MEAT & LIVESTOCK AUSTRALIA

On farm

Design of Gene Mapping Experiments

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Feedbase and Pastures

1. Terms of Reference

1. Review the sheep QTL experiments conducted in Australia, assess the strength of the evidence for each QTL and advise MLA/AWI on the QTL that are the highest priorities for future research aimed at identifying the QTL to meet MLA priorities.

2. Advise MLA and AWI on the flocks that would be necessary to identify QTL that have already been mapped and to find additional QTL.

2. Introduction

The two terms of reference have already been addressed in two milestone reports and these are included here as appendices. As pointed out there, we have been only partly able to complete the project because we have been unable to get access to the results of all Australian sheep gene mapping projects that have been carried out. The main purpose of this final report is to present recommendations to AWI/MLA on the design of experiments that might identify the gene and polymorphism that are the QTL for traits of economic importance.

3. Aims of MLA and AWI in future QTL research

QTL research, by definition, is concerned with naturally occurring variation in genes that affects traits of economic importance. MLA and AWI plan to invest in this field as part of their genomics program. The genomics program will also carry out research into the function of genes. This functional research is outside the scope of this report, but it is important that the two approaches (use of naturally occurring variants and gene function) be tightly integrated. The traits that MLA and AWI have decided are top priority are resistance to internal parasites, muscle growth and wool growth. For meat sheep we believe fertility traits, including lamb survival, should be added to the list.

4. Recommendation A

The benefits to the sheep industry from this research could be:

1. Faster genetic improvement in the sheep flock, by the use of DNA tests for desirable variants of genes affecting traits of economic value.

2. The design of artificial gene constructs that can be introduced into transgenic sheep to make them more profitable.

3. Non-genetic methods to manipulate the physiology of the sheep such as vaccines, growth promotants, or diets.

4. Increased understanding of sheep genetics and physiology which will flow into future research and lead to outcomes that cannot at this stage be foreseen.

The design of the research program depends a little on the relative importance given to the 4 possible industry benefits. If we think that better methods of sheep selection are an important outcome from the research, this implies that we design experiments that can

detect and identify genes of more modest effect (0.3 to 0.7 σ), for traits that are economically important but difficult to improve at present, and give emphasis to genes segregating in commercial sheep. If better methods of sheep selection is not an important outcome then we might focus research on genes with a large effect on any trait but found only in sheep that are unlikely to be used for sheep production in Australia.

The future benefits from marker assisted selection are difficult to quantify. If MAS is added to existing selection programs with little other change, the benefits depend heavily on the proportion of genetic variation in profit that is explained by the DNA markers. The power of the technology to find genes is increasing every year and world-wide there is a huge investment in this research, although little of it specifically in sheep. Consequently, in 20 years time we anticipate there will be a list of, say, 100 known genes affecting sheep production and that these will explain about half of the genetic variance in profit. In that case, MAS might add 0.5% per year to income from sheep production. If sheep production in Australia is worth $4B per year, the net present value of an indefinite rate of gain of 0.5% pa starting in 20 years time, at a discount rate of 10%, is $200m. The uncertainties involved in such predictions are huge, but even a modest increase in rate of genetic gain would be worthwhile. However the benefits are likely to be greater than this as a result of other changes in sheep breeding. If MAS is combined with early breeding, so that the generation interval is shortened, the benefits of MAS are much greater because one cannot select on phenotype at a very early age. The benefits from MAS will also be greater due to discovering and using non-additive effects of genes. We will be able to design crossbreds that have exactly the right genotype for a specific purpose. Therefore, although the benefits from finding a single QTL may not be great, we believe the long term benefits from MAS will be large. In the medium term we expect that some DNA based tests will reach the market for sheep breeding as they have already for dairy and beef cattle and pigs.

The other benefits to the sheep industry from genomics research might eventually be greater than from MAS, but they are even longer term and more unpredictable (A new growth promotant might or might not be discovered, it would take 10 years to reach the market, might or might not be profitable to use and might or might not be accepted by consumers).

5. Recommendation B

Therefore, we recommend that AWI/MLA pursue a mixed portfolio, in which some research is expected to yield DNA tests for MAS and some is not. Fortunately, it is likely that some of the research can generate both type of benefit.

To deliver these benefits, the outputs from the research could be:

1. Identification of a naturally occurring variation in the DNA sequence of a particular gene that causes an effect on an economic trait (ie identification of the gene and polymorphism causing a QTL).

2. DNA sequence variations that have no effect but are tightly linked to the DNA sequence change listed in (1) above.(ie DNA markers)

3. DNA sequence variants that have no effect but are loosely linked to the DNA sequence change listed in (1) above (ie DNA markers).

Outputs of type 1 (ie identification of the gene and polymorphism in it) would be the most useful because

- They would make selection for the gene most effective. However other markers that were in complete linkage disequilibrium with the functional mutation would be almost as good in practice.
- They represents possibly valuable IP.
- They open the pathway, of which the gene is a part, to functional genomic or physiological research that may lead to non-genetic methods of manipulation and greater knowledge of sheep biology.

Outputs of type 2 are useful for marker assisted selection and could also be used as tools in functional studies. Outputs of type 3 are mainly used as a stepping stone towards outputs of type 1 or 2, but could also be used as a tool in functional studies. Therefore we recommend that

6. Recommendation C

The aim of the research is to find genes and polymorphisms causing variation in the target traits or markers very closely linked to these genes.

7. Research Strategy

The recommended strategy to identify QTL has three steps:

- Linkage analysis (LA) to map the QTL to a broad chromosome region,
- Linkage disequilibrium (LD) mapping to map the QTL more accurately, and
- Test positional candidate genes and specific polymorphisms within those genes.

To implement this strategy requires many decisions regarding the design of the experiments. These decisions have already been discussed in Milestone Report 2 and here we will concentrate on the recommended course of action with minimal justification for the decisions implicit in the recommendations.

Three broad strategic decisions affect the rest. They are the need to detect additional QTL, whether experiments are specific for one trait or multi-purpose, and the size of QTL that we aim to find.

Findingadditional QTL. Several part or whole genome scans have been conducted in sheep and so one might hope that they had detected many QTL and therefore there was no need to carry out a further genome scan. Unfortunately the review team has not had access to all the results of genome scans conducted to date so we cannot confirm this conclusion. It does appear that many QTL have been detected and that these could form the basis for future research. However, it is unlikely that all valuable QTL have been found due to the limited size of past experiments and the fact that some valuable traits have not been recorded. Our recommended course of action is based on identifying the QTL that have already been detected but, fortunately, the optimum design for this purpose is also very efficient for a genome scan and hence detecting additional QTL. Therefore we

recommend that AWI/MLA consider scanning for additional QTL, but acceptance or rejection of this recommendation does not affect the rest of the design.

7. Recommendation D

Consider using the half sib families to conduct a genome scan. If this was considered necessary for only a few traits (eg parasite resistance), it could be done very efficiently by using pooled, selective genotyping.

Multi-purpose flock. Resources will be used most efficiently if the same sheep can be used for many or all traits. This saves some costs in sheep breeding and rearing, in genotyping and phenotyping. Also it has the advantage that when a QTL is found we will have already measured the sheep for many traits and so can easily assess the pleiotropic effects of the QTL on other traits including profit.

However, some costs will be proportional to the number of QTL targetted and so will not be reduced by use of a multi-purpose flock. Some traits are incompatible and so cannot be measured on the same animals. Therefore a case could be made for a specific trait flock in some circumstances.

8. Recommendation E

As far as possible AWI/MLA should plan an integrated QTL flock designed to be used for all traits. However this does not mean that all sheep will be measured for all traits as discussed below.

Size of effect of QTL targeted. In a typical trait in a typical population, about 50% of the genetic variance is due to genes with a gene substitution effect between 0.3 and 0.7 phenotypic standard deviations (s). Genes of much larger effect do occur but they are rare. (Genes with smaller effect are harder to detect, need very large families and are less beneficial in MAS). Therefore, we need experiments which can find genes of this size, otherwise we risk finding nothing or only genes with a rare detrimental allele of large effect that will be of no practical use.

9. Recommendation F

Design experiments to find QTL with a gene substitution effect of > 0.3 standard deviations.

Steps in the research plan

1. *Select 6 chromosomes that have been found to carry QTL in past experiments.*
   Criteria for selection are:
   - High significance level
   - Found in multiple families
   - Low false discovery rate
   - Found in multiple experiments
   - Large effect
   - Traits that are economically important and
   - Traits that are difficult to select for at present (this is not essential)
   - Segregating in commercial sheep

A QTL does not need to meet all these criteria to be selected. We anticipate the 6 chromosomes might contain say 9 QTL worth pursuing. The choice of 6 chromosomes is a compromise based on the cost of identifying one QTL and the resources available. The cost to identify one QTL is hard to predict because it depends on luck when choosing candidate genes and polymorphisms, but it might be $1m. As the research progresses some QTL may be dropped and/or others added to the list as further evidence on each QTL accumulates.

2. *Verify the chosen QTL on these 6 chromosomes and re-estimate their effects.*
   All past experiments have significant false discovery rates (20 –100%) and a tendency to grossly overestimate the effects that appear the largest, so this is necessary to avoid wasting resources on a false positive or a QTL whose effect is so small that it is impossible to map accurately. The best way to verify chosen QTL is to produce more progeny from the sire(s) that are thought to be heterozygous for the QTL. This strategy also allows more accurate mapping of the QTL through linkage analysis.

3. *Determine if these QTL segregate in commercial sheep.*
   This can be done in two ways. The best method is to find commercial rams that are heterozygous for the QTL based on mapping the QTL in their offspring. Additional evidence can come from LD between markers and the QTL in maternal chromosomes of the experimental offspring.

4. *Map the QTL to < 5cM.*
   This is a challenging objective because often genome scans lead to 95% confidence intervals for the QTL that cover the entire chromosome. However it is necessary because otherwise the number of potential candidate genes is too large. Use of combined LA and LD leads to the most accurate position of the QTL and also minimises the occurrence of false positives that can occur with both LA and LD mapping. In the recommended operation plan (below) a strategy is put forward to achieve this precision with minimum genotyping cost.

5. *Identify the QTL and the mutation causing the phenotypic effect.*
   In this step, LD mapping and testing candidate genes merge into each other. Both require us to find multiple polymorphisms within the confidence interval of the QTL. The choice of candidate genes is based on knowledge of the physiology of the trait and, if possible, the physiology of this QTL (this implies integration between the mapping and function research on the trait). However, it is important to test many polymorphisms, because otherwise it is impossible to be sure whether a polymorphism is the correct mutation or even in the correct gene. Even with good functional information, one may not find the correct polymorphism or even the correct gene. Then we are reliant on LD mapping to get closer to the QTL. For LD mapping we do not yet know how dense markers must be. A measure of LD, r2, equals 1/(4Nc + 1). If effective population size (N) in sheep is 10000, then markers 0.1 cM apart (c=0.001), yield r2 = 1/41. This implies the need to test many SNPs or other markers. Fortunately, we can do this on a very small number of sheep provided we can identify sheep whose QTL genotype is known with reasonable certainty from the LA-LD mapping. This is most likely for sires, so the strategy is most effective when enough sires have been tested to find at least 4 heterozygotes. However, dam haplotypes that are consistently associated with either negative or positive effects on the trait can also be used.

6. *Confirm the polymorphism discovered in step 5, re-estimate the effect of the gene and estimate its effect on other important traits and hence on profit.*
   This can be done by testing the putative polymorphism across a large population that have good phenotypic records. This could be the mapping population used for steps 1-

5, but it could also be another well defined population such as a collection of rams evaluated in central progeny tests and with Lambplan EBVs. Accurate EBVs provide a more accurate estimate of the QTL effect than the same number of phenotypic records. However the pedigree structure of such a collection of rams is unlikely to be useful for linkage analysis so it cannot replace the mapping population.

Comment [j1]: The term 'well defined' might need to be explained. It seems to me that we rely here less on pedigree structure, animals should be very unrelated.

Operational Plan

1. *Select 4 sires that are heterozygous for QTL on the 6 chosen chromosomes from existing experiments. (eg one each from Awassi cross, Romney x Merino, Golden ram flock and meat sheep experiment). Produce an additional 400 lambs each from these sires.*

   The progeny will be used to verify the existence of the QTL, re-estimate their effects and map them more accurately.

2. *Select 15 industry sires and breed 200 offspring each.*

   This will test whether some QTL are segregating in commercial sheep and increase the precision of mapping the QTL. If some of these sires are found to be heterozygous for the QTL, they can be used for testing candidate SNPs as described in steps 10 and 18.These sires could be selected based on:

   - Evidence from phenotypic databases that they are heterozygous for a QTL of large effect
   - Industry relevance
   - Availability of semen
   - Existing offspring available

3. *The ewes to which these 19 rams are mated could be:*
   - *Ewes from within the existing gene mapping flocks*
   - *Purebred ewes on commercial properties or research stations*
   - *Merino x Border Leicester ewes (especially if a DNA sample from the Border sire is available, this would add information from the segregation of merino vs border alleles).*

The chromosomes from the ewes are principally used for LD mapping. However where they can be traced to a small number of founders (eg the Awassi cross flock or the Romney x merino flock), they can also contribute to the linkage mapping. However, for fine mapping based on LD, it is desirable that most of the ewes are as unrelated as possible, and from a number of different subpopulations.

4. *Record a standard, minimal set of phenotypes on all 4600 lambs and genotype them for 30 microsatellite markers covering the 6 selected chromosomes.*

   Linkage analysis will verify the QTL, provide an unbiased estimate of their effect, and determine whether they are segregating in commercial sheep. Depending on the number of segregating families, the 95% CI for the QTL will be about 30-60 cM. It may not be possible to measure even a minimal set of traits on all sheep, but this should be the aim.

5. *Map the QTL to within about 10 cM. For families that are heterozygous for a QTL, select the offspring that have recombinations in the sire chromosomes within the 95%*

confidence interval (CI) of the QTL and that are extreme in phenotype, and genotype them for an additional 5-10 markers covering the confidence interval.

This is a very cheap way to reduce the confidence interval for each QTL to about the minimum possible for a given number of sheep in segregating families (N) and size of effect (a). For instance, if out of the 4600 lambs, 1200 are in families that segregate for a QTL with effect 0.5 standard deviations, the minimum 95% CI that can be achieved is $3000/(N a^2)$ = 10 cM. Using this strategy, this will be achieved by genotyping approximately ¼ (extreme phenotypes) * 1200 sheep * 1/3 (recombinants) = 100 sheep.

6. *Define the phenotypic effect of the QTL by measuring more expensive phenotypes on a sample of lambs in segregating families, where it is known, from the markers, which sire chromosome the lamb inherited.* Among the offspring of a heterozygous sire, there will be some that have unambiguously inherited the positive and others the negative sire allele. These lambs can be compared for more detailed phenotypes, to integrate the mapping and functional research. This should help to define the function of the QTL and thus guide the choice of candidate genes and suggest hypotheses to be tested in further functional research. It may also increase the power of later stages of mapping by indicating phenotypes on which the effect of the QTL is largest. The number of lambs sampled can depend on the cost of the measurement and it can be done in stages ie a small sample first and, if results are promising, a larger sample.

7. *Reduce the confidence interval for the QTL to < 5 cM by LA-LD mapping.*

The above steps are all based on linkage analysis. Now utilise linkage disequilibrium. Phenotype additional lambs for the traits identified in step 6 as highly informative. Genotype some or all progeny for 10 markers covering the CI of each QTL that is still being pursued. To reduce costs this genotyping can be restricted to phenotypically extreme offspring but, if several QTL are being pursued, this leads to nearly all lambs being genotyped. It can also be restricted to lambs with the appropriate phenotypes measured if this is not all lambs. Analysis of this data will reduce the CI for each QTL to <5 cM. This will also show the dam populations in which the QTL is segregating and hence are useful for further experimentation and implementation of marker assisted selection. The assumption here is that the QTL does segregate in the dam population.

8. *Select candidate genes in the correct region, find and test polymorphisms in these candidates, using sheep of known QTL genotype.*

Use the human and bovine maps to identify all genes within the CI. Select candidate genes and find polymorphisms in these genes. It is desirable to test many genes and many polymorphisms per gene because it is unlikely that one will find the causative mutation and this stage. That is, we are still using LD. A great saving in cost is possible by genotyping only animals whose QTL genotype is known with high probability ie known heterozygous sires and dam haplotypes that have been found to have a large effect. This reduces the problem from mapping a QTL to mapping a mendellian gene. Only a few animals need to be typed for multiple SNPs. From this we detect SNPs that are in strong LD with the QTL.

9. *Type these SNPs across a wide number of offspring to confirm that they are in strong LD with the QTL.*

Phenotypically extreme animals can be used in the first instance to reduce genotyping costs.

10. *Refine the CI for the QTL and find additional SNPs especially those that appear to be functional and identify the causative mutation.*

11. *Genotype additional sheep for the putative, causative mutation and estimate the effect of the 3 genotypes and interactions between QTL or between QTL and the environment on the principle trait and on all recorded traits and hence on profit.*

Polymorphic markers to support this strategy

The strategy requires that dense markers can be found in any part of the sheep genome in which they are required. Markers on the sheep map will not adequately meet this requirement. Additional markers can be microsatellites and SNPs. Microsatellites can be found from the homologous part of the bovine map since most bovine microsatellites work in sheep. New methods to find SNPs may become available in the future. At present the most efficient method is perhaps as follows:

> Find the homologous region of the human or bovine genome based on genes and markers mapped in both. Identify all genes in the homolous human or bovine region. Use bovine and human genomic sequence and ESTs to define intron- exon boundaries and hence design primers to amplify introns and exons. Sequence these introns and exons in a small number of sheep and detect SNPs. (See McEwan et al 2001 AAABG)

Having found ovine SNPs, we still require an efficient method for typing sheep but several are already available and more are likely in the near future.

Integration with functional genomics

An integrated research program will have benefits in both directions. The mapping research will benefit from functional knowledge of the trait which will inform the choice of candidate genes. The functional research may use animals that differ in QTL genotype(s) as a tool to discover physiological pathways affecting the phenotype. In some cases this integration would be enhanced by using the same sheep in both mapping and functional research. It is beyond the scope of this report to detail how this might be done and dependant on the details of individual traits, QTL and research programs.

10. Milestone Report 1

Review of Australian Sheep QTL mapping experiments

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Milestone 1

Review the sheep QTL experiments conducted in Australia, assess the strength of the evidence for each QTL and advise MLA on the QTL that are the highest priorities for future research aimed at identifying the QTL to meet MLA priorities.

Introduction

Five genome scans covering much of the genome have been carried out (Table 1). There is one about to start (Golden Ram experiment) and one scan covering only five selected chromosome regions is under way (UNE meat sheep). The traits covered in these experiments are mainly wool characteristics, growth and carcase traits and worm resistance which match the priorities of MLA (carcase traits and worm resistance) with the addition of the AWI priority (wool traits). We are not aware of any QTL mapping experiments covering fleece rot and fly strike (although suitable sheep no doubt exist if such an experiment was desired). This report does not cover genome scans in other countries or candidate gene experiments. However where these support the results from the Australian genome scans they will be covered in the final report.

Of the 7 experiments only those of Beh and Robinson have been completed and published (Beh) or had a final report submitted to AWI (Robinson). We did not have access to the results from the other experiments although Herman Raadsma provided a summary of results that did not indicate the chromosome on which QTL had been found.

Design of the 5 completed or near complete genome scans

The 5 experiments are summarized in table 1.

The VIAS experiment was designed to examine wool traits. It used 5 half sib families of approximately 130 offspring each. Three sires were crosses of Merino and Border Leicester and two are superfine Merino x strong wool Merino. No sheep or DNA remain.

One CSIRO experiment use F1 rams that were a cross of the resistant and susceptible Trichostrongulus selection lines. Results for worm resistance and wool traits were published by Beh et al. No animals or DNA remain.

The second CSIRO experiment was designed as a gene mapping resource and as a QTL mapping experiment focussing on wool and skin traits but also measuring worm resistance and ovulation rate. Two Merino x Romney sires were used to breed two half sib families which included some full-sib families. Results are incomplete, as the analysis is still being carried out, and were not made available to this review team because the research was funded by CSIRO and AWI. Some sheep and DNA exist and there is the capacity to generate additional animals in the same families.

University of Sydney Awassi x merino experiment concentrating on wool, milk and growth traits. Four F1 rams were used to breed half-sib families. One family contains 502 sheep while the other three contain about 150 each. Only the largest family has so far been genotyped. The analysis is still being carried out. Detailed results were not made available to the review team because the experiment was funded by University of Sydney and others (not MLA). Some sheep and DNA exist and there is the capacity to generate additional animals in the same families.

University of Sydney Javanese x Merino experiment concentrating on worm and fluke resistance. There are 4 families of about 100 and six of about 50 offspring, all from Javanese thin tail x Merino sires backcrossed to Merino ewes. About 50 offspring in each of 7 families have been genotyped. The analysis is incomplete and detailed results were not made available to the review team because the experiment was funded by University of Sydney and others (not MLA). The animals are in Indonesia which limits their use for on-going experimentation such as identifying the genes responsible for the QTL.

Criteria for assessing strength of evidence for QTL

A large number of significance tests is typically carried out in a genome scan (number of markers x number of families x number of traits). Consequently, one expects some of these to reach significance just by chance. Three approaches can be taken to address this problem:

- Adjust the significance level to allow for the multiple testing. To achieve a probability of $p<0.05$ that any QTL will be found in the whole genome when none exists (ie a genome wide significance level of 0.05) typically requires a single-family LOD score of about 3.0 which is a equivalent to a single test with $p< 0.0005$. This is a very stringent criterion and its use will result in many real QTL being declared non-significant. An alternative criterion is based on a chromosome wide significance test. A LOD score of 2.0 corresponds approximately to a point wise $p< 0.002$ and a chromosome wide $p<0.01$. This means that if significance tests on 100 chromosomes that contained no QTL were carried out, one would expect 1 false positive. However genome wide and chromosome wide significance tests still do not correct for the number of traits tested, so that, when all traits are considered, the p-value is still greater than 0.05.
- Calculate the proportion of the reported QTL that are likely to be false positives. This is called the false discovery rate (FDR). If 20 QTL are reported where only 4 would be expected by chance, the FDR equals 20%. That is, we expect that 80% of the reported QTL are real.

The experiments reported do not adopt a consistent approach to testing significance but we have tried to calculate the significance and FDR from the information available.

- Evidence for the same QTL in independent studies also increases the likelihood that they are real. Unfortunately we did not have access to the chromosomal position of reported QTL in all studies considered here.

Criteria for assessing priorities for future research

We assume that the aim of future research by MLA and AWI would be to identify the genes underlying the mapped QTL so that they can be used in practical breeding programs and in other research. As well as the strength of statistical evidence for the QTL, the other criteria we have used are:

- Importance of the traits
- Nature of the sheep in which the QTL was found and
- the opportunity that exists to carry out experiments to identify the gene responsible.

Logically the size of the effect would also be a criterion but the effects reported are likely to be grossly overestimated, making this an unsatisfactory criterion.

Wool traits

Robinson and Carrick (1998) report 20 chromosome-trait combinations with LOD scores above 2.0 (point wise $p < 0.002$ or chromosome wide $p<0.01$) affecting wool traits such as fibre diameter, greasy fleece weight, standard deviation of fibre diameter over the body. Some QTL exceeded a LOD score of 3.0 and so reached genome wide significance. However at least 9 traits were considered over 5 families, so one might expect 9 traits*5 families*26 chromosomes = 1170 * 0.01 = 12 significant results by chance alone, so the FDR = about 60%. In one case two families are both significant for a QTL on chromosome 19 affecting CV of fibre diameter. The combined LOD of 4.57 is very highly significant even when allowance is made for the second degree of freedom. They tested some of the chromosome regions in small families of fine wool merinos bred by CSIRO and confirmed QTL for fibre diameter on chromosome 3 and standard deviation of fibre diameter on chromosome 5.

Beh et al (2001) report 18 QTL with LOD scores above 2.0 affecting 11 traits such as clean fleece weight, yield, resistance to compression. No LOD reached 3.0. They calculate the FDR to be 0.65.

Herman Raadsma presented preliminary analyses (without chromosome location) of the Awassi x Merino experiment. In the one large family that has been genotyped, for 9 wool traits, there were 17 chromosome –trait combinations with LOD > 2.0 (chromosome wide $p<0.01$) compared with 26*9*0.01 = 2.3 expected by chance. Therefore the FDR = 2.3/17 = 13%. In fact some QTL are very highly significant (LOD>4.5) and probably represent genes with large effect as was hoped by crossing a hair sheep to Merino. When more complete results are available, a decision will have to be made concerning if and how to pursue these QTL. If the aim was only to identify the gene because it is scientifically interesting, it would be best to perform further experiments in the Awassi crossbreds. However it is unlikely that wool genes from Awassi are useful in Merinos. Therefore, if the aim is to find alleles that are beneficial in Merinos, the next step would be to see if there is variation in these chromosome regions in Merinos.

There is at best weak agreement between the results of Beh et al and Robinson and Carrick. For instance, both studies found QTL affecting either fibre diameter or its standard deviation on chromosomes 6 , 12 and 19.

Neither of the experiments of Beh or Robinson had sufficient power to find QTL of the size that might explain typical variation in quantitative traits. It is disappointing that no major genes were found but not surprising given that a similar NZ experiment (Henry et al 1998) also failed to find major genes. Because each experiment lacks power, it is not surprising that that did not confirm each others results. In fact, the evidence for QTL is reasonable. Robinson and Carrick found some QTL with genome wide significance and provided evidence that some QTL detected in their cross were segregating in CSIRO fine wool Merinos. Several QTL have sufficient support to justify further experiments. However it would seem premature to make decisions on future experiments when the CSIRO and University of Sydney experiments will hopefully present results within 6 months.

Body composition traits

There are no published results for these traits. Herman Raadsma presented preliminary analyses (without chromosome location) from one large family of Awassi crosses. 27 QTL from 30 traits had a chromosome wide p< 0.01 compared with 30*26*0.01 = 8 expected by chance. Therefore the FDR = 8/27 =30%. The significant effects included the amount of carcase bone, fat and lean and internal fat. The effects are not as large or as significant as the effects on wool traits in the Awassi crosses. Still there is one chromosome region with a genome wide significant effect on fatness and hence on proportion lean.

Priorities for future research depend on whether the aim is to identify the gene without directly using it in selection of commercial sheep, or whether the aim is to use the gene in selection programs. In the former case one might use the Awassi crosses and in the latter case commercial meat sheep. This decision might be aided if the UNE meat sheep experiment happened to confirm any of the QTL found in the Awassi. Unfortunately this is unlikely since the choice of chromosomes to genotype at UNE was not based on the Awassi results.

Worm resistance

Beh et al found 6 QTL for 2 traits that were significant at p<0.05 point-wise (about p<0.125 chromosome wide) when 2*26*0.125 = 6 would be expected by chance. Therefore the FDR was 100%. This is a very disappointing result from what appears to have been a well designed experiment. It is possible that there were technical problems not apparent from the publication, so it would be premature to give up on finding QTL for worm resistance. More results are expected from the Javanese cross experiment and perhaps from the CSIRO gene mapping flock. Since the Javanese cross sheep are in Indonesia, the ability to use them in experiments to identify the genes responsible is limited.

Power of the experiments already conducted

Hayes and Goddard (2001), using data from dairy cattle and pigs, predict that the distribution of gene substitution effects, for an average quantitative trait, is approximately an exponential distribution with a mean of 0.1 phenotypic standard deviations. This distribution implies that only 5 of the 100 segregating QTL, for a typical trait, have a bigger effect than 0.3 standard deviations but these QTL explain 43% of the genetic variance. A single family of 291 offspring has a 50% power to find a QTL of size 0.3 standard deviation as significant (p<0.01 point-wise) if the sire is heterozygous for that QTL. Thus, with the benefit of hindsight, only the large family in the Awassi cross experiment had the power to find QTL of ‘typical’ size. Not surprisingly, it is this family that appears to have been most successful, based on the limited information available to date. Of course QTL of larger size do exist as shown by the booroola gene and others. However it appears they are rare. There is no guarantee that a large QTL is segregating in a particular population or that any of say 10 sires used in an experiment will be heterozygous for that QTL. Use of F1 crossbred sires may not greatly increase the chances of finding QTL of large effect. Only the Awassi cross for wool traits appears obviously to have found QTL of large effect and the difference between merino and Awassi sheep in wool is very marked.

Priorities for future research

Milestone 2 of this consultancy, to be completed by 30/3/2003, included advise to MLA on the flocks necessary to identify mapped QTL and the flocks necessary to map additional QTL. We understood that the intention was to use this report when planning matings to be made in autumn 2003. In formulating these milestones it was assumed that the five QTL mapping experiments were complete and their results were available to the review team to use in writing the first milestone report. This has proved not to be the case. The results of three of the experiments are incomplete and are not available to the review team.

Given these facts we anticipate that it will be difficult to complete the review in a manner that would lead to confident recommendations for QTL to be pursued and hence mating to be made in Autumn 2003. In some respects it would be better to delay part of the review until July 2003 when experiments at Sydney, CSIRO and UNE will be more complete and agreement has been reached to disclose all results.

However it must be realised that CSIRO and Sydney are planning matings for 2003 according to their own assessment of the priorities for future research. Thus if MLA delay decisions until after June 2003 they will delay their plans by 1 year and may find that some irrevocable decisions have already been made.

Our recommendations are

- to proceed with the current review and assess the current information as it is available to us
- to hold back part of the budget and corresponding time commitment until July 2003, so that the review can be updated with the benefit of additional data
- during the next 6 months, for MLA to negotiate with the owners of this data for access to it
- to convene a meeting of interested parties before 2003 mating plans are finalised to discuss them with an aim to make useful decisions but to hold some resources uncommitted until 2004 matings when more complete experimental plans will have been drawn up.

To complete milestone 2, the review team need guidance from the MLA and AWI on the outcome sought. If the aim is to identify a gene so that the physiological mechanism can be investigated, then experiments using crosses, such as Awassi, are satisfactory. However, if the aim is to use the beneficial allele in commercial sheep, then Awassi crosses may be unacceptable to the sheep industry.

Conclusion

There is strong support for QTL affecting wool traits especially in Awassi crossbreds but even in merinos. There is good evidence for body composition QTL in Awassi crossbreds. There is not convincing evidence for QTL for worm resistance.

Future experiments to identify the genes for these QTL will have to consider the sheep to be used (wide crosses vs. commercial sires) in light of the aim of the research.

Future experiments should aim for greater power than those already carried out, mainly by increasing the size of the half sib families used.

It would be desirable to delay 6 months before planning the next phase of research so that the results of the currently incomplete experiments can be considered. By June 2003 the University of Sydney and CSIRO data will be analysed and hopefully an agreement can be negotiated to make the results available to MLA and AWI. Also the UNE meat sheep experiment may be finished but the Golden ram experiment is not due for completion until October 2003.

Table 1 Genome scans in Australian sheep

Institution	Project leader	Genotype of sires	No. of sires	No. of sheep/sire	Traits wool	carcase	worms	other
CSIRO	Franklin	Romney x Merino	2	190	++	+/-	+	fertility
CSIRO	Beh	Merino (high x low Trich lines)	6	170*	+		++	
VIAS	Robinson	Merino and Merino x BL	5	120	++			
Uni of Sydney	Raadsma	Awassi x Merino	1 - 4	144-502	+	++		milk
Uni of Sydney	Raadsma	Javanese x Merino	10	50-100	+	+	++	fluke
UNE	VanDer Werf	Merino (Golden ram)	4	180	+	+	++	
UNE	vanDerWerf	Meat sires	12	100		++		eating quality

* number of genotyped sheep for wool traits was only about 70 per sire.

11. Milestone 2 Report to MLA and AWI

Sheep Resources Needed for QTL Mapping

Mike Goddard, John Henshall and Julius Van der Werf

Milestone 2

Advise MLA and AWI on the flocks that would be necessary to identify QTL that have already been mapped and to find additional QTL.

Introduction

The first milestone in this project was to review the QTL mapping experiments carried out in Australia. In our report on this milestone we noted that much of the data was not available to us because the experiments had not been fully analysed and because agreement from the owners to release their data was not always available. We recommended that the completion of this milestone be delayed until after July 2003. We assume this recommendation has been accepted by MLA and AWI. Consequently it is impossible to complete milestone 2 in detail. For instance, we cannot recommend the resources necessary to identify a particular QTL because we have not been able to assess which QTL should be pursued. However we believe it is helpful to set out the flock resources that will be necessary regardless of the specific QTL that are targetted, so that this can be taken into account in 2003 matings and so that sufficient time is available to negotiate and plan a complete research program to be implemented in 2004.

Aims of MLA and AWI in future QTL research

MLA and AWI plan to invest in this field as part of their genomics program. The traits that they have decided are top priority are resistance to internal parasites, muscle growth and wool growth. For meat sheep I believe fertility traits including lamb survival should be added to the list.

The objectives of MLA and AWI for the next phase of QTL research is Australia are:

1. To identify some of the genes that have been mapped as QTL. Identifying the gene and the mutation that causes phenotypic variation would be the best outcome because:
   - It would make marker assisted selection for the gene most effective. However other markers that were in complete linkage disequilibrium with the functional mutation would be almost as good in practice.
   - It represents valuable IP.
   - It opens the pathway, of which the gene is a part, to physiological research that may lead to non-genetic methods of manipulation.

2. To verify the existence of these QTL and to determine if they segregate in commercial Australian sheep. This is largely a precursor to (1).

3. To find additional QTL for valuable traits. Although many QTL have been detected in current experiments, there are likely to be highly valuable QTL that have not yet been mapped.

Choice of QTL to target

Many QTL have been detected in existing experiments. The criteria by which we would select those we will attempt to identify are:

- High probability that QTL is real,
- QTL of large effect,
- QTL affects economically valuable traits, that are difficult to select for at present
- QTL is segregating in commercial sheep.

A discussion of the relative importance of these criteria and their effect on the design of future research follows.

Evidence that the QTL is real comes from the significance test but, because so many tests are made, some significant QTL will turn out to be false positives and others will have a much smaller effect than originally estimated. Corroboration between experiments will help but in practice it is often difficult to be certain that two QTL reported in different experiments are in fact the same. Therefore it may be desirable to confirm the existance and size of QTL before investing too much money in attempting to identify them. This could be done by taking additional offspring from the sire where the QTL was detected or by testing sires from the commercial population. If we find nothing in the commercial sires it doesn't prove that the original finding was faulty. However if we do find the QTL in the commercial sires then we have shown that it segregates in commercial sheep.

Genes with large effects on economic traits do exist but they are rare and their discovery unpredictable. For instance, the Booroola gene has a gene substitution effect of about 2 σ. More typically, the largest 5-10 genes, that explain 50% of the genetic variation in the trait, have an effect = 0.3 to 0.7 σ.
An important strategic question is :
"Should we target only genes with very large effects or should we target the genes that explain the normal genetic variation that occurs in sheep and which are the material on which conventional selection operates?" We would always prefer to find genes with a very large effect, like the Booroola gene, but should we design our experiments so that more typical genes of more modest effect cannot be detected or identified?

The answer to this question depends in part on whether you expect a benefit from this research to be more effective selection of sheep. If this is the case, the benefits are roughly proportional to the amount of genetic variance in the breeding objective explained by the identified or mapped genes. This implies that we must aim to detect genes that explain a significant fraction of the genetic variance in important traits and typically this means several genes with effects in the range 0.3 to 0.7 σ. On the other hand, if one expects most of the benefit from genomics research to come from other means (eg transgenic sheep, new vaccines or drugs), then it becomes unimportant to explain a significant fraction of normal genetic variance. Our view is that these latter benefits are very high risk and long term whereas better methods to select sheep may be a more achievable benefit in the medium term.

Regardless of the answer to the above question, we should target QTL of large effect if we detect them because they are more valuable and more amenable to research. Unfortunately, as explained below, genes that appear to be of large effect are likely to be overestimated, and so we would need to verify the large effect in an independent experiment before being confident of it.

As suggested in the previous paragraph, the size of the effect of the QTL is important in two different ways. A large effect is more amenable to research, including identifying the gene and, for this purpose, it doesn't matter on what trait the effect is large. Thus a useful research strategy is to find a trait, that is not routinely measured, but on which the QTL has a direct and large effect. However, for a QTL to be valuable for sheep selection it must have a large effect on profit. This means we will have to estimate the gene's effect on a range of traits that are economically important. Even genes with large effects on some traits may not have a large effect on profit. Consequently, at some stage of the research, we will inevitably be working with genes that have at most a modest effect on our primary trait ie profit.

Genes for traits that are easy to select for by existing means, such as fleece weight, give less benefit in a selection program than genes for traits that are difficult to select for such as ewe fertility. This is not important if the benefits from the research are not expected to come from better sheep selection methods. Even if the benefits do come from better sheep selection, knowledge of genes for, say, fleece weight, could be valuable in the future. A major increase in rate of genetic gain could come from combining DNA based selection with techniques for early reproduction such as JIVET.

We will assume that the economic value of the trait is always an important criterion for deciding which QTL to target.

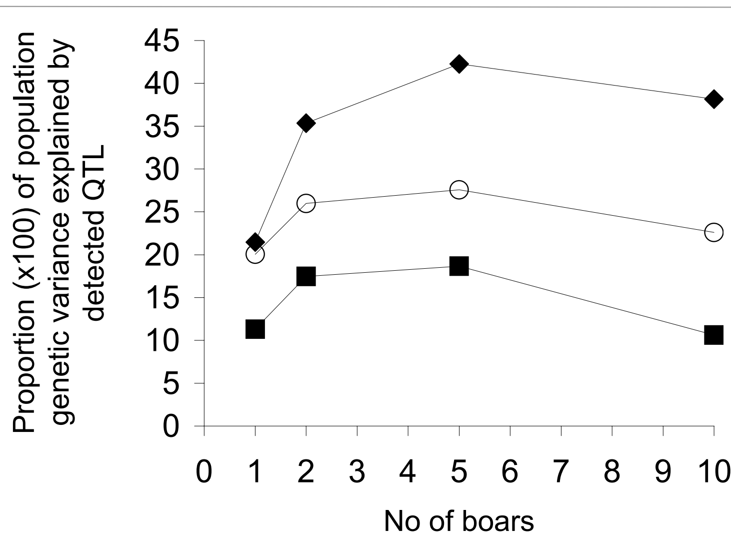
Segregation in commercial sheep. Genes that are discovered in an experimental cross, but are not segregating in commercial sheep, present two disadvantages. Firstly, they would be difficult to use for selection of superior sheep. If a favourable allele existed in an experimental population it could be introgressed into commercial sheep, although this is a long and costly program. If the commercial sheep already contained the favourable allele, there is no benefit from this particular polymorphism. However, it is possible that other polymorphisms in the same gene do exist in commercial sheep. Secondly, it would be difficult to isolate the gene responsible. In an experimental population, such as a wide cross, there are inevitably only a few founder animals. This means that their descendants will inherit one of only a few chromosome segments that existed in the founders and consequently all genes in the segment will be inherited together, making it impossible to say which gene is responsible for the phenotypic effect.

In several of the criteria discussed above, the method by which we expect the industry to derive benefits from genomic research, affects our choice of targets. If we think that better methods of sheep selection are an important outcome from the research, this implies that we design experiments that can detect and identify genes of more modest effect (0.3 to 0.7 σ) , for traits that are economically important but difficult to improve at present, and give emphasis to genes segregating in commercial sheep.

Research strategy

The recommended strategy to identify QTL has three steps:

- Linkage analysis (LA) to map the QTL to a broad chromosome region,



- Linkage disequilibrium (LD) mapping to map the QTL more accurately, and
- Test positional candidate genes and specific polymorphisms within those genes.

Linkage analysis (LA) for QTL requires very large experiments. An experiment with 20 half sib families of 225 offspring each (ie 4500 sheep in total) has 95% power to detect QTL with a gene substitution effect of 0.5 σ (phenotypic standard deviations) but only 50% power to detect QTL with an effect of 0.3 σ (Bouvenhuis and Schrooten 2003). The genes above 0.5σ explain approximately 20% of the genetic variance in a typical quantitative trait, while those above 0.3 σ explain approximately 50% (Hayes and Goddard 2001). The main determinant of the power of the experiment is the total number of offspring (see figure 1 for the benefit of 2000 over 1000). However for a fixed total number of offspring, the power is greater with a small number of large half-sib families eg 5 families of 900 each, even though this increases the risk that a rare QTL allele will not be detected, because it is not present in any of the 5 sires chosen. (Figure 1). However as the total number of offspring increases, the loss from using more than 5 families decreases ie the curve becomes flatter, allowing more flexibility in choice of number of families. Note that if traits that are only expressed in ewes are important, the number of offspring born must be twice as large.

Figure 1 Optimum number of half sib families for LA of QTL

With large families it is possible to use selective genotyping, that is, only the sheep with the highest and lowest phenotype are genotyped. This can reduce the cost of genotyping to about 1/3 of the normal cost, but it is only possible if focus is on a single trait (as it might be when attempting to identify the gene responsible for a particular QTL).

LA does not have to use half sib families. For instance, a complex pedigree can be used where we can trace the chromosome segments, by markers, back to a small number of founders eg in the Golden ram flock.

Even with large experiments, the accuracy with which a QTL can be mapped by LA is poor. With dense markers, the 95% confidence interval in cM for the position QTL is $3000/(N\ a^2)$, where a = the effect of the QTL in phenotypic standard deviation units, N= number of offspring from a heterozygous sire. For instance, if N=800 and a = 0.5σ, the 95% CI is 15 cM. It may not be necessary to genotype all 800 offspring for all markers to achieve this level of accuracy. For instance, one could genotype only the phenotypically

extreme animals that showed evidence for a recombination, based on 2 markers 30 cM apart. This might be only 0.25*0.3*800 = 60 sheep. This strategy of selective genotyping is better suited to designs with few, large families than many small families.

In small experiments, the QTL that appear to be the largest, tend to be overestimated. For instance, if a QTL is estimated to have an effect of 1.0 σ with standard error 0.25 σ, its most likely true effect is about 0.3 σ (Hayes and Goddard 2001), but could be larger or smaller. This standard error is that expected in an experiment with 64 offspring from a heterozygous sire.

How might we design future experiments to find QTL of large effect? We could look for evidence of large genes in phenotypic databases such as Lambplan. Then we would use rams that have a high probability of being a heterozygote in a QTL mapping experiment. Our opinion is that this method would have little power but might still be worthwhile if the cost is low. Alternatively we could make wide crosses between different breeds or selection lines. If two breeds have been isolated from each other for many generations and subject to different selection pressures, a mutation may have arisen in one breed and been driven to high frequency or even fixation by selection. An example may be the divergence of Awassi sheep from Merinos in genes controlling wool vs hair growth. However in most cases we believe the breeds or lines will not have diverged this greatly and consequently wide crosses will not necessarily lead to the discovery of genes of large effect. In support of this belief, we do not see a big decrease in genetic variance in selection lines or a big increase in genetic variance when breeds are crossed.

LD mapping and association studies of candidate genes are less sensitive to family structure provided a large number of alleles that are not closely related are sampled from the population. LD mapping also requires large total population sizes. In theory, less sheep are needed for a candidate gene experiment where only 2 alleles and 3 genotypes are compared. However, in practice when this stage is reached, many supplementary questions become important eg is the effect of the gene the same in all sexes and in all breeds? Our experience in beef cattle research is that 1000's of animals are necessary to be able to answer these practical questions before advocating the use of a DNA based test to select breeding stock.

LD mapping will require dense markers. We do not know yet the marker density that is needed because there has been little attempt to estimate LD in sheep. However 1 marker per cM is suggested. In many regions, there will not be enough markers on the sheep map to achieve this. However additional microsatellite markers can be found from the bovine map and SNPs can be discovered in genes from a particular region of the sheep map.

This density of markers makes LD mapping expensive if the position of the QTL is known only vaguely. Therefore, it is desirable to map the QTL as accurately as possible with LA before starting LD mapping.

LA and LD designs can be combined by using large half sib families. The paternal gametes are traced from sires to offspring for LA and the maternal gametes of the offspring form a sample from the population for LD or association studies. Simulation studies suggest that the power of the design for fine scale mapping of QTL is not very sensitive to the size of the families allowing some flexibility in the design. However, if a scan of whole chromosome(s) is to be performed with LA, then large families are still necessary.

For both LA and LD the QTL must be segregating in the gametes that are to be used eg sires for LA and dams for LD. If the gene shows dominance the recessive homozygote must be compared with the heterozygote.

From an operational point of view, testing positional candidate genes is the same as LD mapping. That is, in both cases one is looking for an association between the gene or marker and phenotype that occurs across the whole population. The unique feature of positional candidate genes is that there is a physiological reason why they might affect the trait. Therefore, an important part of the research is bioinformatic analysis of functional data that might lead to the choice of good candidates. Experience to date is that some QTL are obvious candidate genes and some are not. If a QTL is mapped to a broad region (eg 50 cM) as is usual, there are inevitably many possible candidates in such a large region.

To verify the existence of a QTL, it is important to use a powerful experiment. Otherwise there is a high risk of failing to confirm a real QTL.

To determine if a QTL is segregating in a new population we could use LA or LD or the gene itself depending on what is known. If the QTL has been mapped to only a broad chromosome region, it will be necessary to use LA. If the QTL has been mapped to about 20 cM, LD could be used. Obviously if the mutation causing the phenotypic effect is known, one can test directly for that. The best LA strategy depends on the frequency of the rare allele. At one extreme are cases like the 'Golden ram' gene for worm resistance. We are very keen to find this specific gene that has a large effect, and since the gene was found in a merino, it is probably segregating but at very low frequency. In this case we might need to use a large number of sires to detect one heterozygote. At the other extreme we might have 10 QTL that were detected in Awassi crossbreds and we would like to know if any of them are segregating in commercial sheep. In this case a smaller sample of sheep might be used because the gene may not be segregating at all or it may be segregating at intermediate gene frequency. If some of the 10 are present but at low frequency it is not disastrous if we fail to detect them.

12. Strategic decisions for the design of future experiments

Decision 1. Do we need any additional sheep?
It is possible that the sheep already bred in existing QTL mapping experiments are sufficient. We do not believe this is the case for a number of reasons. Existing experiments lack the total sheep numbers to accurately estimate the size and position of QTL. Also many use crosses such as Romney or Awassi x Merino which are not ideal for LD mapping and which don't tell us whether the QTL segregates in commercial sheep.

Decision 2. Do we need further linkage analysis?
We believe the answer is yes because we need to verify the QTL found to date, estimate their size and position more accurately, determine if they segregate in commercial sheep and probably find additional QTL.

Therefore we need to design experiments that can support LA , LD mapping and candidate gene studies. Fortunately the same design can suit all these requirements : it is

a design with a number of large half sib families. The key decisions then became the choice and number of sires and dams to be used to breed the new sheep.

Decision 3. Should we use sires that are 'known' to be heterozygous for a QTL or sires from the commercial population?
The advantage of sires that have been found to be heterozygous in existing experiments is that we can verify the existence of the QTL, and estimate its effect and position more accurately. The advantage of commercial sires is that we may discover if the QTL segregates in commercial sheep. If so the other advantages of estimating position and effect are also realised. We recommend a mixed strategy with use of both types of sire.

Decision 4 How many sheep are needed?
To obtain sufficient power to detect, verify and map the position and estimate the size of the QTL we recommend 4600 sheep. Although genes of large effect could be detected with a smaller number, this would not be enough for many purposes. Many QTL, that appear to have a large effect in the existing experiments, will have been overestimated and their effect will shrink when it is re-estimated. Without a powerful experiment we will be left wondering why the QTL vanished or worse still, invest resources to identify it when we lack to power to achieve this end. The effect of the QTL on all economic traits is important and requires a large number of animals. To commence LD mapping we need to have mapped the QTL to about a 20 cM or smaller interval. For a gene with true effect 0.5 σ, this requires 600 offspring from heterozygous sires. (note that this assumes multiple sires segregate for the same QTL given the family sizes recommended in decision 5).

Decision 5. How many sires and how many offspring per sire?
This must be a compromise. Very large numbers of offspring per family have many advantages. In theory data from multiple families can be combined but we can be far more confident that we are mapping only one QTL if we use data from a single family. If we combine data from 2 families that are segregating for different QTL on the same chromosome, we will get an incorrect estimate of the position of the QTL. Also large families have more potential for selective genotyping, genotyping only recombinant offspring, and progressive genotyping and phenotyping in which additional offspring are used if initial results are favourable. However, to determine if a QTL is segregating in commercial sheep, we would like to survey a larger number of sires. Therefore we recommend that 4 sires from past experiments be used to sire 400 additional lambs each and that 10 commercial sires be used to sire 200 lambs each.

Decision 6. Choice of dams.
The dams will contribute the bulk of the LD information, but can also contribute to the LA mapping. In either case the ewes must be segregating for the QTL. As with the sires, we can use dams from existing QTL mapping flocks or commercial ewes. If we use ewes in which chromosome segments can be traced, using markers, to a small number of founders, the ewes can contribute to the LA information. For instance, this could be the case in the Awassi cross ewes that all trace to one F1 sire or the Golden Ram flock ewes that all trace back to Goldie. By mating these ewes to rams carrying the same chromosome segments, we can generate homozygous offspring and these will add additional information. However, these ewes are not ideal for LD mapping because they are too closely related and therefore contain large segments of chromosome in common. Consequently, it may be impossible to tell which of several linked genes is the QTL.

Therefore the bulk of the ewes should be commercial ewes that are not closely related. It is an advantage to have more than one breed represented because the linkage phase will be different in different breeds and this will help isolate the gene that is the QTL. However, we need substantial numbers from each breed, because we must estimate LD between the QTL and markers within breed.

We recommend that ewes from existing QTL mapping flocks be used but that the bulk of the ewes be commercial ewes say Merinos, Poll Dorsets and Border Leister x Merinos.

Decision 7. Genotyping strategy
Not all lambs need to be genotyped for all markers. The details of the strategy must be worked out when we have chosen which QTL to target. However, a probable course of action might be as follows. Select 6 chromosomes that contain QTL that we wish to pursue. (These 6 chromosomes will probably contain more than 6 QTL allowing some to be dropped as we progress through the stages of verification, estimating effects and positions, fine scale mapping and choice of candidate genes). Genotype all lambs for sparse markers on these chromosomes (eg 4 markers per chromosome). Conduct the LA and determine which QTL are verified, segregating in sufficient families and of large enough effect. Genotype for extra markers (10 per chromsome) the extreme, recombinant offspring in segregating families to map the QTL by LA as closely as possible. Then genotype the extreme offspring in all families for 10 additional markers in the correct chromosome region and map the QTL by LD. Finally test position candidate genes from this region.

Decision 8. Phenotyping strategy
Again the details depend on the QTL that are targetted. We assume there will be a minimum list of traits for which all offspring are recorded. Additional traits can be recorded on families in which the QTL is segregating. Some traits will be recorded in commercial flocks without our intervention. However, these are likely to be less than even the minimum list of traits and , if the offspring were born some time ago, there will be little opportunity to collect additional phenotypes. Therefore we recommend that most of the offspring need to be on research stations or part of a deliberate on-farm experiment where ability to collect necessary phenotypes is guaranteed.

Decision 9. Should we have a common flock used for identifying several QTL or separate flocks for different purposes?
If the total resource is split into several experiment specific flocks we fear that none of them will be large enough. Also, we anticipate that over time a large part of the chromosomes will be genotyped in any given experiment. Therefore there is a major cost saving if a common resource can be used.

Decision 10. Can existing commercial sheep to be used?
Use of existing large commercial half sib families via Lambplan has the advantage that the breeding is already done, saving time and money. However, in the long term the genotyping costs are greater than the breeding costs. Also we may be limited in the traits we can measure on commercial sheep.

Sheep that might be used

The mapping experiments from which sheep are still available are
1. The CSIRO merino x Romney mapping flocks
2. The Awassi x flock

3. The current MLA meat sheep experiment
4. The Golden ram flock

In addition there are a large number of sires in Lambplan with >400 offspring and link sires in central progeny testing flocks with a large number of offspring.

13. Recommendations

These recommendations can only be preliminary, because once we decide which QTL are to be targeted, the detailed planning will have to be carried out. However, in the meantime decisions are still being made and so it is desirable to have framework for future planning. Based on the key decisions made above, we propose the following:

Breed a common resource that is used for QTL mapping for all traits. This resource will consist of 4 families of 400 each from sires shown to be heterozygous for QTL in existing experiments plus 15 families of industry sires of 200 offspring each. The ewes used to produce these families will include ewes from some existing mapping flocks plus commercial ewes. Most of the sheep will be run on research stations but some could be in commercial flocks or central progeny tests provided it is possible to collect relevant phenotypes. The sheep will be located at a range of sites representing the organisations that take part in the program.

This recommendation implies a substantial cost for producing the sheep, phenotyping and especially geneotyping them. However, if identifying genes at which naturally occurring variation affects traits of economic importance is a major aim of the sheep genomics program, then the approach recommended here is the best way to achieve this aim, and consequently we should be prepared to spend a large part of the sheep genomics budget on it.

14. References

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