MAPPING THE HORNS LOCUS IN SHEEP

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SUMMARY

The horns locus has been mapped to a 200kbp region in sheep using a segregating (Merino x Romney) x Merino resource. This region contains a single candidate gene. Maximal LODs exceeded 110 for the best location. Thirty-two SNPs spanning 400kbp were subsequently genotyped over 1000 sheep from a wide variety of polled and horned breeds. A 17 SNP haplotype was associated with the polled locus which had near perfect (>97%) concordance with phenotype. The gene is currently being sequenced.

INTRODUCTION

Undomesticated sheep typically have horns. Horns are present in most *Bovidae* and resemble modified hooves. In wild populations they are used in males for mate competition in the breeding season and for feed competition in females in the peri-parturient period (Clutton-Brock and Pemberton 2004). The frequency of hornless (polled) sheep originally increased during domestication 7000-9000 yrs BP, and dramatically in English breeds after 1500 AD. Many breeds are now polled, although certain breeds, such as the Merino, still have horns. Previous work provided evidence of a Horns (Ho) locus on sheep chromosome OAR10 (Montgomery *et al.* 1996). Previous work at the Ho locus suggests it has at least three alleles: normal horns (H^+), sex limited horns (H^L), and polled (H^P). The phenotype depends on sex and alleles inherited (Figure 1). The expression of the H^+ and H^L allele can also be modified by castration as horn growth ceases immediately at this time (Marshall and Hammond 1914). Horns are generally considered an undesirable trait by sheep farmers and are selected against in most breeds. A DNA test for this locus would therefore be beneficial for two reasons. Firstly, normal Ho locus expression appears to be masked by modifiers present in dual purpose composite NZ breeds resulting in unpredictable inheritance. It is thought that these modifiers were originally present in Scandinavian breeds (A. Bray pers. comm.). Secondly, wool shedding and extended breeding season traits are being introgressed into dual purpose animals from horned breeds such as Wiltshires and Dorset Horn. These breeders want an efficient method of retaining the desirable traits while eliminating horns.

A

	Ho^LHo^L ♀ Scurred	
Ho^LHo^P ♂ Horned	Ho^PHo^L ♂ Horned	Ho^LHo^L ♀ Scurred
	Ho^PHo^L ♂ Horned	Ho^PHo^L ♀ Polled

B

	Ho^+Ho^+ ♀ Horned	
Ho^+Ho^P ♂ Horned	Ho^PHo^+ ♂ Horned	Ho^+Ho^+ ♀ Horned
	Ho^+Ho^+ ♂ Horned	Ho^PHo^+ ♀ Scurred

Figure 1. Inheritance of horns adapted from Clutton-Brock & Pemberton (2004): two models; sex-limited horns (A), and normal horns (B). Ho^PHo^P is polled for males and females in both models. P: polled, L: sex-linked horns, and +: normal horns.

MATERIALS AND METHODS

Microsatellites. The seminal report that mapped Ho locus to OAR10 used female offspring from Merino X Romney rams backcrossed to Merino dams i.e. (MxR)xM (Montgomery *et al.* 1996). Only polled and sex limited horn alleles were segregating in this resource. We screened a superset of this resource with additional years of female (MxR)xM progeny with six microsatellite markers, three of which had been used in the initial study, in order to localise a region of interest.

There were 360 F2 female offspring genotyped along with their four F1 sires and six available paternal grandparents. Horns were scored using the Dolling scale (Dolling 1970) on their presence and size.

Subsequently, 71 (MxR)xM females that had a recombination event in the region of interest (breakpoint panel) were then genotyped with a further 24 microsatellite markers spanning the region identified. In addition a further 22 animals from a separate breed resource of horned (Merino, Dorset Horn and Wiltshire Horn) and polled (Texel, Gotland, Romney) animals were also genotyped. These animals were identified by breed but often did not have individual horn phenotypes recorded.

An additional resource ("breed standards", n=698) were then genotyped with the four most informative markers. This resource consisted of animals from the following horned (NZ Wiltshire, Dorset Horn, Soay, Spanish Merino, French Merino, Awassi, Finnish Landrace, and Bighorn) and polled (Texel, Poll Dorset, Coopworth, Romney, Suffolk, Perendale, Corriedale, East Friesian, Cheviot, Gotland, and Fleischschaf) breeds. Horns status was determined from known breed characteristics. Microsatellite markers were developed from the bovine genome. The data was analysed via multi-point linkage analysis using CRI-Map in order to position the Ho locus.

SNPs. Thirty-two SNPs spanning the most likely 400kbp region containing the Ho locus were then genotyped over the breakpoint panel and breed standards. These SNPs were developed from sequence generated from initial 454 ISGC ovine genome sequencing (McEwan *et al.* 2009), and three ovine BACs selected to span the region of interest. The combined data set was then analysed via multi-point linkage analysis using CRI-Map in order to refine the location of the Ho locus.

Sequencing. Some ovine sequence for our gene of interest was available from the previously described sequencing. This was then used to design primers to sequence the mRNA and DNA isolated from a polled (Coopworth x Texel) and horned (NZ Wiltshire) ram. The last seven and the first exon of the gene was sequenced using DNA. The ovine mRNA was converted to cDNA and also sequenced.

RESULTS AND DISCUSSION

Microsatellites. From the six markers genotyped, the Ho locus was localised to a 14Mbp region (Maximal LOD 108). The second and third rounds of fine mapping with an additional 24 markers narrowed the Ho locus region down to a 200kbp region (maximal LOD 110; figure 2). Within this region a four marker haplotype was observed that best explained polled versus horned animals. This haplotype was validated on the breed standard animals and was estimated to have 92% accuracy.

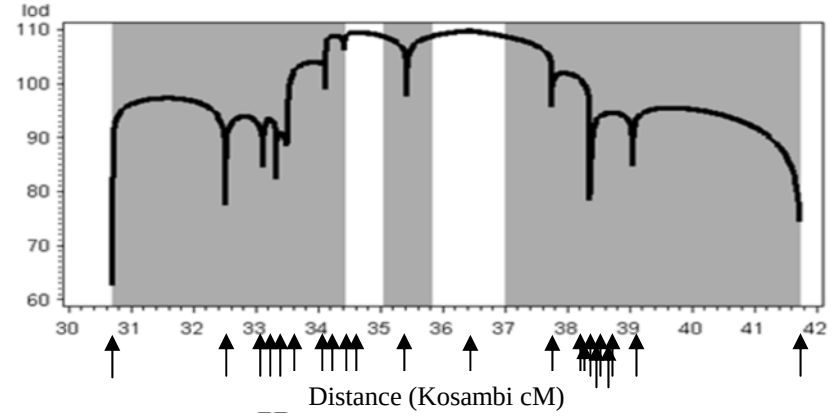

Figure 2. Multi-point linkage graph of the horns locus on OAR 10, after microsatellite genotyping. Marker BM6108 was the origin and arrows indicate microsatellite positions. White region indicates the 1 LOD score drop-off region (200kbp).

SNPs. Using the thirty-two SNP markers genotyped, the Ho locus was further localised to a 50kbp region (maximal LOD 110, figure 3). Within this region resides a single candidate gene. Analysis of breed phenotypes generated a haplotype of 17 markers for the polled haplotype; with a >97% concordance with phenotype. This haplotype extended over the 3' end of the candidate gene.

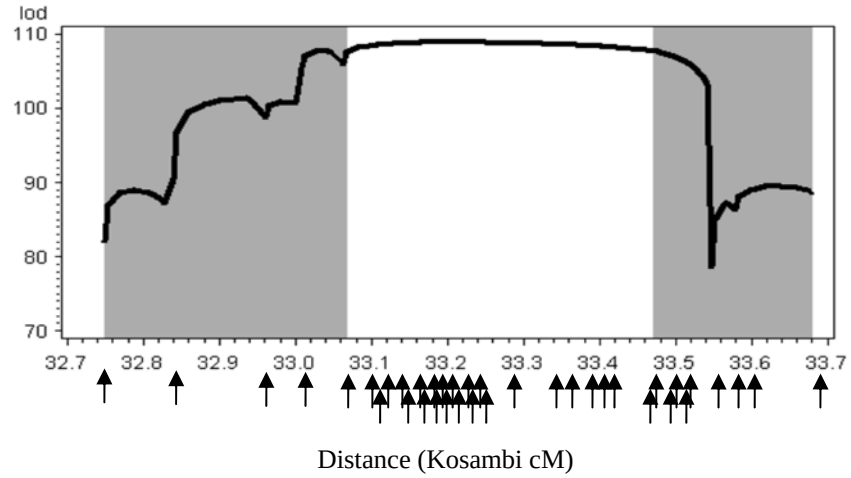

Figure 3. Multi-point linkage graph of the horns locus on OAR 10, after SNP genotyping. Marker BM6108 was the origin and arrows indicate relative SNP positions. White region indicates the 1 LOD score drop-off region (50kbp). Minor differences in scale are due to additional markers used to build the linkage map.

Sequencing. Initial sequencing identified a large ~3kb insert in the non coding 3' untranslated region of the candidate gene. This insert seemed to be present only in polled animals. The sequence of the insert indicated that a complete functional mRNA has been retrotransposed in the

reverse orientation. Comparison of the Coopworth sequence with bovine, human and Wiltshire identify a flanking thirteen base pair direct repeat. This is characteristic of a target site duplication commonly associated with the integration of a transposable element. Studies are now underway to validate this in a variety of breeds and horned phenotypes. Insertions of this nature are essentially unique within a species and it may be that the majority of polled animals in the world descend from a single common ancestor.

CONCLUSIONS

The horns locus has been fine mapped to a 50kb region in the sheep genome and predictive haplotypes for the polled H^P allele identified. A single candidate gene underlies this region and preliminary work has identified a large retrotransposed insertion in the 3' untranslated region of this gene in polled animals. This insertion appears to be functional, would be transcribed in the opposite orientation and is flanked by 13bp direct repeats. Our hypothesis is that a polled mutation has occurred in a single ancestor and this has subsequently been selected to near homozygosity in a wide variety of polled breeds. In contrast horned animals still display the full range of ancestral variability in this region.

ACKNOWLEDGMENTS

The various breeders and researchers who collected and provided the DNA samples utilised. Gemma Payne for the BAC sequencing. This research was supported by Ovita.

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