WOOL KERATIN GENE POLYMORPHISMS AND PRODUCTION CHARACTERS IN AUSTRALIAN MERINOS

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SUMMARY

Wool is a fibre made up of many proteins, which are arranged in several levels of structure. The number of proteins is difficult to estimate precisely, but it is probably 50-100. They can be divided into keratin intermediate filament proteins, designated type I and II, and keratin-associated proteins, within which eight families are presently recognised. The availability of genomic and cDNA probes has allowed the definition of Restriction Fragment Length Polymorphisms (RFLPs) and microsatellite markers for these genes. Polymorphisms have been found for genes in seven of the eight keratin-associated protein families. We give data which indicate that keratin gene markers might define some genetic variation in economically important characters of wool.

INTRODUCTION

Since the physical properties of wool differ between animals and between breeds and since some of the variation is heritable (Mortimer, 1987), there is clearly considerable potential economic benefit in being able to define the molecular and genetic basis of this variation. Selection based directly upon DNA sequence could be a great deal more efficient than traditional methods based upon external phenotype (Beckman and Soller, 1983; Gelderman *et al*, 1985). Genes which affect characters of interest to animal breeders are often called quantitative trait loci or QTLs. For wool, the most obvious candidate genes for QTLs are those which specify its structural components. However, although wool is a protein fibre, the study of genetic variation in the structure and/or levels of its proteins has until recently been impractical. Pure wool proteins have proved difficult to isolate. None the less, the amino acid sequences of some wool proteins and of hair proteins from other organisms were determined in the 1970s and early 1980s. These data have provided the springboard from which modern molecular techniques have been used to clone and sequence wool protein genes. The existence of these cloned sequences makes it possible to test the hypothesis that wool protein genes are QTLs, variation in which is responsible for inherited differences in the physical properties of wool. We are undertaking a research program funded by The Australian Wool Research and Promotion Organisation (AWRAPO) to test this hypothesis, and here summarise some of our findings on sequence variation defined by wool protein gene probes and a preliminary search for QTLs.

WOOL PROTEINS, THEIR GENES AND CHROMOSOMAL LOCATIONS

Wool proteins consist of two broad groups, the hair keratin intermediate filament (IF) proteins and keratin-associated proteins, designated KRT and KRTAP respectively in the most recent nomenclature (Powell and Rogers, 1994). Some of the characteristics of their genes are shown in Table 1. KRTAP proteins lack introns, a most unusual feature for the genes of higher organisms. In the wool fibre cortex the IF keratins are found in the innermost microfibrillar component and the cysteine-rich and glycine-tyrosine-rich keratin-associated proteins are found in the surrounding matrix component. One family of cysteine-rich keratin-associated proteins (KRTAP5) is located in the outermost or cuticle layer. The genes concerned map to at least three different chromosomal locations in sheep (Table 2) and, interestingly there is some clustering. KRT1 and KRTAP1 are both on sheep chromosome 11 while a KRTAP6 gene and the KRTAP8 gene are near each other (recombination fraction of 15%) on sheep chromosome 1. However the chromosomal location of five of the families of genes remains to be determined.

Table 1. Characteristics of sheep wool protein gene families

Nomenclature New	Old	No. sequences*	Introns	No. amino acids
KRT1	IF type 1 keratin (IF Type I)	M	Yes	392 - 416
KRT2	IF type II keratin (IF Type II)	M	Yes	479 - 506
KRTAP1	High sulphur B2 (HSB2)	M	No	151 - 181
KRTAP2	High sulphur BIIIA (HSBIIIA)	M	No	130 - 132
KRTAP3	High sulphur BIIIB (HSBIIIB)	M	No	94 - 97
KRTAP4	Ultra high sulphur cortex (UHS cortex)	M	No	~ 150
KRTAP5	Ultra high sulphur cuticle (UHS cuticle)	M	No	168 - 181
KRTAP6	High glycine tyrosine type II (HGTII)	M	No	79 - 82
KRTAP7	High glycine tyrosine type I-C2 (HGTIC2)	U	No	81
KRTAP8	High glycine tyrosine type I-F (HGTIF)	U	No	84

* M = multigene family; in most cases the exact number is not fully characterised in sheep.
U = unique sequence. Data summarised from Powell and Rogers, 1994.

Table 2. Current Data on Chromosomal Locations of Sheep Keratin Genes

Family	Information available	Reference
KRT1	Sheep chromosome 11 by *in situ* hybridisation	Hediger (1988)
KRT2	Sheep chromosome 3 by *in situ* hybridisation	Hediger (1988)
KRTAP1	Genetic linkage to growth hormone, which is on chromosome 11	Parsons *et al*, 1994a
KRTAP2	NI	
KRTAP3	NI	
KRTAP4	NI	
KRTAP5	Cross hybridising sequences map to human chromosome positions 11q13 and 11p15	Mackinnon *et al* (1991)
KRTAP6	Sheep chromosome 1, by genetic linkage to KRTAP8	Parsons *et al*, 1994a
KRTAP7	NI	
KRTAP8	Sheep chromosome 1, using somatic cell hybridisation	Wood *et al* (1992)

NI = no information

RFLP VARIATION IN MERINO SHEEP

We have carried out an investigation of RFLP variation in wool protein genes in a medium Peppin flock maintained by the CSIRO Division of Animal Production in Armidale. Polymorphisms have been found in genes for seven of the ten gene families (Table 3). No variation has so far been found for the keratin intermediate filament genes themselves. This is in line with investigations in other species, where RFLP variation has been difficult to find. The keratin-associated protein genes appear to be more variable; all seven polymorphisms are for these genes. This, combined with their unusual amino acid composition leads us to think that the keratin-associated protein genes are good candidates for QTLs which affect characteristics of wool. They could do this in two ways, through variation in DNA coding sequences which affect the amino acid sequences of the wool protein itself, or in control regions which regulate the amount of protein produced.

Table 3. Results of RFLP searching in sheep

Gene family/gene	Polymorphisms with RE*	References
Keratin IF proteins		
KRT1 (IF type I)	None found	
KRT2 (IF type II)	None found **	
Keratin-associated proteins		
KRTAP1 (HS B2)	Taq I, Eco R1, Msp 1,BstEII	Geraldine Rogers (pers. comm.)
KRTAP2 (HSBIIIA)	None found	
KRTAP3 (HSBIIIB)	Bam HI, Pst 1, Rsa I, Hind III	Parsons *et al*, (unpub.)
KRTAP4 (UHS cortex)	Bam HI, Taq I, Hind III	Parsons *et al*, (unpub.)
KRTAP5 (UHS cuticle)	Bam HI, Bgl II, Hind III, Rsa I	Parsons *et al*, 1992
KRTAP6 (HGT2)	Bam HI	Parsons *et al*, 1993
KRTAP7 (HGT1C2)	Bgl II, Msp I	Parsons *et al*, (unpub.)
KRTAP8 (HGT1F)	Microsatellite polymorphism	Wood *et al*, 1992

* RE = restriction enzyme, ** CA repeat known; to be investigated.

PRODUCTION CHARACTERS

We have analysed genetic linkage between DNA markers for KRTAP genes and possible wool QTLs in ten half-sib families. The characters investigated include wool fibre diameter, greasy wool weight and clean fleece weight. The two most interesting findings are linkage between both the KRTAP6 and KRTAP8 gene loci and wool fibre diameter in one half-sib family (Table 4; Parsons *et al.*, 1994b). These two KRTAP genes are linked on ovine chromosome 1 ($\theta = 0.15$, lod score = 5.0; Parsons *et al.*, 1994a).

The question arises - how would such linkage information be used in a practical breeding program? A lot will depend upon the ease with which rams could be typed for marker genes which help define the QTLs. We have hitherto used blood samples to obtain the DNA necessary for genetic typing. Blood sampling is cumbersome and expensive, and its transport to laboratory facilities is also costly. We are therefore contemplating developing PCR (Polymerase Chain Reaction) methods for DNA typing from the cells of the wool follicle itself. PCR is

extremely sensitive, and the transport of a small sample of wool plucked from an animal would be both convenient and cheap - the cost of a letter, in fact.

Table 4. Results of linkage analysis between KRTAP6 and KRTAP8 and wool fibre diameter.

Locus	Sire	Allele	No. offspring	LSM (μm)*	SE	P-value
KRTAP6	3033	A	5	23.38	0.82	
		B	5	19.62	0.75	0.0002
KRTAP8	3033	A	11	22.02	0.52	
		B	5	19.49	0.73	0.003

* LSM: Least squares mean of wool fibre diameter of progeny grouped on basis of allele inherited.

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REFERENCES

BECKMAN, J.S. and SOLLER, M. (1983). Restriction fragment length polymorphisms in genetic improvement, mapping and costs. *Theor. Appl. Genet.* 67: 35-43.

GELDERMANN, H., PIEPER, V. and RATH, B. (1985). Effects of marked chromosome sections on milk performance in cattle. *Theor. Appl. Genet.* 70: 138-146.

HEDIGER, R. (1988). Die *in situ* hybridisierung zur Genkartierung beim Rind und Schaf. Ph.D. thesis, Zurich.

MACKINNON, P.J., POWELL, B.C., ROGERS, G.E., BAKER, E.G., MACKINNON, R.N., HYLAND, V.J., CALLEN, D.F.and SUTHERLAND, G.R. (1991) An ultrahigh-sulphur keratin gene of the human hair cuticle is localised at 11q13 and cross-hybridizes with sequences at 11p15. *Mamm. Gen.* 1: 53-56.

MORTIMER, S. (1987). Australian estimates of genetic parameters for wool production and quality traits. In: Merino Improvement Programs in Australia (Ed. B.J. McGuirk) *Aust. Wool Corp.* pp. 159-173.

PARSONS, Y.M., COOPER, D.W., PIPER, L.R. and ADAMS, T.E. (1992). Restriction fragment length polymorphisms at the ovine locus for growth hormone. *Animal Genetics* 23: 571.

PARSONS, Y.M., COOPER, D.W., PIPER, L.R. and POWELL, B. (1992). A *Bam*HI polymorphism in ovine wool cuticle keratin sequences. *Animal Genetics* 23: 569.

PARSONS, Y.M., COOPER, D.W., PIPER, L.R. and POWELL, B.C. (1993). A *Bam* HI polymorphism in ovine glycine/tyrosine-rich type II (KRTAP6) keratin sequences. *Animal Genetics* 24: 218.

PARSONS, Y.M., PIPER, L.R. and COOPER, D.W. (1994a). Linkage relationships between keratin associated protein (KRTAP) genes and growth hormone in sheep. *Genomics* (in press).

PARSONS, Y.M. COOPER, D.W. and PIPER, L.R.(1994b). Evidence of linkage between high-glycine -tyrosine keratin gene loci and wool fibre diameter in a Merino half-sib family. *Animal Genetics* (in press).

POWELL, B.C. and ROGER, G.E. (1994). Differentiation in hard keratin tissues: hair and related structures. In: Keratinocyte Handbook (ed. by I. Leigh, F. Watt and E.B. Lane). Cambridge University Press (in press).

WOOD, N.J., PHUA, S.H. and CRAWFORD, A.M. (1992). A dinucleotide repeat polymorphism at the glycine-rich and tyrosine-rich keratin locus in sheep. *Animal Genetics* 23: 391