THE APPLICATION OF GENETIC LINKAGE IN THE SEARCH FOR THE BOOROOLA GENE

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SUMMARY

Genetic linkage analysis can be applied to the search for genetic markers linked to major genes such as the Booroola (F) gene. Results from the identification of RFLP markers show that the frequency of RFLPs is high in New Zealand sheep populations. The flock structures commonly used have limitations for linkage since pedigrees are fragmented or incomplete. These problems can be minimised through the collection and storage of DNA or semen from sires in important flocks, and through the development of highly polymorphic probes to reduce the numbers of individuals required to provide linkage information.

INTRODUCTION

Linkage can be defined as the occurrence of two loci sufficiently close together on a chromosome such that their assortment is recognized as being non-independent. Until recently, linkage in farm animal populations has been restricted by the limited number of genetic markers available. The development of methods to detect individual variation in DNA has provided a powerful new class of genetic marker, known as restriction fragment length polymorphisms (RFLPs, Botstein et al. 1980). Consequently, it is now possible to identify genetic markers linked to genes of economic importance in sheep flocks.

One application of genetic linkage methods is the identification of markers for major genes such as the the Booroola fecundity (F) gene, a gene with a large effect on ovulation rate in sheep (Piper and Bindon, 1982; Davis et al., 1982). This involves identifying a large number of new markers, collecting samples from suitable families or pedigrees where the candidate gene is segregating, scoring all classes of markers (including protein polymorphisms) in the individuals, and analysing the data. The objective of this paper is to discuss results from a programme to identify a genetic marker linked to the F gene and the general application of linkage analysis to livestock selection programmes.

GENETIC MARKERS

A large number of markers are required to give a reasonable chance of detecting linkage. While RFLP markers have been identified in sheep (Montgomery, Hughes, and Hill, 1988), the frequency of RFLPs in sheep is not known. Sheep populations differ from outbred human populations since selection for the improvement of productive traits is primarily directed to the selection of males and the selected males are mated to large numbers of ewes.

In addition, the markers will need to span the entire genome for linkage studies with genes known to show autosomal inheritance. Few sheep genes have been mapped. However, a comprehensive genetic map is available for the human genome and a there is a high degree of conservation between the genetic maps of different mmammalian species. In addition, the sequences of some genes are known to be conserved across species and probes from other mammalian species may be useful to detect RFLPs in sheep genomic DNA.

To assess probes from other mammalian species, identify RFLP markers for sheep, and the frequency of RFLPs in New Zealand sheep populations, we have screened 50 probes from a variety of sources. The procedure now adopted involves screening DNA from 10 unrelated individuals cut with 10 different restriction enzymes. Results show that 10 of the probes detected RFLPs. This is a small number in relation to the number of markers needed for complete coverage of the genome. However, only 50% of the probes tested in this sample showed any homology (bands with one or more restriction enzymes) with sheep genomic sequences. The number of probes detecting RFLPs represents 38% of the probes showing any homology. This proportion compares very favourably with the 30% of probes detecting RFLPs from screening a large sample of human probes against human genomic samples (Schumm et al. 1988). Considering that the probes used in this set of experiments did not give data for all 10 enzymes, and several probes detected RFLPs with more than one enzyme, it appears likely that a higher frequency of RFLPs can be expected in sheep compared with human populations.

A higher proportion of cattle cDNA probes detected homologous sequences than probes of other species. Future work will concentrate on probes from species closely related to sheep. However, probes from other species, for genes of particular interest, will be worth screening for subsequent linkage analysis.

FLOCKS

Linkage between two loci can only be determined from "informative matings"; that is when the alleles present in the offspring show whether recombination has occurred at meiosis. Pedigrees that yield maximum information are large pedigrees of 3 or more generations so that the phase of the candidate loci and markers can be determined and therefore, a higher proportion of individuals contribute information on recombination.

Twinning frequencies are greater in sheep compared with human populations and matings will produce more offspring to score for recombination. However, in most cases, ewes are mated to different rams in successive years. This results in very fragmented pedigrees. We have collected samples from a backcross flock designed to transfer the F gene to the Romney breed. New sires are used in the flock each year and subsequently culled. The net result is that there is usually only one daughter from any single mating and no samples are available from the rams, a situation that is typical of many flocks. In addition, most of the daughters that were not carrying the F gene have been culled out of the flock in the early generations. Consequently, there are very few informative matings. For example, the flock comprises records of 550 individuals in 70 separate pedigrees. We have scored 150 individuals at the haemoglobin (Hb) locus by protein electrophoresis. Only two of the pedigrees from the 23 analysed are informative and show a single crossover between the Hb locus and the F locus.

Despite the limitations of this flock for linkage, it has been essential in defining alleles for new RFLPs. While this is straight forward for simple two allele markers, patterns of bands obtained using cDNA probes and large genomic fragments can be quite complex. These probes must be used in pedigree data where the alleles can be identified.

The other type of flock available for linkage are large half-sib pedigrees from progeny tested rams. These flocks have been generated to identify the F gene phenotype of the rams and the daughters have not been individually identified to their dams. This limits the linkage information that can be extracted, although this is less important if the family size is large. The ram must be heterozygous at both the gene and marker loci. In the case of the progeny test data for F+ rams, all daughters will be informative at the F locus. For a marker with two alleles, approximately half of the daughters will be informative (i.e. those daughters that are homozygous at the marker locus). More daughters are informative where the locus has more than two alleles. For example, in a half-sib family of 35 daughters, 19 of the daughters were informative for haemoglobin (2 alleles) while 27 daughters were informative for transferrin (6 alleles).

FINGERPRINTING

The DNA probes that give individual specific banding patterns commonly referred to as DNA fingerprints or "hoofprints" are highly polymorphic. They have the advantage that many loci can be scored in a single experiment. However, for linkage analysis they have a major disadvantage since it is usually impossible to identify the alleles at each of the loci. Therefore, these probes must be used within a single family. The large half-sib families should be suitable for linkage studies using these probes. Sheep DNA "hoofprints" have been developed in the laboratory and their use for linkage is currently being evaluated. However, they also provide a test of parentage and a rapid audit of the pedigrees. Results from one of the pedigrees collected for our linkage studies clearly showed that a high proportion of the putative daughters of the test ram were not sired by the ram in question.

CONCLUSION

The frequency of RFLPs detected clearly show that it will be possible to find sufficient DNA markers to make linkage studies practical in sheep populations. Current breeding practices place major limitations on the suitabililty of many flocks resulting from the fragmented nature of the pedigrees and short lifetime of the sires that constitute a major part of the flock structures. Designed matings can generate suitable pedigrees, but these will take some time to establish. Collection of DNA samples and/or semen from rams in critical flocks would be an advantage if linkage studies were contemplated in the future. Highly polymorphic sheep DNA markers (e.g. locus specific VNTRs) must be developed to minimise the number of individuals that have to be scored, and lessons should be learnt from human genetics on the importance of identifying critical individuals' combined with cytogenetic studies to identify chromosomal regions implicated in production traits.

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