IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) FOR CARCASS COMPOSITION IN SHEEP USING X-RAY COMPUTED TOMOGRAPHY (CT) SCANNING IN LIVE ANIMALS

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INTRODUCTION

Understanding the genetic basis of biologically complex traits involved in meat production is now possible with contemporary molecular tools. The ability to identify key chromosomal regions flanking loci with large effect (Quantitative Trait Loci-QTL) has seen a myriad of QTL mapping experiments as the start of a pathway leading to gene discovery. Application of advanced knowledge of the genetic basis of complex production traits can lead to novel breeding technologies such as marker-assisted selection (MAS) where animals may be selected for favorable QTL alleles based on markers closely linked to anonymous production genes, or where the gene is known, direct selection for favorable alleles may be possible. Identification of major genes and their function is also a pre-requisite for novel means of gene manipulation or control. MAS and gene manipulation are likely to be of interest for traits of high economic value and where selection is difficult and/or expensive. Body composition and meat quality are complex biological traits where a substantial investment in genetic characterization is warranted to reduce the cost for prediction of genetic merit. Body composition generally refers to the relative amounts of lean, fat and bone, but this definition can be broadened to include partitioning between the fat depots and also distribution within both the fat and lean depots (Thompson, 1998). Carcass composition traits such as carcass weight and fat thickness are moderately to highly heritable in sheep (Simm, 1992), and can be predicted by numerous methods, from very rapid inexpensive and less accurate methods such as visual appraisal to expensive more time consuming methods such as CT scanning. Stanford *et al.* (1998) reported that methods such as CT scanning and NMR (Nuclear magnetic resonance / magnetic resonance imaging) were regarded as the most accurate methods in assessing body composition but the cost, particularly for the NMR scanning would prevent them from being used on a commercial basis. In this study we describe the search for QTL, which may contribute to variation in carcass composition in sheep, on body composition data derived from live animals prior to slaughter.

MATERIAL AND METHODS

The Reprogen sheep mapping resource population (Raadsma *et al.*, 1999) utilizes extreme breed differences between the Merino and the Awassi sheep to map genes for milk, wool and carcass composition characteristics. The two breeds differ in regard to growth rate and meat production with the Merino having a smaller frame size combined with major differences in fat deposition and distribution by comparison with the fat tail Awassi.

The Awassi X Merino population. Four Awassi sires were crossed with medium and superfine Merino ewes to F1 heterozygous males and females. Four F1 ram lambs were selected to represent each of the founder families and were backcrossed to super fine and medium Merino ewes to produce four families with approximately 400, 150, 150, 150 progeny. This experiment utilized the wethers from the first sire family (n = 164).

Genotyping. A genome-wide scan was used for QTL mapping using a panel (245) of pre-selected polymorphic micro-satellite markers. A total of 136 markers were informative. On completion of genotyping, 117 markers provided complete information across all animals covering all 26 autosomes. Genetic marker distances were calculated using CRI-MAP (Green *et al*., 1990) for all 117 markers, and used as an input variable for QTL analysis. The number of markers per autosome varied between 2 and 9, with an average distance between markers in the range of 20-65 Centimorgans (cM) for each chromosome. The average marker density across the genome was 40cM with a predicted genome coverage greater than 70 %.

Phenotyping.

Live animal measures. All wethers from the first mapping family were randomly allocated to two management groups and grown out to early maturity and maturity at slaughter (two years (n = 85) and three years of age (n = 79)). All sheep were grazed on pasture initially. Three and six months prior to slaughter the two groups were fed *ad libitum* on a grain/lucerne diet as part of a feed intake study. Live weights were measured progressively from within 2 weeks after birth until slaughter on a monthly basis.

CT measurements. Prior to slaughter all animals were analysed by Computed Tomography for measurement of body composition. The animals were restrained and scanned three days prior to slaughter. Cross-sectional images were collected every 40mm starting proximal to the femur/tibia articulation finishing at the first cervical vertebra. A total of 24 to 28 images were collected, depending upon the length of the sheep. Each image was copied, cropped (to remove viscera and internal fat) and stored separately. From the information of all processed images, the yield of lean, fat, and bone were predicted using the PC based CT image analysis program AutoCAT (Jopson *et al.*, 1995).

Genetic and Statistical analyses.

Map distance. Genetic marker distances were calculated using CRI-MAP (Green *et al.,* 1990). Marker information was compared with the sex-averaged ovine genetic map for consistency in marker order and distance (Maddox *et al.,* 2001).

QTL analysis. Online QTL analysis was conducted using 'QTL express' available at http://qtl.cap.ed.ac.uk. The half-sib QTL analysis servlet was used, based on the interval regression approach detailed by Haley and Knott (1992). Genotype probabilities for each animal were calculated at 4cM intervals along each of the 26 autosomes. A chromosome wide threshold for statistical significance was calculated for each chromosome based on a permutation test of 1000 iterations. A combined analysis of the two experimental (age) groups was undertaken adjusting for age as a fixed effect. In addition final body weights were used as a co-variate in a separate analysis of the five traits reported on here.

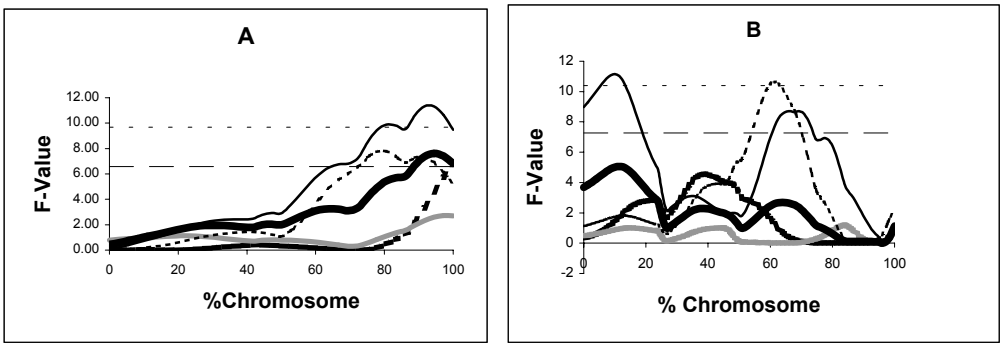
RESULTS AND DISCUSSION

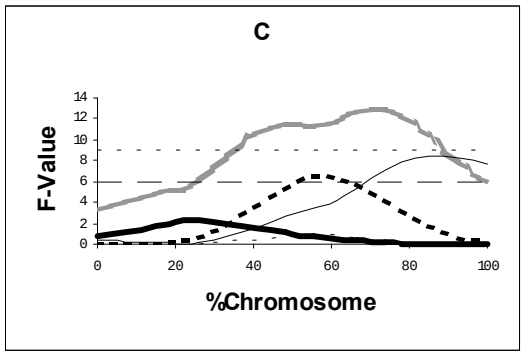
The linkage map used in this study was in agreement with other studies, with the same marker order and similar distances between markers (Maddox *et al.,* 2001). General descriptions of the body composition traits reported in this paper are shown in table 1.

Table 1. Average body weights and tissue composition for each experimental group

Age	2 Years		3 Years	
Trait	Average	St. Dev	Average	St. Dev
Body weight (KG)	45.2	4.89	59.2	6.38
Carcass lean (KG)	14.95	1.6	18.14	1.77
Carcass fat (KG)	7.58	1.38	9.93	2.51
Internal fat (KG)	2.88	0.93	4.74	1.58
Bone (KG)	3.11	0.29	2.7	0.244

Our initial analysis detected the presence of 31 QTL for carcass lean, carcass fat, internal fat, carcass weight, and bone quantity ($P < 0.05$). Of these, seven reached chromosome wide significance ($P < 0.01$) for predicted carcass lean, and bone content (figure 1). Adjustment for body weight as a co-variate, resulted in a different subset of QTL (13) of which four were in common with unadjusted yield traits described above and seven reached chromosome wide significance ($P < 0.01$) for internal fat, carcass fat, carcass lean and bone content. The procedure to use CT information in analysis of body composition for QTL detection without need for slaughter of animals opens the possibility to screen for QTL in growth rate and tissue yield at different stages of the growth phase. The presence of QTL for body composition provides the first stage in gene identification, or marker identification to allow selection for body composition at an early age.





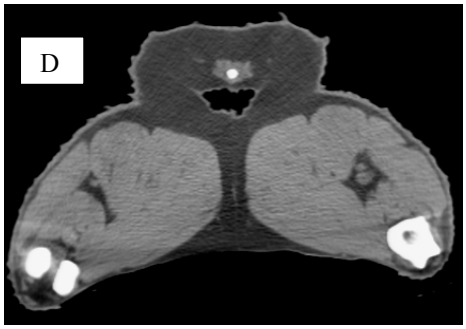


Figure 1. (A-C) : An all traits over plot of F-ratio from interval regression analysis of marker genotype along the chromosome and five body composition traits (internal fat : ▪▪▪▪▪▪, carcass weight : ▬▬ , carcass lean :—— , carcass fat : ▬▬ , bone : -----). The x-axis indicates the relative position along a chromosome, and y-axis represents the F-ratio. Two thresholds are shown for 5 % chromosome-wise (––––––) and 1 % chromosome-wise (-----) significance. A and B show two chromosomes analysed without adjusting for body weight and C is an output for a different chromosome after adjustment for body weight. (D) : Cross-sectional image of CT scan used to obtain estimates of body composition in live animals

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