

## Review Article

# Genomics of Sheep

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### Abstract

During the last five years, advances in livestock genome mapping have been remarkable. Species-specific genetic maps exist for cattle, sheep, pigs, fowls and horses, with marker intervals of 5-20 cM. These maps have been essential for the identification of genes and genetic markers associated with economically important traits in livestock, thereby having a significant impact on world-wide livestock production. In addition, many aspects of livestock genome projects will contribute to human genetic research.

### Introduction

Traditional plant and animal breeding practices have made great progress in improving the original wild-type species, resulting in the domesticated varieties, lines and breeds we have today. These improvements were accomplished by selecting plants and animals possessing desirable phenotypes and eliminating those that were undesirable. In recent years, the genetic analysis of organisms has been revolutionized by recombinant DNA technology, allowing the direct identification and manipulation of DNA sequences, and the application of a marker-assisted selection (MAS) to complement traditional selection methods.

### Genome maps

Central to the identification of DNA sequences controlling traits of economical interest is the development of genome maps. These maps contain assignments of markers and genes to specific regions along the chromosomes and are useful for organizing systematic searches for chromosome regions containing interesting genes. Once these regions have been identified, additional markers in the area can be used to locate the locus of interest with better resolution. About 200 equally-spaced markers are necessary to completely scan the genome of livestock species (Georges and Massey, 1991). Because of marker clustering, many more markers will be needed to achieve an adequate coverage for a basic 10 to 20 centimorgan (cM) genetic map. Additional markers will be essential to increase map resolution in order to proceed with the isolation of economically important genes and to apply MAS strategies.

The development of the genome map for sheep has advanced rapidly. A first-generation map was published in 1995 (Crawford *et al.*, 1995) and contained 246 markers (86 ovine microsatellites, 126 bovine microsatellites, 1 deer microsatellite and 33 known genes). Total coverage of the map was 2070 cM (about 75% of the genome) and markers were assigned to all 26 sheep autosomes. This map was constructed using the AgResearch International Mapping Flock (IMF), which included nine three-generation pedigrees. The number of informative meioses possible in the IMF was 222.

Three years later, a second-generation ovine genetic map was published (de Gortari *et al.*, 1998). The map was developed by merging data from the IMF and USDA mapping flocks. It contained 519 markers (402 bovine microsatellites, 101 ovine microsatellites and 16 known genes) and spanned 3063 cM across the autosomes, with an average marker spacing of 6.5 cM. The USDA reference flock included four pedigrees produced from matings of F1 rams and Romanov ewes. A third generation map is currently under development (J. Maddox, personal communication). This map will contain at least 900 markers and is a compilation of genotype data generated by over 11 laboratories using the IMF population.

A prerequisite for the development of a genome map is a well-characterized karyotype for the species. Sheep have 54 chromosomes, with 26 pairs of autosomes and two sex chromosomes. Most of the chromosomes are telocentric, with the exception of three pairs of large metacentric chromosomes (1, 2 and 3) and a very small Y metacentric chromosome. In 1995, a standard G-band karyotype was agreed upon at the 9th North American Colloquium on Domestic Animal Cytogenetics and Gene Mapping. The ovine and bovine standards have been correlated based on similar band patterns and mapped gene loci. Human paints on ovine chromosomes or sheep paints on chromosomes of other species have not yet been reported; the results from such experiments would further identify chromosomal segments with conserved synteny between sheep and other species.

The total number of physically mapped loci on the sheep genome is greater than 200 (Broad *et al.*, 1997). About two-thirds of the assignments were made using somatic cell hybrids and the rest by *in situ* hybridization. Although several somatic hybrids have been developed, most physical map assignments have been made using hybrids from Saidi-Mehtar *et al.* (1979) and Burkin *et al.* (1991). Ovine yeast artificial chromosomes (YAC) (Broom and Hill, 1994) and bacterial artificial chromosomes (BAC) (Gill, I. 1998; Vaiman, personal communication) libraries have also been constructed. However, an ovine radiation hybrid (RH) panel has not yet been produced. While the bovine radiation hybrid map (Womack *et al.*, 1997) is useful for

ordering conserved gene sequences within an ovine contig, an ovine-specific RH panel would be preferable when mapping ovine sequence-tagged sites (STS).

An informational database that includes mapped loci in sheep is now available. SheepBase contains an up-to-date compilation of published data from sheep genome mapping projects and provides both physical and linkage maps of the sheep genome, together with information on individual loci and associated references. The database has recently been converted to an ARKdb format and can be accessed through AgResearch (New Zealand): [<http://www.zaphod1.agresearch.cri.nz:8002/>] and the Roslin Institute (United Kingdom): [[http://www.ri.bbsrc.ac.uk/genome\\_mapping.html](http://www.ri.bbsrc.ac.uk/genome_mapping.html)].

### Important traits

Genome scanning projects have been initiated for several economically important traits in sheep. Chromosomal assignment of single gene traits include the Booroola fecundity gene (OAR6; Montgomery *et al.*, 1994), *callipyge* muscle hypertrophy (OAR18; Cockett *et al.*, 1994), *Carwell* muscle hypertrophy (OAR18; McEwan *et al.*, 1998), Belgium Texel double muscling (OAR2; Marcq *et al.*, 1998), horns (OAR10; Montgomery *et al.*, 1996) and Spider Lamb Syndrome (OAR6; Cockett *et al.*, 1999).

The identification of quantitative trait loci (QTLs) has been reported for several traits including parasite resistance (Beh *et al.*, 1998), facial eczema (Paterson *et al.*, 1998), wool production traits (Parsons *et al.*, 1994; Jenkins *et al.*, 1998), milk traits (Diez-Tascon *et al.*, 1998) and dagginess (MacDonald *et al.*, 1998). The development of additional resource flocks is ongoing for traits such as parasite resistance, out-of-season breeding, carcass traits and wool production.

In 1994, the Booroola fecundity gene, *FecB*, was assigned to ovine chromosome 6 (Montgomery *et al.*, 1994). The *FecB* mutation is codominant for ovulation rate and partially dominant for litter size, with one copy of *FecB* increasing litter size by approximately one extra lamb. Twelve pedigrees were used in the linkage study; each pedigree included a heterozygous sire (*FecB*/+) carrier mated to homozygous wildtype (+/+) dams. The ovulation rate of the female offspring was determined by laparoscopy, allowing the females to be classified as either *FecB*/+ or +/+. After screening 144 markers through the pedigrees, linkage to *FecB* was first detected with the microsatellite OarAE101, with a maximum lod score of 17.33 at a distance of 13 cM. Using linkage information for OarAE101 from the ovine genome map, a second marker, OarHH55, was tested and found linked to *FecB* at 20 cM with a maximum lod score of 9.38. These markers, and by inference the *FecB* locus, were later assigned to sheep chromosome 6 (Crawford *et al.*, 1995). Comparisons to human chromosome 4q, the evolutionary homologue, have not revealed a position candidate for the *FecB* gene.

The *callipyge* gene is a mutation in sheep responsible for pronounced muscle hypertrophy of the fast twitch muscle fibres; however, the hypertrophy is absent at birth and develops only after approximately three weeks of age. *Callipyge* animals produce leaner, higher yielding carcasses but there is some concern with decreased tenderness of the loin. Genetic characterization of the locus has demonstrated a unique mode of inheritance termed 'polar overdominance' (Cockett *et al.*, 1996), in which only heterozygous offspring inheriting the mutation from their sire express the phenotype. The three other genotypes are normal in appearance. Progeny data indicate that reactivation of the maternal *callipyge* allele occurs after passage through the male germ line, although this reactivation is not absolute. The locus has been mapped to the distal end of ovine chromosome 18 (Cockett *et al.*, 1994) and positional cloning experiments are ongoing.

The characterization of another gene responsible for an increase in the rib eye muscle of lambs has been recently reported (Banks, 1997). The effects of the *Carwell* gene are less dramatic than for *callipyge*, with the increase in muscle mass limited to the *longissimus* and detectable only with ultrasonic scanning. Using microsatellite markers from ovine chromosome 18, the *Carwell* locus was localized to approximately the same position as *callipyge* (Nicoll *et al.*, 1998), suggesting that *Carwell* is allelic to *callipyge*.

Spider Lamb Syndrome (SLS), or ovine hereditary chondrodysplasia, is a recessive genetic disorder causing skeletal deformities in young lambs. Common features include abnormally long, bent limbs and curvature of the spine. Since the late 1960s the disorder has spread to several black-faced sheep breeds within the USA and Canada, as well as Suffolks in Australia and New Zealand. Researchers at Utah State University, in collaboration with the University of Illinois, have mapped the Spider Lamb Syndrome locus to the distal end of ovine chromosome 6 (Cockett *et al.*, 1999). Comparative analysis of genome maps between sheep, cattle and humans, combined with the results of knockout studies in mice, identified fibroblast growth factor receptor 3 (FGFR3) as a positional candidate for the disorder. Genomic and cDNA sequencing of ovine FGFR3 has revealed a single-base mutation causing a non-conservative (non-polar to charged) amino acid substitution in the tyrosine kinase domain of the receptor. Population studies including more than 1000 sheep of differing SLS genotypes demonstrates that this is the causative mutation in SLS. It is most likely that the mutation leads to loss of receptor function with improper regulation of bone growth. There is also a suggestion that heterozygous animals have increased bone growth, making them taller in stature than their homozygous normal counterparts. This might be an explanation for the high frequency of the Spider Lamb Syndrome allele in show-ring animals (Beever, unpublished data).

While comparative mapping has been beneficial in the Spider Lamb Syndrome gene search, it has not been effective for traits unique to the sheep, such as Booroola fecundity, *callipyge*, *Carwell*, daggs, and horns. Researchers are seeking to identify these genes using positional cloning techniques, thereby leading to the discovery of genetic mechanisms not yet explored.

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