek *et al.* 2001). Protein phosphorylation is the best understood process involved in regulatory metabolism networks. About one-third of eukaryotic proteins can be modified this way, and up to 5% of genes code for protein kinases or phosphatases (Kaufmann *et al.* 2000). Variations in protein abundance found on 2-D PAGE result from transcriptional and translational regulation, mRNA half-life and protein turnover.

These modified proteins, of which some hundreds of thousands exist in a sheep, are the functional parts of the living cell. Only a fraction of these proteins may be found in a given tissue type. The chemical characteristics of these functionally important proteins can only be determined by proteomic techniques. This has been recognised in plant science where one approach has been to consider the amount of a specific protein as a quantitative trait (PQLs) to which QTL mapping methods can be applied (Lander and Bolstein 1989) to find the loci controlling the abundance of the protein and to discover candidate proteins that explain the phenotype (Thiellement *et al.* 1999). For example, in maize, three PQLs were detected for a drought-induced protein, and were mapped to the same chromosomal regions as QTL for growth under water deficit, on three chromosomes.

The potential of protein markers for selecting wool- and meat-quality traits in livestock has not been reported. At present, the cost of determining a protein marker on a multi-gel PAGE system is over $A180/sample, and so at present the technique is only a protein discovery tool. The direct use of protein markers in applied breeding programs will only occur if proteins can be determined more cheaply on protein arrays or chips, similar to DNA microarrays (Jenkins and Pennington 2001) and, at least initially, is more likely to be of use for very high-quality products, e.g., protein bioactives, pharmaceuticals or future protein-based biomaterials. Their more likely use is to discover specific proteins that are related to a desired trait; then their protein amino acid sequences can be used as a template to determine likely cDNA probe sequences for genes that code for the desired proteins. As noted above, this is not straightforward due to post-translational modifications that can occur and the degeneracy of base pairs at the third codon. This can also work in reverse, i.e., express proteins (detect the gene marker for a trait, isolate mRNA from the tissue of interest, prepare cDNA by standard methods, clone the cDNA by PCR or other strategy, express the protein in a suitable culture medium), then isolate and characterise the proteins of interest by proteomic techniques.

We think it unlikely that the presence or absence of an allele of a single gene coding for a single (wool or meat) structural protein product will have a major effect on any polygenic quality traits. There may be individual (enzymatic) proteins whose presence

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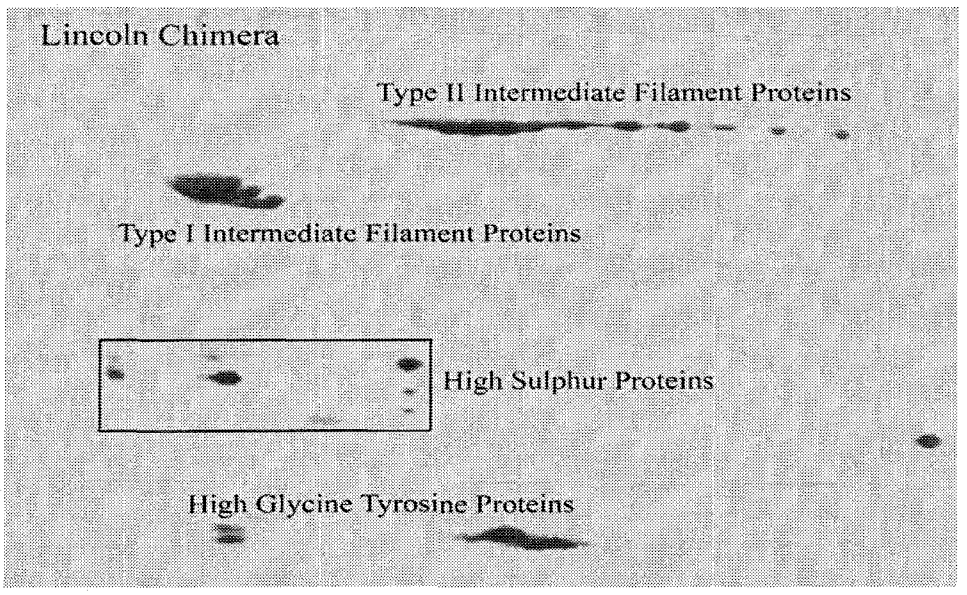
or absence influences the metabolic pathways involved in forming structural proteins and hence affects their relative abundance, e.g., genes effecting the development of the wool follicle bulb and fibre cells. At present, DNA or protein microarray techniques are only of use for detecting the presence or absence of markers, but they may become quantitative in future. If the relative abundance of protein is important, 2-D PAGE or sophisticated high-throughput, mass spectrometry methods (Griffin *et al.* 2001; Smith *et al.* 2002) are required. Protein marker possibilities for wool include KAP 6 and KAP 8 (fibre diameter); intermediate filaments KAP 1.1, 1.3 (staple strength); KAP 6, KAP 8 and KAP 1 (colour); KAP 1, KAP 3.2 and KAP 4.1 (crimp); ASP (dark fibre); KRT1 and KRT2 (tenacity, elasticity); and KAP 1, KAP 2 and KAP 3 (extension, dyeability). Myostatin and calpastatin proteins are candidates for protein marker work for meat-quality traits.

Fig. 1 2 D PAGE gel showing keratin classes



Work in WRONZ laboratories has concentrated on improving the use of 2-D PAGE methods to detect protein markers for wool colour and crimp (Fig. 1). Colloidal Coomassie Blue G250 has satisfactorily stained all protein classes. Labelling reduced keratins with ^{14}C-iodoacetamide followed by detection by autoradiography has resulted in a protein map with low background and all protein spots stained positively (Plowman *et al.* 2000). Work to date suggests that several major high-sulfur proteins may be associated with fibre crimp. From MALDI-TOF mass spectral peptide mapping, one of these proteins has been identified as being from the B2A family from the high-sulfur protein class (Flanagan *et al.* 2002). The search for a colour protein marker (Plowman

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et al. 1999) was not successful, in that proteins extracted from the fibre did not appear to correlate with colour. The colour resided mainly in the non-extractable residue, which included the wool cuticle in which the proteins are reported to be polymerised by glutamyl-lysine bonds (O'Connor *et al.* 1995; Swift 1997).

Higher-crimp wools have a higher proportion of paracortical cells. It is thought that there are either unique protein classes in high-crimp wools or some proteins in the matrix of the paracortex in higher abundance. Data from immunolocalisation studies and work with cRNA probes (Powell *et al.* 1994; Powell 1996) in hair follicles has shown that genes coding the high-glycine-tyrosine proteins are activated in cells of the orthocortex and that, soon after, those coding the high-sulfur proteins are activated in the other half, the paracortex. Further up the follicle, most cortical cells produce both classes; but two sheep cysteine-rich keratin gene families, belonging to the ultra-high-sulfur proteins, remain largely restricted to the paracortex. This has been confirmed by an electrophoretic study of the proteins of the ortho- and paracortical cells of Merino wool (Dowling *et al.* 1990). The paracortical cells were shown to contain more ultra-high-sulfur protein than the orthocortical cells and, in addition, were observed to have a high-sulfur protein not found in the orthocortex.

Conclusion

For sheep selection, using protein markers detected by the use of 2-D PAGE technology is probably too expensive. However, by discovering which specific proteins are expressed in superior animals, the process of gene detection and location of SNPs within the genes that can be used as DNA markers will be greatly assisted. DNA or protein chip arrays to detect the presence of DNA or protein markers will make the process less expensive. Relative abundance of certain structural proteins or protein types may be more important than the presence or absence of individual proteins in determining quality-trait phenotypes. However, genetic control of these traits could also be exercised through different metabolic pathways involved in the formation of structural proteins by the presence or absence of individual key enzymes coded by genes. Protein expression work may also assist in determining which genes to silence or knockout to develop superior wool- or meat-quality genotypes.

The crimp protein marker work represents the start of a major proteomics initiative at WRONZ to discover useful markers for wool-quality traits. The Proteomics Discovery Centre Ltd. (www.proteomicsnz.com), a subsidiary company of WRONZ, has been launched with a Q-TOF mass spectrometer for microsequencing proteins and peptides and determining post-translational modifications. Enquiries from other research groups with related interests are welcome.

Acknowledgments

We would like to thank Cynthia Bottema and Alan Pearson for commenting on the manuscript.

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