PRELIMINARY RESULTS OF A WHOLE-GENOME SCAN TARGETING QTL FOR CARCASS TRAITS IN A TEXEL X ROMANOV INTERCROSS

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INTRODUCTION

Texel sheep are recognized for their good conformation, muscularity and leanness. In Belgium Texel sheep have been selected for an extreme form of muscular hypertrophy - sometimes referred to as "double-muscling" by analogy with the bovine condition (Leroy, 1989). While this trait is recognized to be inherited, its precise mode of inheritance remains unknown. In order to clarify the heredity of this trait, a crossbreeding experiment involving Belgian Texel and Romanov was carried out. A large number of carcass traits were measured in these crosses in order to more precisely characterize this ovine muscular hypertrophy.

MATERIAL AND METHODS

Pedigree Material. Respectively three and 143 (Romanov x Texel) F1 rams and ewes produced and intercrossed over four breeding seasons (1997-2000) to produce a total of 278 F2 animals. In addition the F1 rams were backcrossed to 70 Romanov ewes to produce a total of 228 Romanov x (Romanov x Texel) offspring. All animals were reared at the Domaine de Langlade in Toulouse (INRA).

Phenotypes. F2 and backcross offspring were raised and slaughtered at a fixed weight : 33 kg for females and 39 kg for males. The day after slaughter, carcass measurements were performed (Laville *et al.,* 2002). The carcass was weighed and the carcass yield was computed as the ratio between carcass weight and body weight at slaughter. The length of the carcass and the width of the carcass and the leg were measured with a meter rule and a calliper square, respectively. The round of the leg was estimated by the angle at the ankle joint by image analysis (figure 1). The conformation score was pointed on a 10 points scale. The retail cuts were obtained following the French standard cut : shoulder, leg and saddle joint, loin, expressed in percentage of the carcass weight. Shoulder muscle was separated from the bone, and muscle percentage was computed in shoulder. The loin area was measured by image analysis. On a leg cut, the area of the external part area (outside) was estimated by image analysis (figure 1). In the external part of the *vastus lateralis* muscle, a core was removed for heavy myofiber chain typing by electrophoresis (Sayd *et al.,* 1998).

Microsatellite genotyping. Microsatellite genotyping was performed according to standard procedures (Georges *et al*., 1995). Ovine and bovine microsatellite markers were chosen, based on their polymorphism and chromosomal location.

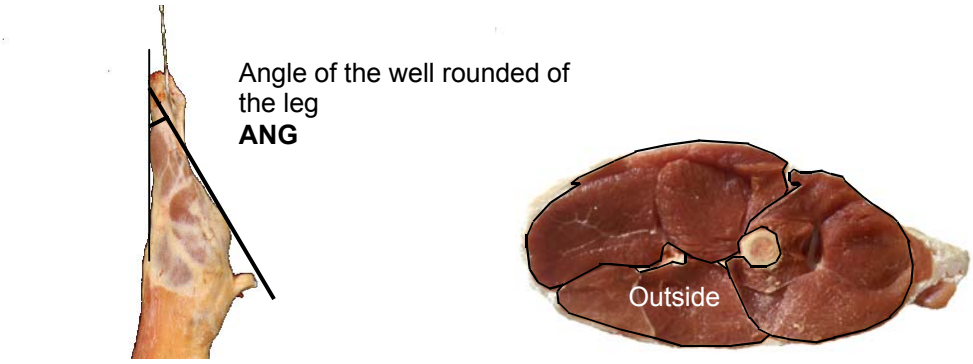

Figure 1. Image analysis measurements

Map construction. Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package (Lander and Green, 1987).
Statistical analysis. Applying a typical "interval mapping" strategy, conventional QTL mapping was performed using a multipoint maximum likelihood approach. The applied model assumed one segregating QTL per chromosome, and fixation of alternate QTL alleles in the respective parental lines : Texel (*T*) and Romanov (*R*). Evidence in favor of QTL was expressed as LOD scores. Following Lander and Kruglyak (1995), we assumed significant lod score at the threshold of 4.3.

RESULTS AND DISCUSSION

A QTL scan in a Romanov x Texel intercross reveals a major effect on ovine chromosome 2. At this point, we have completed genotyping of 16 ovine chromosomes for a total of 118 markers and performed analysis for six of these (OAR1, 2, 9, 14, 18 and 21). Four of these were selected to initiate the whole genome scan as they are known to harbor genes that were shown in other studies to affect muscular development (OAR2 (*MSTN*), OAR14 (*CRC*), OAR18 (*CLPG*), OAR21 (*IGF2*)). So far, only chromosome 2 yielded very strong evidence for a QTL influencing several of the carcass traits. The corresponding location scores are shown in figure 2A. At least 12 phenotypes measuring muscularity gave highly significant lod scores ranging from 4.5 to 13, therefore providing strong evidence for a QTL affecting muscular development in this chromosome region. The associated ML estimates of the genotype means and residual variances are given in table 1. This QTL explained between 6 and 22 % of the phenotypic variance in the F2 generation.
Absence of evidence in favor of a structural or regulatory anomaly of the ovine MSTN gene. The most likely position of the QTL on chromosome 2 coincides virtually exactly with the MSTN gene, making it an obvious positional candidate for the observed effect. To verify this hypothesis we sequenced approximately 10 kb of the Texel and Romanov MSTN alleles, including 3.6 Kb of upstream sequence, the complete MSTN gene, and 1.9 Kb of downstream sequence. No single polymorphism that could explain a loss of MSTN function could be identified. We then analyzed the expression of the MSTN gene at the mRNA level in skeletal

muscle of Texel and Romanov sheep. So far, we have not found evidence for any significant difference in the MSTN expression profile between these two breeds. Further studies are underway to elucidate the contribution of the ovine MSTN gene to the observed QTL effect.

Table 1. Characterization of the identified chromosome 2 QTL effects : maximum likelihood genotype means (TT : Texel/Texel, TR : Texel/Romanov, RR : Romanov/Romanov), part of the phenotypic variance explained by the QTL(% var) and lod score

	TT	TR	RR	% var.	Lodscore
Muscle wt (g)	51.98	-7.79	-37.85	22.05	13.42
Muscle %	1.74	-0.111	-1.372	19.15	11.68
Hindquarters wt (g)	194.22	-10.797	-143.13	18.82	11.36
Conformation score	0.52	0.03	-0.60	14.38	8.95
Carcass width (cm)	0.412	-0.062	-0.243	16.98	8.27
Bone %	-0.61	-0.008	0.546	14.67	7.23
Dressing %	0.977	-0.124	-0.635	10.46	6.32
Fat %	-1.218	0.128	0.846	9.34	5.38
Carc. compactness	0.819	-0.029	-0.642	10.30	5.05
Hindquarters %	0.398	0.020	-0.361	7.57	4.41
Shoulder wt (g)	31.090	-8.470	-21.324	7.48	4.18
Fat wt (g)	-16.808	0.569	11.978	6.92	3.96

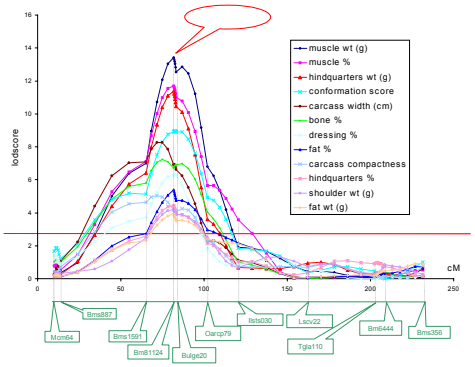

Figure 2A. Lod score profiles obtained in the F2 for 12 traits along the chrom. 2 map

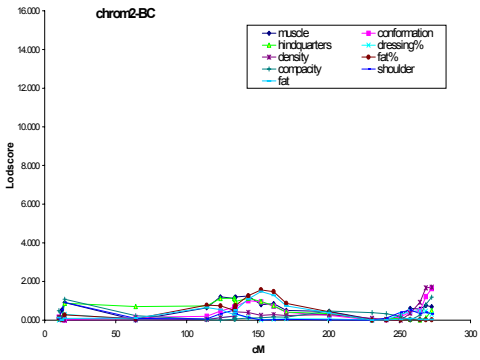

Figure 2B. Lod score profiles obtained in the BC for 9 traits along the chrom. 2 map

Analysis of the chromosome 2 QTL effect in Romanov x (Romanov x Texel) backcross. We genotyped our backcross population for a series of markers spanning the MSTN locus and performed a conventional QTL analysis. Unexpectedly, the effect of the MSTN region on carcass traits was much less pronounced in the backcross when compared to the F2 population

(figure 2B). A linear model applied to the F2 and backcross populations combined revealed a statistically significant interaction between QTL effects and cross (F2 versus BC). We hypothesized that this could be due to a parent-of-origin effect and that only the maternal Texel allele would be effective in improving carcass merit. We therefore analyzed our F2 population using an imprinting model as previously described (Nezer *et al*., 2002). However, no evidence was found in favor of an imprinting effect in the F2 population. An alternative hypothesis is that the effect of the Texel MSTN requires the presence of a recessive Texel allele at another locus. Our whole genome scan should allow us to test this hypothesis

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