

Searching for genes for parasite resistance in sheep

Julius van der Werf, School of Rural Science and Natural Resources, UNE

Breeding of livestock will be increasingly influenced by information generated from new molecular technologies. With the advent of such technologies, DNA tests can be carried out, giving the exact genotype of different individuals. Such tests can be useful to determine parentage and in selection of superior genotypes. Genetic tests are likely going to be a part of the selection tools available to breeders. At UNE, projects are underway that search for genetic markers for economically important traits. One of those aims at finding genetic markers for parasite resistance in sheep. The project aims at finding the location of a putative major gene that has been suspected in the UNE's "Golden Ram" flock since 1984.

The Golden Ram flock

Already for many years, "the Golden Ram" has dominated the parasite resistance work at UNE. The key paper that started most of the excitement appeared in 1987. Under the supervision of Prof. Stuart Barker, a group of researchers tested progeny groups of 60 Merino sires for worm resistance. They challenged the progeny with larvae from the most common gastro-intestinal parasite in New England, *Haemonchus contortus*. It appeared that the progeny mean from one ram was extremely far below the average for faecal egg count (see Albers *et al.*, 1987). Figure 1 shows that the progeny of this particular ram had a much lower egg count and showed therefore an extremely high level of resistance. For obvious reasons, the ram became known as the "Golden Ram", or more affectionately just "Goldie".

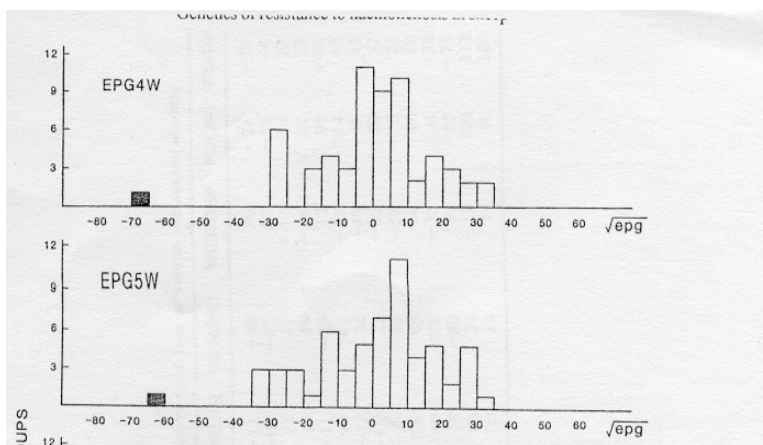


Figure 1: Frequency of progeny group mean deviations for faecal eggs per gram (square root transformation). The mean of The Golden ram progeny are shaded at the right (Albers *et al.*, 1987)

Not much is known about the origin of Goldie. He was part of a group of sheep that had been randomly sampled from a number of commercial sheep properties from different

parts of Australia, and was originally used by the animal physiology group. The exciting discovery of this super ram imparting such great resistance to intestinal parasites to its progeny was the start of a number of follow up projects at UNE. The main driver of this work was Douglas Gray. After 1997 this was taken over by Steve Walkden-Brown, who, together with Lewis Kahn and Jim Lea, has kept the Golden Ram project alive. The superior worm resistance of sheep related to Goldie was repeatedly demonstrated, and therefore there was overwhelming evidence that there was a genetic base for this resistance. A major gene was suspected, but could never be demonstrated convincingly. A second important paper was published in 1999. Bruce Tier and John Hensall had developed new software at the Animal Genetics and Breeding Unit (AGBU). This software, which was named “Gene Detective”, was very powerful for carrying out a segregation analysis. Segregation analysis aims at finding evidence for segregation of a major gene based on phenotypic data. Some traits have such large genetic effects based on single major genes that from the pattern of inheritance its segregation can be clearly observed. For example, if some sires have progeny that can be clearly distributed in ‘good ones’ and ‘bad ones’, than there is an indication of segregation of a major gene. Susan Meszaros and Sue Burgess looked into the history of the Golden Ram flock and retrieved data on several generations from hundreds of animals having been tested for parasite resistance (based on FEC). Such data is very suitable for segregation analysis, and indeed, a significant major gene effect was demonstrated (Meszaros et al., 1999). Animals having two copies of the desirable allele brought about by ‘Goldie’ were more than 50% lower in FEC than animals having none of these alleles. This was the start of a new project that would aim to find the location of this major gene. The advent of DNA markers technology provided an obvious route to track down the location of this major gene. In other words, DNA markers can be used to map the gene.

New DNA technologies

Recent developments in genetic research have led to the detection of genetic markers. Genetic markers are pieces of DNA that can be identified relatively easily in individual animals through a “DNA-test”. The genetic variation that exists can be demonstrated by cutting or amplifying the DNA at particular sites and letting the variation show up from fragments of different sizes. These fragments are sorted by electrophoresis showing a typical pattern of different bands. Figure 2 illustrates such a pattern. The first column shows two bands for a certain sire, indicating that the sire has two different alleles at the site under investigation (it is heterozygous). If animal 2 is a progeny from the sire, we can see which of the two alleles it has received from its sire. Animal 3, which is also a progeny, has received the other allele. Animal 4 cannot be related to the sire, as it has two different alleles.

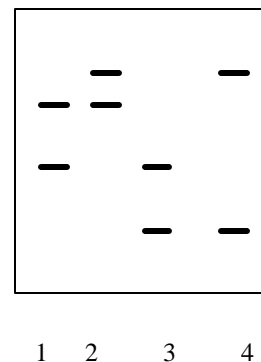


Figure 2: Example of a “DNA test”, an electrophoresis sorts different DNA fragments, here for 4 individuals

Such DNA tests can in principle be carried out for functional mutations in important genes, for example 'black wool' in sheep, red coat colour in cattle, double muscling mutation in cattle etc. However, genes are hard to find, and most DNA tests are for genetic markers that have no biological role themselves. Such genetic markers can still provide information, as they can be located close to a gene of interest. Although the exact location of the gene may not be known, the genetic markers do allow us to follow the Mendelian segregation at that gene locus. For example, if we know that the desirable allele in the sire is linked to the marker allele highest in the gel (Fig 2.) we know also that progeny no. 2 has most likely received that same allele, but progeny 3 has most likely *not* received it. In other words, genetic markers are useful in finding out which of the alleles were inherited by progeny from their parents. DNA markers are also used in parentage testing.

Using DNA markers to map major genes

The principle of finding a major gene for a quantitative trait such as parasite resistance is illustrated in Figure 3. Suppose a ram has two different alleles for a DNA marker, say the alleles are 'blue' and 'yellow'. If the progeny of a ram with a 'blue' marker have persistently more resistance (lower FEC counts) than progeny with a 'yellow' genetic marker, then there is an indication that this particular marker is located on the same chromosome and close to an important gene for parasite resistance. This gives then the approximate location of the major gene as we know the positions of markers, and such locations are termed "quantitative trait loci" (QTL). Having found an approximate location is already useful because nearby genetic markers can help determine

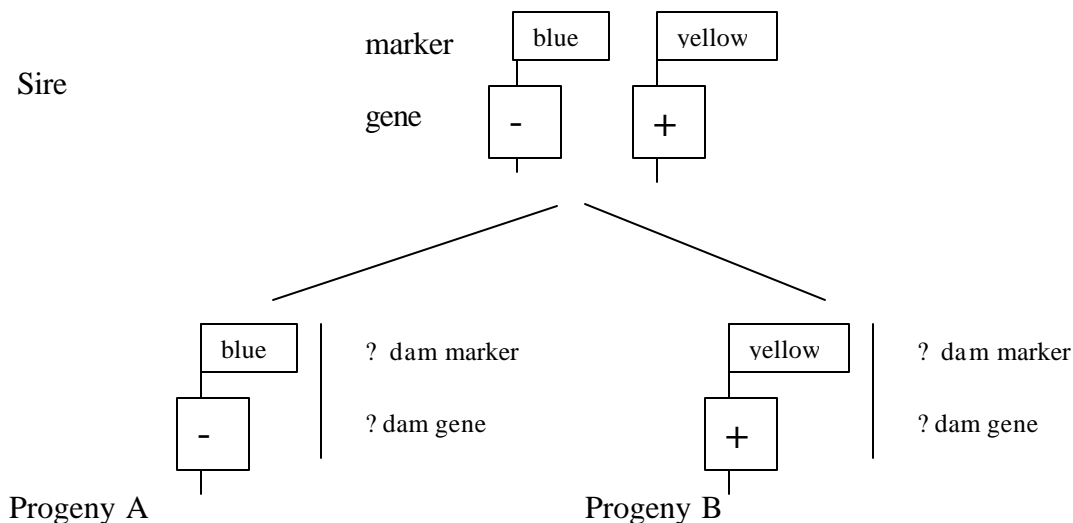


Figure 3: A sire two genes transmitting either his good gene (+) or his inferior gene (-) to a progeny. Inheritance can be followed by linkage of the gene to a nearby marker. The blue marker is associated with the +-gene, and the yellow marker is associated with the inferior gene

which of the QTL alleles was inherited by progeny from their parents. If it has been established, based on a progeny test, that the blue marker is associated with more resistance, then for future progeny we can directly select those with a blue marker. Note that such markers can be detected at a very early age, and therefore, can be very useful in selection, especially when it is not easy to measure the trait. Parasite resistance is not easy to measure without much environmental noise, and it requires the presence of disease to be expressed, therefore selection based on DNA markers could be particularly useful.

With a linked marker, as in Figure 3, it is not always guaranteed that the blue marker is associated with the desirable allele. In another sire family the association may be the other way around (yellow with “-”) as a result of a recent recombination. Which marker allele associates with which of the major gene alleles is indicated as linkage phase. With linked markers, the linkage phase between marker and resistance alleles has to be established for breeding families, before the marker information can become useful. In many breeding programs, the number of families is not very large and our current work at UNE is looking into effective genotyping strategies, allowing maximum information about an animal's probability of carrying desirable alleles to be determined with a minimum number of genotypings on breeding animals.

Finding the actual gene

After determining the location of QTL (major genes) using markers it might seem easy to find the actual mutation, and develop a marker test for the actual gene. Such a ‘direct marker’ test is very advantageous, as it is possible to determine an animal's genotype without genotyping of relatives. Why not try to find the actual gene? The answer is that this is quite a task, requiring many research dollars. Finding the approximate position and target selection with genetic markers is an obvious first step. A chromosomal region of 10 centi-Morgans may seem small, but it still contains about 10 million base pairs and about 100 genes. When the region is small enough, it could be sequenced, and the sequences could be compared with the human genome map. After all, cows and sheep have about 95% of their DNA in common with humans! If on the human genome we could find genes in the corresponding region with some functionality that could be related to parasite resistance, there is a chance we could be successful. However, this will take some more years of research. Until now, only a few genes have been found through such a process of comparative genome mapping. Examples are the double muscling gene in cattle and the thyroglobulin gene in cattle. The last gene affects marbling and a DNA test for this gene is marketed in Australia as “Gene Star. Finding genetic variation at the level of the actual gene is not only helpful in selection, but also helps much more in understanding the regulation of key processes, which in its turn often leads to better ways of improving or controlling a trait.

Marker assisted selection

Marker Assisted Selection combines the information at genetic markers with the phenotypic measurements on breeding animals and their relatives. The improvement in selection efficiency would be mostly dependent on the importance of the genes that are found close to the genetic markers, on whether the trait to be improved is easy to measure on breeding animals and on the type of breeding program. Genetic improvement of traits that are not easy (or cheap) to measure is accelerated more by using genetic markers than that of easily measured traits. Genetic markers would mostly be useful if there is a desire to select animals at a younger age as this speeds the rate of genetic improvement that is achieved annually. Marker assisted selection is therefore particularly useful when combined with reproductive technologies such as AI, embryo transfer and IVF. Model studies have shown that if we were able to use genetic markers in selection of livestock, the rates of genetic gain could be improved by 5 up to 40%.

Selection for improved parasite resistance

Parasite resistance is the major health problem in Australian sheep, and the estimated cost of production losses through this problem are close to 300 million dollars annually. Drenching of animals has been for long the preferred method of combat. However, there is an increased resistance of parasites to chemicals and new solutions need to be found. Selecting animals that are more resistant against intestinal parasites is one way to reduce the problem. The Nemesis project, which is coordinated from CSIRO at “Chiswick”, Armidale is designed to introduce such selection based on phenotypic measurement.

Seedstock producers have selected their animals on visual and subjective scoring and in more recent times they have often moved to more formal trait measurement and genetic evaluation to use estimated breeding values (EBV's) as a selection tool. Such EBV's for parasite resistance are provided in the Nemesis project. Such selection could be improved even more if a major gene with a large effect on parasite resistance could be identified and targeted with genetic markers.

Research project at Kirby

In order to find the location of the major gene for parasite resistance, a project has started this year at UNE, using Merino ewes at the Kirby research station. In April 600 ewes were inseminated by laparoscopic AI with semen from 4 rams. These rams were chosen based on having a very high probability of being heterozygote for the major gene. Heterozygosity is required in order to distinguish in the progeny the “haves” and the “have-nots”. The progeny will be genotyped for genetic markers scattered over all chromosomes. Furthermore, the same progeny will be measured for growth and wool and they will be challenged with 11,000 *Haemonchus contortus*, and faecal egg counts will be measured in the subsequent weeks. Those genetic markers that are associated with a progeny difference in FEC are likely linked to a major gene for parasite resistance. The

different families can be used to confirm the existence of such a major gene. Provided the first step is successful, a next step in the project will investigate whether the same major gene is likely segregating in commercial breeding flocks. If it is, breeding for the major gene in commercial studs could be enhanced by information from genetic markers. If the gene is not found in commercial flocks, the UNE Golden ram gene is unique, and can potentially be bred into commercial flocks. Here, genetic markers can be even more useful, as only Goldie's' parasite resistance gene, and not his wool or muscling genes are interesting to a commercial wool or lamb producer.

References:

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