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on New Zealand farms. For the foreseeable future, the volume requirements for raw wool as a feedstock for keratin extraction are likely to be modest as a proportion of the total wool clip. Nevertheless, new products based on specific keratins are likely to provide an incentive for the development of specialised flocks producing wool matched to the requirements of the processing biotechnology and hence of higher value to the grower. Both of these outcomes would contribute to a future where diversification, direct supply chains and production to specification were the rule rather than the exception.

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Gene-markers for wool fibre traits

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ABSTRACT

Selective breeding has been used for thousands of years to improve the quality and weight of wool, but surprisingly there is still a large amount of genetic variation present at the loci involved in the "blueprint" for wool synthesis. This genetic heterogeneity results in considerable variation within, and between individual fleeces. This has both positive and negative implications. Where a wool processor requires uniformity in raw stock, both to gain efficiency in processing and to make a product to specification, it has a negative effect. Where specific traits convey unique properties it opens up opportunities for differentiated products. Markers for genes or knowledge of individual gene effects may be useful for identifying sheep with desirable wool traits that are difficult or expensive to measure under field conditions. The discovery of genetic variation in valuable fibre traits could allow the development of gene-marker tests which would assist breeding for more consistent wool.

Keywords: gene-markers; fibre characteristics; sheep breeding.

INTRODUCTION

The wool fibre consists of three distinct cell types; an external cuticle, a mass of cortical cells and, in coarser wool, a medulla (Höcker, 2002). The cortex comprises approximately 90% of the wool fibre and consists of microfibrils of intermediate filament keratins (KIFs), embedded in a protein matrix of keratin intermediate filament-associated proteins (KAPs) as reviewed by Plowman *et al.* (2006). Both the structure of the fibres, and the mean and variation in their properties affect processing performance and the suitability of any given wool for a particular end-use. In this review, we focus on some of the genes that have an impact on the structure and colour of the wool fibre and how variation in these genes may be developed into gene-markers to assist in the breeding of sheep that produce wool better suited to a processor's requirements.

GENES ASSOCIATED WITH THE WOOL FIBRE

Fibre protein composition

The genetic control of the protein composition of wool is complex with several distinctive multigene families encoding each group of matrix components. Type I and Type II KIFs are encoded by the KRT1.n and KRT2.n gene families respectively (Powell & Rogers, 1994). The *KRT2.10* and *KRT2.13* genes have been mapped to chromosome 3 and found to be tightly linked (McLaren *et al.*, 1997). One member of the KRT1.n family, *KRT1.2,* has been mapped to chromosome 11 and is linked to the cluster of high sulphur KAP genes (KAP1.n and KAP3.n) found at that location (McLaren *et al.*, 1997).

KAPs are divided into three broad groups based on their amino acid compositions; high-sulphur, ultra-high sulphur and high-glycine-

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tyrosine KAPs (Powell & Rogers, 1994). Genes encoding high glycine–tyrosine matrix proteins have been shown to be positioned on chromosome 1 (Wood *et al.,* 1992; Parsons *et al.*, 1994a; McLaren *et al.*, 1997). The high-sulphur proteins are encoded by three multigene families, KAP1.n, KAP2.n, and KAP3.n, with the members of the KAP1.n and KAP3.n families located on chromosome 11 (McLaren *et al.*, 1997). The ultra-high sulphur family on the other hand consist of at least two multigene families, the KAP4.n family and the KAP5.n family (Powell & Rogers 1994). *KAP5.1* has been localised to ovine chromosome 21 (McLaren *et al.*, 1997).

A high level of amino acid sequence variation has been reported in the proteins produced from the genes described above. Many of the genes have multiple alleles, or are polymorphic and this would suggest that these genes underpin some of the variation in the wool fibre and hence are good candidates for use in the gene-marker assisted selection of wool for more consistent composition.

Wool proteins are characterised by a high overall content of sulphur. As this sulphur is supplied from dietary sulphur-amino acids, the supply of sulphur-amino acids entering the duodenum for digestion has a major effect on regulating wool growth rate as well as other bodily processes. Abomasal infusion of supplementary sulphur-amino acids and/or their analogues, can induce marked increases in wool growth rate (Radcliffe *et al.,* 1985; Hynd, 1989). This would suggest that genes involved in sulphur uptake and metabolism may also be related to wool growth rate and associated dimensional characteristics in addition to the variability caused by environmental and physiological factors (Kendall *et al.,* 2006). The nature of the involvement of these genes is poorly understood. Opposing selective pressures with respect to utilisation of a limited supply of dietary sulphur-amino acids may partly explain the high level of genetic diversity present in wool production of our current "developed" breeds.

Pigmentation

The pigmentation patterns of the fleece have been subject to scrutiny. As with wool follicle proteins, pigmentation patterns have been found to be influenced by a number of loci (Sponenberg, 1997). One of the most important of these is the *agouti* locus found on chromosome 13. This encodes a protein which binds to the melanocortin receptor (MC1R), thus preventing the melanocyte stimulating hormone (αMSH) from binding. This prevents the production of eumelanin, which is responsible for yellow/brown colour in the fleece (Parsons *et al.*, 1999). Recently, it has been suggested that a second, *agouti*-like locus exists, an allele of which differs from exon 2 of the ovine *agouti* gene by a 5 bp deletion. This allele has been found in white-faced but not black-faced sheep breeds (Smit *et al.*, 2002; Purvis & Franklin, 2005). A comprehensive summary of alleles that result in distinct pigmentation patterns has been published by the Committee on Genetic Nomenclature of Sheep and Goats (Lauvergne *et al.*, 1996). Many of these alleles interact and are expressed at different stages in the lifetime of the sheep. This, in addition to the recessive nature of colour alleles, has made it difficult to determine the pigmentation genotype solely from studies of the phenotype (Purvis & Franklin, 2005).

Despite our understanding of the genetics of pigmentation in sheep, farmers have difficulty in breeding rams whose offspring always produce white fleeces. We do not however understand the inheritance of the presence of occasional pigmented fibres, commonly referred to as "black fibres", within the fleece. The presence of black fibres in a consignment of white wool affects the dyeing potential of the wool and as a result may incur a price discount for the wool grower.

Fibre dimensions

The influence of genetics on the dimensions of wool fibres is obvious when observing between-breed variation. However, only a few single genes are known which have a major effect on an individual wool trait. One of these is the Halo Hair 1 (HH1) gene (also known as the "N-type" or "Drysdale" gene) which produces an abundance of medullated halo hairs in the lamb birth coat (Dry, 1955). The mature fleece is also highly medullated and is used as speciality carpet wool. Several alleles of this gene have now been identified and used to develop the Tukidale ($HH1^T$), Carpetmaster ($HH1^J$) and Elliotdale (HH2) breeds (Sides and Banks, 1987; Wickham, 1978). These mutations have pleiotropic effects, causing the formation of horns in females as well as males, altering primary follicle morphology and primary fibre growth rate. Bray *et al*. (2002) have also reported the effect of a single gene that produced increased fibre length growth rate in the lamb fleece of Finn sheep.

Other attributes

Another gene which exerts a major effect on a single wool trait is the Lustrous Wool (LW) gene. A dominant allele in this gene (called LW^L), identified in Merino sheep, causes lustrous, light yellow wool (Short, 1958). This mutation is also associated with significantly reduced follicle density and fleece weight. Similar mutations called

$LWM1^L$ and $LWM2^L$ have been identified in Romney flocks and Romney-cross flock respectively (Blair, 1990).

Two genes have been characterised that control the presence or absence of fibre. A gene was first identified over 30 years ago in Poll Dorset sheep, mutations of which result in a complete or partial absence of wool at birth (Dolling & Brooker, 1966), and a gene with a similar effect, the *hairless, hr,* gene has since been identified in a Sicilian sheep breed (Finochiarro *et al.*, 2003).

In contrast, most wool fibre traits show quantitative variation controlled by many loci, which are typically under the influence of environmental factors.

GENE-MARKERS FOR WOOL TRAITS

A number of studies have been conducted to identify whether there are associations between different genetic markers and wool quality traits. These associations could be exploited to develop marker-assisted breeding strategies. The use of genotypic data in conjunction with wool phenotypic data could provide more accurate information for selecting livestock for breeding. Replacement sheep destined to enter a breeding flock are normally selected when about one year of age at yearling shearing. Fleece weight and mean fibre diameter reach a plateau at 3-4 years of age while staple length growth rate begins to decrease from one year of age (Brown *et al.*, 1966; Sumner & Upsdell, 2001). The use of gene-marker tests has the potential to provide information on a sheep's genetic potential for aspects of their adult wool production much earlier in life such that sheep could be selected for adult wool production at a young age. Additionally, from a scientific viewpoint, associations between gene-markers and wool quality traits may further increase our understanding of the biology of wool growth.

METHODS OF IDENTIFYING GENE-MARKERS

There are several methods of identifying gene-markers for production traits. Two commonly used approaches are genome scanning and candidate gene analysis. Genome scanning uses maps of DNA markers to describe the chromosomes. This in turn assists in scanning the genome to identify regions likely to contain genes that affect a given trait. These are subsequently called quantitative trait loci (QTL) and the process is frequently called "QTL-mapping". A sheep chromosome map has been published that represents 1062 unique loci made up of 941 anonymous loci and 121 genes. It spans 3500 cM (sex-averaged) for the autosomes and 132 cM (female) on the X chromosome, with an average spacing of 3.4 cM between autosomal loci (Maddox *et al.*, 2001).

The genome scanning approach is particularly useful when there are no obvious candidate genes for a specific trait. A weakness of the approach is that large numbers of sheep are required to detect association. Equally, the associations may not be seen if a gene influencing a particular trait is a long way from any markers and hence the gene and the marker may recombine frequently and not be perceived to be linked. Once associations are identified, a great deal of work is still required to narrow the region down to a size that can be used reliably in marker-assisted breeding. Few QTL discoveries have been published to date. However as associations are likely to be commercially valuable they may not have been made public. QTL experiments in sheep have been reviewed by (Crawford, 2001).

Candidate gene analysis is employed where either biochemical or physiological analysis, or a comparison with other species, has identified genes which are likely to be linked with production traits. In working to alter wool characteristics, good candidate genes that might underpin the variation seen in wool would include those that encode the structural components of wool fibre; the KIFs and KAPs.

WOOL CHARACTERISTICS THAT ARE IMPORTANT IN PROCESSING

The processing performance of wool fibres is dependent on aspects of their mean dimensions, length, diameter and crimp and their variation along and between fibres. While fleece weight is the most important factor economically, mean fibre diameter has historically been the main determinant of price premiums for Merino and mid-micron wools, as it dictates the lightness and softness of the resultant yarn. Where there is a reduction in diameter along fibres, tensile strength is reduced so that they are liable to break during processing. Fibre length, in association with tensile strength, impacts on fibre loss during processing and also the strength of the resulting yarn. Yarn strength is also related to the mean and variation in the length of fibres in the yarn.

The identification of gene-markers for wool traits is particularly difficult as wool traits interact. For example fibre diameter, and fibre diameter variation between and within fibres, impact on staple strength. This problem is further compounded by the observation that many of the genes that may underpin wool characteristics are

TABLE 1: Summary of identified gene-markers linked to individual wool characteristics. Adapted from Purvis and Franklin (2005).

Trait	Approach	Breeds	Location	Marker	Ref.
Mean fibre diameter	Candidate gene	Peppin merino	Chr. 1	KRTAP6 and KRTAP8	Parsons *et al.*, 1994b
	Genome scan	Merino x Romney		Linked, but not named	Henry *et al*., 1998
	Segment mapping	INRA 401	Chr. 6	Segment OARAE101 (20 cM)	Ponz *et al.*, 2001
			Chr. 25	Segment IDVGA8 to midpoint with IDVGA088	
Fibre diameter variation	Candidate gene	Halfbred x Merino	Chr. 11	KRT1.2 and KAP1.3	J.K.R. Abbott and J.G.H. Hickford, unpublished data
	Within sire regression	INRA 401	Chr. 4	McM218	Allain *et al*., 1998
	Segment mapping	INRA 401	Chr.7	Segment ILST005 (20 cM)	Ponz *et al.*, 2001
			Chr. 25	Segment IDGVA8 – IDVGA088	
Staple strength	Candidate gene approach	Romney	Chr. 11	KRT1.2, KAP1.1 and KAP1.3	Rogers *et al*., 1994
	Candidate gene approach	Halfbred x Merino	Chr. 11	KRT1.2 and KAP1.3	J.K.R. Abbott and J.G.H. Hickford, unpublished data
Fibre length	Segment mapping	INRA 401	Chr. 3	Segment BMC1009 – OARVH34	Ponz *et al*., 2001
			Chr. 7	Segment ILST005 (20 cM)	
			Chr. 25	Segment IDGVA8 – IDVGA088	
	Candidate gene	Halfbred x Merino	Chr. 11	KAP1.1	J.K.R. Abbott and J.G.H. Hickford, unpublished data
Yellowing	Candidate gene	Halfbred x Merino	Chr 3	TGLA77	McKenzie, 2002
				*Oar*FCB193	

clustered closely together and hence are probably frequently co-inherited. This makes it doubly important to investigate the influence of these loci on wool traits, as an increased understanding of the effect of variation in these genes and interactions between these loci will help to reveal the mechanisms by which these genes function. This in turn may help in breeding for traits with antagonistic correlations and increase the speed with which growers could respond to changing processing and market requirements.

Wool characteristics which impact on processing performance for which potential QTLs or genes have been identified are discussed individually below with the gene-markers summarised in Table 1.

Fibre diameter

Parsons *et al.*, (1994b) used a candidate gene approach to identify an association between high-glycine-tyrosine keratin gene loci and wool fibre diameter in a Peppin Merino half-sib. Progeny groups from a sire heterozygous for the KRTAP6 gene showed a mean difference in fibre diameter of 3.8 μm. An association was also seen between KRTAP8 and fibre diameter, with an average difference of 2.5 microns between the two progeny groups. Both of these differences are of processing significance (Hunter, 1980).

A candidate gene approach was also used by Robinson *et al* . (1995) who identified an association between the acidic fibroblast growth factor, FGF1, and fibre diameter. This locus was also found to be associated with differences in fleece weight. A genome scan approach in a Merino x Romney flock identified a 1.7 μm difference between progeny inheriting one of two forms of an unnamed marker (Henry *et al.*, 1998) and a segment mapping approach found that regions of ovine chromosomes 6 and 25 accounted for approximately 20% of genetic variance in fibre diameter in INRA401 half-sibs (Ponz *et al.*, 2001).

Fibre diameter variation between and along fibres

Fibre diameter is normally quoted as a mean value obscuring the inherent variation in fibre diameter between and along individual fibres. Studies have shown approximately two-thirds of the variation in fibre diameter within a line of wool is associated with between and along fibre variation (Stobart *et al.,* 1986) with within-fibre variation in

fibre diameter being between two and ten times the between-fibre variation (Sumner & Revfeim, 1973; Stobart, *et al.,* 1986), depending on the fleece type, environmental effects and management of the sheep (Kendall *et al.* (2006). Consequently the genetic control of variation in fibre diameter is likely to be complex. Several genes are currently being investigated.

Using within sire regression procedures Allain *et al.* (1998) showed McM218 on chromosome 4 to be linked to fibre diameter variation within a sample of fibre snippets measured for mean fibre diameter.

In our research, the keratin gene cluster on chromosome 11 has been identified as a potential locus that may affect variation in fibre diameter. Specifically, in a large half-sib family, two alleles of the KIF gene, KRT1.2 (alleles A and B) were found to define two progeny groups of significantly different fibre diameter standard deviation (A = 3.7 μm vs B = 3.4 μm, P = 0.008), despite the mean fibre diameter not being significantly different (A = 17.0 μm vs B = 16.8 μm, P > 0.1) (J.K.R. Abbott, T. Itenge-Mweza, and J.G.H. Hickford, unpublished data). This suggests this locus may be worthy of further investigation if we want to better control variation in fibre diameter.

A significant reduction in fibre diameter variation within an individual flock could be potentially beneficial in a direct supply chain where smaller quantities of wool are produced for an end-use requiring uniformity.

Staple strength

Individual fibres are subjected to considerable tensile stress during carding, combing and spinning. This results in varying degrees of fibre breakage with fibres tending to break at their weakest points, commonly where there are marked reductions in fibre diameter. Short broken fibres are removed by these processing steps generating waste.

As the strength of individual fibres is expensive to measure objectively, an assessment of the tensile strength of a group of fibres, as in a staple of wool, is used instead.

A region of sheep chromosome 11 has been reported as a potential QTL for wool staple strength in Romney half-sib families (Rogers *et al.*, 1994). Three loci were identified within this region and these were considered to be strong candidates for linkage with wool quality traits. One locus encodes the keratin intermediate filament protein (KRT 1.2) and the other two loci encode matrix proteins (KAP1.n and KAP3.n). Analysis of the pooled progeny data for allelic variation at the three loci revealed a significant genotype-within-sire effect for staple strength. Subsequently, two sires heterozygous for these loci showed a specific genotype effect with a difference in staple strength within the half-sib groups of 16 N/ktex and 24 N/ktex respectively. Differences in staple strength of more than 5 N/ktex are of processing significance (Hunter, 1980).

Unpublished work by J.K.R. Abbott, T Itenge-Mweza and J.G.H. Hickford has indicated that variation in KRT1.2 shows the strongest association (P<0.01) with staple strength with the mean difference in staple strength between progeny groups from a single heterozygous halfbred Merino sire of 3 N/ktex. Preliminary work genotyping half-sib groups from a super-fine Merino flock has thus far confirmed these associations between alleles of the KRT1.2 gene and staple strength.

Fibre length

Individual fibres in a staple grow at different rates with the coarser fibres tending to have a faster growth rate resulting in the "tippy" appearance of fleeces that have been growing for several months. There is no single relationship between mean fibre length and staple length with the ratio varying from 0.9 to 2.1 between different fleece types (Hunter, 1980). The variation in fibre length can increase up to five-fold because of breakage during processing (Bownass, 1984).

Our research has revealed an association between staple length and two alleles of KAP1.1, with a mean difference in length of 4.4 mm (P<0.05) in a Merino half-sib family (J.K.R. Abbott, T. Itenge-Mweza, and J.G.H. Hickford. unpublished data). Investigation of this locus in a flock of super-fine Merino sheep also identified association (P<0.05) between alleles of this gene and fibre length with different alleles showing a significant association with fibre length. Differences in fibre length of processing significance within either carded sliver or top vary with the mean diameter and mean length of the fibres. Differences in fibre length of 4 to 5 mm for fine Merino wool are of processing significance (Hunter, 1980).

Unscourable wool yellowing

The presence of unscourable colouring in wool affects its potential to be able to be dyed to a range of colours, particularly pastel shades. Yellow discolourations are associated with long fleeces being exposed to warm moist conditions while the fleece is growing on the sheep. Individual sheep differ in their susceptibility to develop yellow discolourations while grazing on the same farm. Wool yellowing is linked to the relative outputs from the sebaceous and sweat glands that are

associated with wool follicles in the skin (Sumner and Craven, 2005).

A QTL for wool yellowing has been identified on chromosome 11 (McKenzie, 2002). A marker on chromosome 11, *Oar*FCB193 has been linked in a (Merino x Romney) x Merino backcross flock, with the development of wool yellowing in warm moist conditions. Subsequent half-sib analysis of this *Oar*FCB193 marker did not provide further support for the association (McKenzie, 2002).

Where to from here?

Many economically important wool traits are under the influence of multiple gene loci and are also influenced by environmental factors (Kendall *et al.,* 2006). Many of the traits also have been revealed to be interrelated, or to interact either positively or negatively, and many of the genes encoding the wool proteins are linked, as discussed above, making it difficult to dissect the influence of any one gene on fibre characteristics. What is more, extensive polymorphism has been found in many of the genes, which could also affect the wool fibre and wool traits by altering protein expression, structure or post-translational modifications.

Despite all these compounding and interacting effects, many wool traits show relatively high heritabilities and hence are relatively easy to breed for. This may be because the key wool genes are clustered; meaning that selection for one gene will result in indirect selection for a haplotype of alleles at the proximal or clustered loci. In this respect, further analysis of these clusters should enable us to advance breeding for wool with more closely controlled specifications.

The potential impact of the gene-markers described here is difficult to evaluate. From the preliminary results described above, quite large differences in traits such as mean fibre diameter, fibre diameter variation and staple strength have been shown to be associated with variation in a number of genes and loci. The identified effects are of processing significance and may equate to price differences for raw wool, where that wool is purchased for a specific end-use.

To be of practical significance these gene-markers would need to reliably predict variation in these traits in different sheep breeding operations, and within and between breeds and to be cost-effective relative to other areas of activity. If they could achieve this consistently, with a minimal cost of measurement, the technology would assist growers in providing raw fibre to meet the specifications required by a particular processor to produce a specific product with reduced wastage.

Gene-marker-assisted selection techniques are currently utilised by ram breeders in New Zealand when breeding sheep for increased tolerance to footrot and rams to produce lambs with improved survival capabilities. Currently over 400 New Zealand sheep farmers use the footrot gene-marker test on a regular basis with independent surveys of the technology indicating multi-million dollar savings (Greer *et al.,* 2004). With confirmation of the location and identity of appropriate marker genes for important wool characteristics and demonstrations of their cost effectiveness, gene-marker-assisted selection techniques could assist ram breeders to select for particular wool characteristics.

Environmental and physiological mechanisms underlying wool growth rhythms in coarse wool sheep

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ABSTRACT

Coarse-woolled sheep breeds such as the Romney exhibit a pronounced annual cycle of wool growth with concomitant changes in fibre length growth rate and mean fibre diameter with maxima in summer and minima in winter. This cycle, which is entrained by seasonal changes in daylength via changes in circulating prolactin, parallels wool growth rate in Romney sheep. The cycle is also influenced by complex interactions between nutrient supply and hormones associated with reproductive status. A marked depression in wool production occurs during early pregnancy, which cannot be completely ameliorated by supplementary feeding, and does not directly involve prolactin. Additionally, in ewes, experimental and pharmacological increases and decreases in the prolactin concentration at parturition and during lactation are associated with