Recent Advances in Sheep Genome Mapping

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ABSTRACT : The rapid development of the sheep genetic linkage map over the last five years has given us the ability to follow the inheritance of chromosomal regions. Initially this powerful resource was used to find markers linked to monogenic traits but there is now increasing interest in using the genetic linkage map to define the complex of genes that control multigenic production traits. Of particular interest are those production traits that are difficult to measure and select for using classical quantitative genetic approaches. These include resistance to disease where a disease challenge (necessary for selection) poses too much risk to valuable stud animals and meat and carcass qualities which can be measured only after the animal has been slaughtered. The goal for the new millennium will be to fully characterise the genetic basis of multigenic production traits. The genetic linkage map is a vital tool required to achieve this. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1129-1134*)

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INTRODUCTION

In the last five years a large scale world-wide effort has produced genetic linkage maps of most important livestock species and sheep are no exception. The primary genetic linkage map of the sheep genome was published in 1995 (Crawford et al. 1995) and a second generation map is currently in press. The current map (De Gortari et al. 1998) is comprised of a total of 519 genetic markers covering approximately 3000 cM.

A sufficiently dense linkage map gives us the tools to follow the inheritance of every piece of every chromosome in any pedigree, breed or selection line cross. In addition the methods that enable us to analyse the genes and DNA contained within the chromosomes have undergone many incremental improvements so that it is now a realistic research goal to find and characterise genes responsible for multigenic or quantitative traits in livestock species.

Providing we have pedigrees in which genes which have an important influence on the trait of interest are segregating, we can systematically identify the regions of the genome involved. This process has become known as a genomic scan (Lander and Botstein 1989) and has become a powerful new method for identifying genome regions and eventually finding genes involved in multigenic production traits. This paper will describe the development of the sheep linkage map and its use in the discovery of genes affecting production traits in sheep.

THE SHEEP GENETIC LINKAGE MAP

When markers are near each other on the same chromosome they are not inherited independently, but tend to segregate together at meiosis. This phenomena is called genetic linkage, and was first described in Drosophila about 90 years ago. Providing there is a high enough density of markers spread over each chromosome, the markers can be assembled into a linkage map of the genome (Botstein et al., 1980). Markers that tend to segregate together are placed into linkage groups, with the proportion of recombinants (crossover events) detected between linked markers being used as a measure of the distance between them. The greater the proportion of crossovers detected the greater the distance is between markers. The requirements for efficient linkage mapping are therefore, large numbers of informative genetic markers (see below) and a common set of pedigrees so that the inheritance of these markers can be followed. The development of DNA based genetic markers, as opposed to classical phenotypic (e.g. Horns) and protein markers (e.g. haemoglobin), has provided sufficient markers to assemble a linkage map for sheep.

The first DNA markers, called restriction fragment length polymorphisms (RFLPs), are two allele systems that detect the presence or absence of a cut site for a restriction endonuclease. Because they are relatively uninformative and require large quantities of DNA for their analysis, the speed of linkage mapping using these markers was slow. It was the discovery of microsatellites, a new type of DNA marker, that was

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responsible for the rapid development of the genetic linkage maps. The first microsatellites were described in 1989 (Weber and May, 1989) with the first microsatellite from livestock published a year later (Crawford et al., 1990).

Microsatellites, which are also known as STR's (simple tandem repeats) or SSR's (simple sequence repeats) were a new type of DNA marker that are not only very informative but are relatively easy to type and score. At the heart of any microsatellite is a simple sequence, either a mono-, di-, tri-, or tetra-nucleotide, that is generally repeated between 10 and 50 times. Virtually all of the microsatellites that have been found for sheep and other ruminants have the sequence (AC/GT) as the repeat unit. The reason for this is not that the other types don't exist but that this type is the most abundant within the ruminant genomes and hence they are much easier to find and characterise. The variation between alleles of the microsatellites is due to variation in the number of simple sequence repeats. The way microsatellites are typed is to design primers to the unique DNA sequences on either side of the repeat and, using the polymerase chain reaction (PCR), to amplify the region containing the repeat. The size of the PCR amplicon is then measured, usually by electrophoresis on a DNA sequencing gel which will resolve two basepair differences in DNA size.

The genetic linkage map for sheep prior to 1994 contained only 17 markers assigned to 7 linkage groups (Rasmusen, 1966; Tucker et al., 1980; Leveziel et al., 1991; Penty and Montgomery, 1991a; Crawford et al., 1992). Following a search for the Booroola fecundity (*FecB*) gene (Montgomery et al., 1993), microsatellites and RFLP's used in the study were analysed for co-segregation in half-sib pedigrees. This search identified 19 linkage groups, comprising a total of 52 markers (Crawford et al., 1994). Of the 19 linkage groups, 13 could be assigned to sheep chromosomes.

The primary linkage map comprising 246 markers covering 2070 cM of the sheep genome in which linkage groups were assigned to all chromosomes was published in 1995 (Crawford et al., 1995). This map was constructed by linkage analysis using the specially developed AgResearch International Mapping Flock (IMF). The IMF comprises nine three generation pedigrees. Five breeds contribute to the pedigrees; Texel, Coopworth, Perendale, Romney and Merino. The different breed crosses were used to try and maximise the heterozygosity of the F1 generation. The total number of informative meioses possible in the IMF is 222. DNA from these animals has been distributed to 14 laboratories worldwide so that all markers can be mapped in a common pedigree and a linkage map constructed from all contributed markers. A linkage map of the sheep X chromosome developed by Galloway et al. (1996) used a flock designed to examine segregation of the X chromosome in addition to the IMF animals.

The primary sheep linkage map was developed in a collaboration with twelve laboratories across the world. In particular, there was close collaboration between the AgResearch Molecular Biology Unit and USDA, Clay Centre which swapped sheep and cattle primers so those markers that could be amplified and were informative in both species could be placed on both the cattle (Kappes et al., 1997) and sheep linkage maps. The second generation linkage map (De Gortari et al., 1998) has seen a continuation of this collaboration with the USDA using both the AgResearch IMF and there own linkage mapping flock to increase the marker density and ability to order these markers. The new map contains 519 genetic markers in 27 linkage groups with each linkage group assigned to a sheep chromosome and covering >3000 cM. In addition to the publications the linkage map can be found at the following web sites:

AgResearch, New Zealand
[http://zaphod.invermay.cri.nz]
National Agricultural Library, USA
[http://tetra.gig.usda.gov:8400]
Roslin Institute, Scotland
[http://www.ri.bbsrc.ac.uk/sheepmap/]
USDA Meat Animal Research Center, Nebraska
[http://sol.marc.usda.gov/]

The third major contributor to the sheep genetic linkage map has been the Centre for Animal Biotechnology, University of Melbourne. They have added a further 300 markers to the linkage map. Compilation of a third generation map, containing approximately 800 genetic markers is currently underway. (J. Maddox, personal communication). Fifty RAPD markers have also been added to the map (Cushwa et al., 1996).

One significant finding of the sheep linkage mapping to date has been the demonstration that sheep are genetically very heterogeneous. The average number of co-informative meioses using adjacent framework markers in the IMF was 118 (Crawford et al., 1995). This compares favourably with the cattle reference population of Bishop et al. (1994) which was approximately twice the size, but contained an average of only 81 co-informative meioses per marker. The estimate from Bishop et al. (1994) includes all the markers and not just framework markers. If we include all the markers in the estimate for sheep we expect an average of 88 co-informative meioses per marker which is still a much higher percentage than in cattle.

Table 1. Major genes affecting production traits in sheep

Trait	Gene name	Chromosome	Reference
Fecundity	Booroolla	6	Montgomery et al. 1993
Fecundity	Inverdale	×	Davis et al. 1991
Horn development	Horns	10	Montgomery et al. 1996
Muscular hypertrophy	Callipyge	18	Cockett et al. 1994
Wool fibre diameter	Drysdale	unknown	Dry 1955
Wool colour		unknown	Barsch 1996

The IMF was generated using a variety of breeds in order to produce a high level of genetic heterogeneity. Subsequent studies (Buchanan et al., 1993) have shown that the extent of genetic diversity within the NZ Romney population and the Australasian Merino populations was similar to the genetic diversity found in a mixed population of 6 different breeds including the five used to generate the IMF. The situation in cattle is different, since *Bos taurus* purebred populations have a significantly lower level of heterozygosity than crossbreeds or *B. taurus/B. indicus* crosses (Kappes et al., 1997).

SEARCHING FOR GENES CONTROLLING PRODUCTION TRAITS

A. Major genes

There are certain genes which have such a major effect on the phenotype of an animal that their inheritance can be followed by simply observing the phenotype of the offspring. A good example of this is the Horns locus or Polled gene where the inheritance can be followed by simply observing horn formation, or the lack of it. Table 1 lists the major genes that have been identified in sheep. The list does not include the genetic disorders that have been identified but confines itself to those traits affecting production. A database, Mendelian inheritance in Animals, compiled by Prof. F. W. Nicholas, University of Sydney, lists all the genetic disorders of animals and can be found at the following web site: http://www.angis.org.au/Databases/BIRX/omia

B. Quantitative or multigenic traits:

Most traits that influence production, such as growth rate, wool and carcass characteristics, and resistance to disease show continuous variation within the population and are not controlled by single genes. The genes or loci that affect such quantitative variation are known as quantitative trait loci (QTL). The reason for the continuous variation is that many genes and environmental effects contribute to the phenotype being observed. This hasn't stopped animal breeders making very good progress in selecting for complex traits over the last 50 years. They have ignored the underlying discrete nature of the genetic cause but estimated, instead, the similarity between relatives compared with non-relatives. Armed with this information and accurate and efficient phenotypic information, they used sophisticated statistical techniques (e.g. BLUP) to help them decide which animals to use as parents of the next generation. The success of this approach can be seen by the genetic progress of some group breeding schemes in the New Zealand sheep industry and in many single trait selection experiments (Clarke and Johnson, 1993).

The development of a genetic linkage map doesn't replace this technology but will allow us to begin to try and unravel the complex mixture of genes likely to be responsible for variation in desirable traits. Eventually, we may understand enough about each one of the estimated 100,000 genes that make up a sheep to be able to decide which is the best animal for breeding based only on the analysis of its DNA but this is in the distant future. Of more immediate benefit is to use this technology in the selection for those traits which at the moment are too expensive to measure on a regular basis or entail potentially harmful and ethically debatable procedures. Examples of these are:

1. Resistance to diseases: There are some breeders who measure resistance to internal nematode parasites but the challenge regime is both costly and risks harming valuable animals. If we could develop a DNA test to identify those animals resistant to parasites we could save exposing these valuable stud animals to a parasite challenge and hopefully reduce the cost of selection. A test for resistance to other diseases such as Johnne's disease, Facial Eczema, foot rot and brucellosis would all be useful.

2. Meat tenderness: This trait is important and quite easy to measure but you must kill the animal first before you can measure it. Unless one collects gametes, prior to killing and testing the animal, it is not possible to use it for breeding. Alternatively, progeny testing can be used but this is very expensive.

3. Leanness: Ultrasound measurement of backfat is

a useful and relatively cheap breeding tool but better more accurate measurement using a CT scanner is very expensive. Once again a genetic marker may be quite cost effective in selecting for leaner sheep.

The generation and phenotypic measurement of pedigrees designed to show segregation of the trait of interest is the first requirement for any genome scan. The most common designs involve large half-sibships where the sire (normally a breed or selection line cross) is mated to either unselected dams (an outcross pedigree) or dams from the breeds or selection lines (a backcross pedigree). The phenotypes of all the progeny are recorded and the progeny genotyped at markers spread at approximately 30 cM intervals across the genome (Darvasi and Soller, 1994). To try and reduce the substantial cost involved with genotyping often only the phenotypically extreme progeny are genotyped. After the genotypes have been obtained one then attempts to link the inheritance of a particular region of the sires chromosome(s) with the inheritance of the phenotype (Janss et al., 1995)

Linkage studies with this design are simplified because you are examining the meioses of a single animal, the sire of the half-sib pedigree. There is only one opportunity for crossing over (recombination) to disrupt the phase of the marker(s) with the QTL you are searching for. This means that markers as far away as 15 centiMorgans (cM) from the QTL show evidence of linkage so that 150 evenly spaced informative markers can be used to search the entire genome for QTL. This contrasts with an association study where a comparison between selected populations with ample opportunity for phase disruption means that the marker must be within 1 cM of the QTL for a significant association, so that 3000 markers will be required for a genome scan.

The search for genes responsible for complex multigenic traits is likely to have different outcomes depending on the trait that is being examined. Some traits may be influenced by a small number of genes with large effects in which case it is likely that the regions containing them will be identified by a genomic scan. If, however, the trait is influenced by a large number of genes, each with a small effect, the genomic scan is unlikely to identify any region linked to the trait. Segregation analysis using programmes such as FINDGENE (Kinghorn et al., 1993; Kerr and Kinghorn, 1996; McEwan et al., 1997) attempt to identify those pedigrees where genes of large effect are present. Given the high cost of these experiments it would be prudent to undertake some form of segregation analysis prior to the commencement of a large scale genomic scan. If, however, one is looking to identify the genetic cause of fixed differences between breeds and strains this analysis is of little use.

There is considerable debate amongst geneticists as to what constitutes significant linkage of a marker locus to variation in a trait (Lander and Kruglyak, 1994). With the large number of marker genotype/trait phenotype comparisons being made in a genomic scan the chance that type I errors (false positives) will occur is very high if inappropriate significance levels are used. The most common method used to estimate the true level of type one errors and significance is using simulations on the actual dataset being used for the study (Churchill and Doerge, 1994; Wright et al., 1996).

A further complication can be the inheritance pattern of the gene involved. It has been estimated that up to 500 mammalian genes are imprinted. Imprinting is a genetic mechanism where only the allele of a gene inherited from a specific parent is expressed. Animal breeding tends to place most selection emphasis on the sire. The possibility of imprinting needs to be considered as it could confound an analysis based strictly on Mendelian inheritance. Other factors such as epistasis or whether the gene contains dominant, co-dominant or recessive alleles must be considered in any analysis of genomic scans

After QTL are detected by a genome scan, further work will almost always be required before the results can be used by industry or even published. Initially, the QTL region may not be well localised spanning a range of 20-40 cM with moderate confidence. To more closely localise the QTL additional steps should be undertaken. These include: genotyping all animals (rather than just the extremes) in the regions of interest, the development of further polymorphic markers specific to the locality in an attempt to make additional pedigrees fully informative, and testing the validity of the results in independently generated populations to both verify and more closely define the likely QTL location. If this work is successful, marker assisted selection (MAS) may become a viable addition to existing genetic selection tools. This methodology uses closely bracketing marker loci if they are available and sufficiently polymorphic, and does not require the identification the actual gene involved (Meuwissen and Goddard, 1996).

The ultimate goal of QTL mapping is to find not only the gene(s) but also the mutation within the gene(s) responsible for the QTL. Finding genes by positional cloning is still a very difficult assignment even when it is a major gene you are trying to identify. Finding QTL by positional cloning where the phenotype of individuals is not so clearly defined will be even more difficult. Fortunately large segments of chromosomes are conserved between the different mammalian species (O'Brien et al., 1993). Providing

we can identify the equivalent region in species such as humans or mice, where the gene map is more highly developed, we can use the maps of these other species to search for candidate genes that could affect the trait we are studying. This approach is called positional candidate cloning and is likely to be the method whereby most of the genes underlying QTL are identified.

The economic benefits of MAS or QTL selection depend on the value of the trait, the inheritance pattern of the QTL identified, the nature of the bracketing loci available and the ease, cost, proportion of the animals needing to be measured and/or genotyped, and age when phenotypic measurement normally occurs. Prior to industry use it is likely that a formal economic evaluation will need to be undertaken for each QTL identified.

CONCLUSION

Predicting the future is always difficult but scientists have already identified the chromosomal regions in which most of the single genes are found. I think it is safe to say that scientists will have identified a number of chromosome regions in sheep responsible for complex traits such as disease resistance and meat and wool quality by the year 2000. It will be in the first decade of the 3rd millennium that we will begin to see the individual genes being identified and simple DNA tests available for sheep breeders.

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