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SUMMARY

The wool textile market is supplied by a traditional industry which processes a variable raw product into increasingly highly specified end-products. A declining demand for wool products has resulted in global rationalisation by processors and product substitution by processors and consumers.

Wool processing plants are expensive to purchase and operate, and only generate low margins. Processing costs may be reduced through use of a less variable raw stock more suited to a specific purpose. Hence, reliance on existing relationships through the middle of the chain will continue to constrain attempts to link differentiated

raw materials to specific consumer propositions. New business models and tightly aligned supply chain relationships are required to support innovative initiatives.

Branding can play a role for part of the clip. Growers can match sheep genotypes to the environment and specific requirements through genetic technologies and breeding strategies. New uses could be found for wool. Regardless of the technological advances, close and open relationships along the supply chain, in which information on specifications, quality and trends is readily communicated, will be crucial for wool to continue to be a viable raw material in the textile industry

SheepGenomics – a trans-Tasman collaboration

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ABSTRACT

SheepGenomics is a major A\$30 million initiative of Meat and Livestock Australia and Australian Wool Innovation Limited supported by 10 leading research organisations in Australia and New Zealand. It aims to discover genes that influence economically important traits in sheep. By using discoveries made within SheepGenomics' research program, sheep producers will be able to start breeding for a wide range of economically important traits with greater confidence than is currently possible. One of the themes in the programme is to improve key aspects of wool quality and focus on genes that regulate follicle formation in the skin of foetal lambs. This paper describes key aspects of the management structure of SheepGenomics, an overview of three of the Subprograms (Muscle, Parasites and Coretech) and a more detailed description of the Wool Subprogram.

Keywords: sheep; genomics; wool; fleece; follicle.

INTRODUCTION

SheepGenomics ("the Program") was initiated in 2003 with the signing of a joint investment agreement committing Australian Wool Innovation Limited (AWI) and Meat and Livestock Australia (MLA) to invest a total of A\$30 million over a 5 year period to June 2008.

Since that time, 10 leading research organisations from Australia and New Zealand have become active participants in the Program bringing a wide range of infrastructure, skills and knowledge to complement the Australian sheep industry's cash investment in the Program.

This paper describes the integrated structure and activities of SheepGenomics with

particular reference to the wool follicle regulation research in the Wool Subprogram.

SHEEPGENOMICS AND AUSTRALASIAN SHEEP INDUSTRY RESEARCH

SheepGenomics' role in the Australian sheep industry is to conduct leading edge gene discovery and function work and commercialise the results of that research for the benefit of the industry. In order to do this, the Program is aligned and interacting with two other key organisations in the Australian sheep research infrastructure, namely Sheep Genetics Australia (SGA) and the Sheep Industry Co-operative Research Centre (CRC).

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SGA was launched in October 2005 through the integration of a number of meat and wool industry databases, including Lambplan™ and Merino Select™. Its role is to provide quantitative genetic information to the industry in the forms of Australian sheep breeding values (ASBVs). SGA is working with SheepGenomics both as a source of information on useful and interesting industry sires for experimentation and as a partner in delivery of genomic information to complement the ASBVs. This partnership is important for the long term future of sheep breeding in Australia to ensure that there is a reliable and consistent source of information on which industry can make breeding decisions.

The Sheep Industry CRC is currently in the process of bidding for a second round of funding. SheepGenomics is working with the rebid committee to ensure a program of research that is mutually complementary and, again, integrated with SGA. If the rebid process is successful then the Australian sheep industry will benefit from a highly interactive and coordinated research infrastructure.

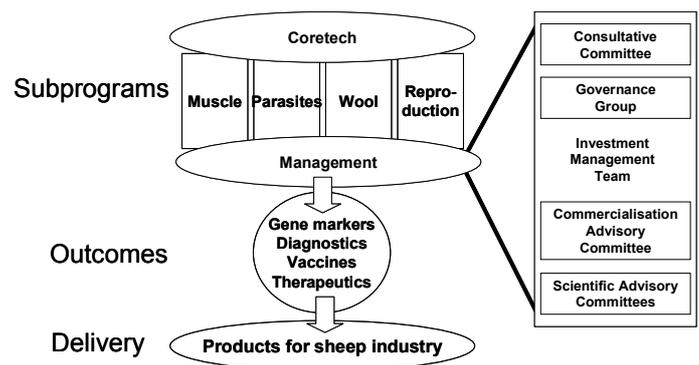
The New Zealand pastoral farming sector currently benefits from the SheepGenomics' research at a variety of levels including

- application on New Zealand sheep farms of relevant outcomes (e.g. markers for wool traits) to improve productivity and market returns;
- distribution of commercial returns among participating organisations;
- support of a New Zealand research capability in wool functional genomics;
- establishment of a trans-Tasman critical mass in genomic science required to address industry issues relevant to both countries.

STRUCTURE

SheepGenomics is an unincorporated research, development and commercialisation vehicle. It has six functional Subprograms (Figure 1). There are 4 industry Subprograms dealing with muscle and energy utilisation (Muscle), wool production (Wool), host resistance to internal parasites (Parasites) and reproductive efficiency (Reproduction). They are supported by the Core Technologies Subprogram and a Management module. With the exception of the Reproduction Subprogram, which is still in planning phase, the activities of each Subprogram are outlined in figure 1.

FIGURE 1: Structure of SheepGenomics



MANAGEMENT MODULE

The role of the Management Module is to coordinate the Program, to plan, contract and manage the research and ensure commercialisation of the outcomes for industry benefit. The operational management is conducted by the Investment Management Team comprising the Subprogram Leaders, Investment Managers from AWI and MLA and a Program Director.

A Consultative Committee provides stakeholder representation and a Commercialisation Advisory Committee provides expertise from stakeholders in regard to the nature and usage of intellectual property arising from SheepGenomics' research.

Scientific Advisory Committees work with the Subprogram Leaders to review the science being conducted within the Subprograms and make recommendations for funding on project applications.

The financial investment is overseen by an in-house joint committee of AWI and MLA and a number of staff from both organisations have ongoing roles in contract and project management, representing a further significant in-kind contribution to the cash investment.

At 31 December 2005, research organisations and third party grants contributed approximately A\$14.0 million against A\$21.3 million of SheepGenomics cash investment.

The intellectual property (IP) arrangements of SheepGenomics merit special mention. In effect, all researchers have access to the results of the Program, wherever produced. One of management's roles is to promote the dissemination of the research information and promote interaction across projects and Subprograms in order to build a true multidisciplinary group. The IP is owned on a project-by-project basis and commercialised by

mutual agreement of the owners. However, there are mechanisms to ensure that:-

- all participants in the program in both New Zealand and Australia have some stake in a successful commercialisation of IP, reflecting the collaborative contribution to its generation; and
- contributions of pre-existing background IP are also differentially recognised.

CORETECH SUBPROGRAM

The role of the Coretech Subprogram is to provide tools, animal resources and services to the four major industry Subprograms. Examples of activities undertaken in this Subprogram to-date are:-

- Evaluation and validation of a range of leading genomic tools for use with sheep samples. These include the Affymetrix bovine array and the Lynx massively parallel signature sequencing (MPSS™) technology.
- Establishment and operation of a 5,500 ewe fine mapping resource being run on AWI's Falkiner Memorial Field Station (FMFS). This flock is being artificially inseminated to deliver approximately 4500 progeny to 21 sires selected from both industry and research flocks as being outstanding for traits of interest to industry.
- Development of an integrated bioinformatics platform and biostatistics services. This major resource has been put together through trans-Tasman collaboration and enables participating SheepGenomics researchers to access a range of databases being generated across the Program as well as analytical tools (including some proprietary tools) and services for experimental design and analysis, especially for microarray experimentation and quantitative trait loci (QTL) mapping work.

PARASITES SUBPROGRAM

The Parasites Subprogram is focused on two worm species – barber's pole (*Haemonchus contortus*) and black scour worm (*Trichostrongylus colubriformis*) as major causes of economic loss in sheep. Two broad experimental approaches are being used, namely:-

- QTL gene mapping: Researchers are working with a number of different flocks to genetically map regions on the genome that control traits for worm resistance and susceptibility. These flocks include

experimental research flocks and the FMFS fine mapping flocks.

- Gene expression: Extensive libraries of tissue samples have been taken at pivotal times during the infection process from both resistant and susceptible sheep. Microarray and real time PCR experiments are being used to identify a series of candidate genes that are differentially expressed in the infection process.

The results from both approaches are being cross-correlated to provide better surety in the identification of high priority gene targets and DNA marker development.

MUSCLE SUBPROGRAM

The primary focus of this Subprogram is the identification and characterisation of traits affecting muscle yield and quality. The first approach was to characterise in detail the effects of the Callipyge mutation in collaboration with Prof Noelle Cockett at Utah State University. To identify gene activity involved in the Callipyge mutation, time-series tissue samples have been collected and used in multiple experiments encompassing a range of techniques including microarray, real time PCR, immunohistochemistry and proteomics. In addition to this, a range of phenotyping protocols have been developed that are currently being applied to animals from industry that exhibit interesting muscling traits.

DNA markers are being developed for known muscling traits, including the Carwell mutation, myostatin and calpastatin as well as for new traits identified in industry animals and QTL regions uncovered by the FMFS fine mapping flock.

WOOL SUBPROGRAM

The wool industry competes in a highly-competitive, global textile marketplace dominated by synthetic textiles (polymers derived from petrochemicals) and natural fibres (cellulosics derived from plants). With the exception of silk, wool is unique in this competition in that it is a protein product derived from animals. A combination of the intrinsic weakness of keratins and variations in the cross-sectional area of fibres along the staple length, contribute to the low strength of wool fibres. This, together with a high level of contaminants and high processing costs reduces the competitiveness of wool despite some positive functional advantages as a textile fibre, namely warmth, drape, handle and moisture absorbance.

TABLE 1: Summary of research in SheepGenomics Wool Subprogram.

| | Gene detection | Gene expression | Gene function | Gene or biochemical manipulation |
|-----------|------------------------------|--|--|--|
| Methods | QTL detection | QPCR (candidate genes) Microarrays | Cell transfection Literature | Pregnant ewe treatment Treatment of foetus |
| Resources | FMFS flock U Sydney flock | Foetal time series Induced shedding model | Follicle cell cultures (dermal papilla and keratinocyte) Grafting <i>in vivo</i> | Embryonic skin culture Dermal papilla cells Keratinocytes Grafting <i>in vivo</i> |
| Outcomes | Markers of wool traits | Candidate genes for testing/manipulation | Identification of biochemical pathways and potential treatments | Novel wool traits Increased fleece weights Reduced fibre diameter |

By all measures wool is competing poorly in the marketplace. It represented 2.5% of total textile fibre use and about 5% of apparel use in 2000 (AWI, 2006). Demand for wool is declining and prices, on average, are only marginally above the costs of production. The Wool Subprogram was devised in the light of this rather-sobering scenario.

The mission of the Wool Subprogram is to identify and establish new ways of optimising the two key profit drivers of fleece weight and fibre diameter and other important wool traits, through a genomics approach. It will do so by increasing the range of tools available for use in genetic selection systems, by identifying genes of major effect for traits determining profitability and by delivering therapeutics to alter certain fibre and accessory gland traits in a non-heritable manner. It was clear that to achieve this would require strong focus on the development of the follicle population and associated appendages during foetal development, as it is at this time that the follicle ‘factory’ is established. Virtually all wool traits of economic importance derive from the characteristics of the follicle factory. These include fibre length, crimp characteristics, fibre diameter, clean fleece weight, staple strength, variation in fibre diameter and length, fibre colour, wax and suint content.

The approach taken by the Wool Subprogram is summarised in Table 1. A pipeline of activities has been established ranging from QTL detection, fine mapping for genes affecting wool traits, analysis of gene expression during critical events in development, investigation of the function of these genes in biological systems, and manipulation of critical biochemical pathways to beneficially alter wool quality and quantity.

1. Detection of QTL for wool traits

Two major gene mapping activities are underway. The first is a component of the Coretech Subprogram’s FMFS gene mapping resource flock. A large number of wool and skin traits are being measured including fleece weight, yield, fibre

diameter and diameter variability, staple strength, staple length, colour, birthcoat score, body wrinkle, pigmented areas, and bare breech area. These traits are additional to the muscling, parasite resistance and reproductive performance traits being measured on the same animals.

The wool sires included in the gene mapping flock were selected on the basis of high estimated breeding values (EBVs) for high fleece weight and/or low fibre diameter. In 2005 an additional Merino sire was included which has a large bare area on the breech with potential to remove the need for mulesing and crutching.

It is envisaged that the gene mapping activities will play two important roles. One is to inform the genomics research by pointing to potential candidate genes and gene clusters regulating follicle development. The second is by direct application to breeding programs by inclusion in the SGA database, particularly for making early selection decisions and selection for difficult to measure traits such as staple strength and pigmented fibres.

2. Gene expression during foetal follicle development and post-natal growth cycling

A comprehensive series of skin samples has been derived by South Australian Research and Development Institute (SARDI) researchers, from Merino sheep during foetal life (days 35 to 150 post-conception) and early post-natal life (days 0 to 100 post-natal), at intervals of 3 days. The samples have been prepared for histological analysis and RNA has been extracted for analysis of gene expression. Gene expression is being quantified by two approaches.

The first is by quantitative analysis of expression of candidate genes selected from literature surveys of molecules known to be involved in development of similar epithelial tissues such as lung, mammary glands, prostate glands, and kidneys. Genes of the Wnt signalling pathway, ectodysplasin and its receptors, and a

number of members of the TGF-beta Superfamily are amongst the candidates. Analysis of changes in the levels of expression of these molecules at key developmental time points will point to the cascade of events triggering the establishment of follicles (primary and secondary), branching of follicles (secondary derived follicles) and establishment of sebaceous and sweat glands.

The second approach is to interrogate a 20,000 expressed sequence tag (EST) cDNA microarray including ovine skin ESTs collected through key stages of the wool follicle growth cycle in Wiltshire sheep (Yu *et al.*, 2006).

Follicle morphogenesis throughout the hair cycle displays many similarities to follicle neogenesis in the foetus, leading many to believe that the signalling mechanisms may be common to both events. AgResearch scientists are investigating gene expression throughout an induced wool growth cycle in Wiltshire sheep using the ovine skin array.

CSIRO researchers are also investigating gene expression in sheep selected for extremes in sensitivity to fleece rot. Susceptibility to fleece rot may relate to differences in immune function or characteristics of the fleece such as suint and wax ratios which relate directly to the establishment of these glands *in utero*.

3. Site of gene expression during critical phases of foetal skin development

As candidate genes arise from the gene expression research, their spatiotemporal expression will be assessed using a combination of *in situ* hybridisation (DIG-labelling) for DNA transcripts and immunohistochemistry to detect protein expression. This approach is being used for candidate molecules in the Delta/Notch cell signalling pathways, known to play a role in epithelial/mesenchymal interactions in developing tissues. A combination of gene discovery, gene expression profiling and *in situ* hybridisation is a powerful tool for unravelling the biological events.

4. Models for testing gene function

The program is utilising a number of *in vitro* and *in vivo* models to test the effects of genes of interest. AgResearch scientists have established an efficient procedure for transfecting follicle keratinocytes derived from the wool follicle, using lentiviral vectors. In combination with cell behaviour analysis (clumping and motility assays) and grafting transfected cells into nude mice, this is a powerful system for determining gene function. University of Adelaide scientists have established an *in vivo* culture system for embryonic mouse skin, which allows the effects of candidate

molecules and inhibitors of key biochemical pathways to be tested for effects on follicle initiation. The culture of skin micro-dissected from mouse embryos at 13.5 days post-conception faithfully mirrors the sequence of events leading to the formation of a fully-functional hair follicle. As candidate genes are identified in the SARDI, CSIRO and AgResearch gene expression studies they can then be tested for function in the *in vitro* and *in vivo* function models.

5. Identification of extreme phenotypes of sheep

Mutant mouse models, both spontaneous and induced, have proven to be powerful tools for identification of biological function. Sheep mutants with unusual wool phenotypes such as “felting lustre”, “silky”, “doggy” and “hairless”, have been described but have not been subjected to detailed analysis. It is likely that many extreme wool mutants exist in the Australian Merino sheep population, but no systematic and extensive attempt has been made to identify them. The Wool Subprogram has initiated a national search for sheep with extreme wool phenotypes and has already identified two new traits of interest.

6. Manipulation of follicles with targeted therapeutics

There are two primary means by which the wool industry will benefit from the wool genomic research program. The first is in the application of gene markers for wool traits (early selection of superior genotypes, selection for genes associated with difficult-to-measure traits). The second is through the development of therapeutics derived from an understanding of gene pathways involved in foetal skin development. University of Adelaide researchers are pre-empting this by manipulating pregnant ewes through direct treatment of the ewe (particularly in early pregnancy prior to placental maturation) or by more invasive direct treatment of the foetus (by intra-amniotic injection in later pregnancy). Preliminary results of trial experiments have provided evidence for the first time that non-heritable changes in skin and wool traits can be produced by treatment of pregnant animals, suggesting that this approach has realistic potential for practical application.

CONCLUSIONS

SheepGenomics is a new, highly flexible model for collaborative research. It is designed to promote cross-institutional and multidisciplinary collaboration on large-scale problems. Its unincorporated nature requires a greater reliance on the internal resources of key stakeholders for

administrative procedures but does allow for significantly more flexibility in interactions with stakeholders and variation of work programs.

The Coretech Subprogram has been invaluable in bringing together the four industry Subprograms (Wool, Muscle, Parasites and Reproduction) with common tools, resources and services.

DNA markers are likely to be the first products from the program that are made available to industry for enhancing breeding programs. Mechanisms have already been put in place to ensure integration with genetic information in the form of SGA's ASBVs.

The biological basis of variation in sheep's fleece cover

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ABSTRACT

Efficient sheep farming of the future will demand reduced labour costs, ease of shearing, and minimised susceptibility to flystrike, while modern markets simultaneously demand reduced chemical use and high standards of animal welfare. Sheep with a reduced area of the body covered with fleece may provide a practical solution to these conflicting requirements. Research has shown that it is possible to select sheep for bare faces, legs, belly and breech, and that at least some of these traits behave as though they are controlled by recessive genes, but that some only become evident later in life. Patterns of fleece distribution define major skin domains in the sheep that are evident in other mammals. We hypothesise that discrete skin domains arise because fibroblasts that induce wool follicle development originate from different embryonic tissues and carry with them differing developmental programmes for each follicle type. Identifying genes underlying variation in fleece distribution would allow desirable distributions to be established in sheep of diverse genetic backgrounds. Markers for distribution traits would enable us to introgress appropriate genes into flocks that do not carry them, and to determine early in life which animals might express the traits as adults.

Keywords: fleece distribution; regional specification; bare breech; bare belly; wool quality.

INDUSTRY NEED FOR ALTERED FLEECE COVER

The profitability of sheep farming is continually being eroded as input costs rise, while returns for wool decline. In the past, high wool prices led to selection for maximum production, resulting in long wool cover on the head, belly and breech in traditional dual purpose sheep breeds (Figure 1a). The associated husbandry requirements, including crutching, dagging and docking, were justified by the high value of the wool. More recently, in addition to falling prices, dramatic increases in shearing costs have occurred (Rennie, 2006). Two major reasons are: firstly, the Accident Compensation Commission is passing on escalating costs due to shearer injuries (ACC, 2003), and secondly, labour costs are driven up as shearing contractors attempt to attract and retain skilled shearers for a physically demanding job. An optimised fleece distribution would reduce these

costs by improving the ease and efficiency of the shearing process. There would also be no cut teats on ewes with a bare belly, and no cuts on the pizzle or scrotum of rams which has advantages from both production and animal welfare perspectives.

Variations within a harvested fleece also undermine the value of the wool (McDermott *et al.*, 2006). Attributes of the fleece that vary between body regions include staple length, staple strength, fibre diameter and follicle density (Sumner & Craven, 2000). In Romneys, as an example of a typical "woolly" sheep, within-sheep variation in wool characteristics is three-fold greater than that between-sheep (Sumner & Revfeim, 1973).

Long belly wool also significantly increases the incidence of microbiological contamination of sheep carcasses during meat processing. Recent high lamb prices relative to low wool prices provide additional impetus to addressing wooliness of the belly. Furthermore, public awareness of animal welfare has increased, and practices such as

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