Mapping and exploiting economically important genes in Australian pigs

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Introduction

Over the past 7 years, a number of Australian institutions, including the University of Sydney, the Victorian Institute of Animal Science and University of Melbourne, the Animal Genetics and Breeding Unit, Armidale, and the Pig Research and Development Corporation and its successor Australian Pork Limited, have financed and participated in a sustained program of porcine genomics research, seeking economically important genes for the pig industry and ways to exploit them. The involvement of Bunge Meat Industries has provided access to the largest piggery in the southern hemisphere with the capacity to quickly breed and evaluate very large resource pedigrees under commercial conditions of production, frequently for traits not normally recorded in commercial pigs. Standard growth and fatness traits have been recorded, but also records of food conversion efficiency, intramuscular fat, androstenone levels, tenderness, stress responses, immune traits and many other characteristics affecting the efficiency of production or product quality have been measured. The University partners have provided molecular genetics expertise and facilities, leading edge quantitative genetic analysis for identification of genetic regions affecting variation in these traits, and economic and genetic evaluation of the potential for the exploitation of the results in the industry. The aim is eventually to develop DNA tests to supplement conventional performance selection to expedite selective improvement and allow its implementation for traits where it is too difficult or too expensive to implement performance selection.

Outcomes

Number and size of quantitative trait loci (QTL) in pigs

QTL are genes affecting any trait of interest. Providing their effects are not too small, they can be detected by gene mapping. The first question that should be asked about QTL is how useful they might be in promoting selective improvement and enhanced economic returns to the pig industry. At one level, this question can be answered by asking how much variation for economically important traits is caused by QTL whose effects will be sufficiently large that we can detect them with reasonable effort and cost. Professor Mike Goddard and Dr Ben Hayes have asked this very question as part of our PRDC program. By applying sophisticated statistical analyses to all published estimates of QTL effects published for pigs, they have estimated that only about 7% of pig QTL have effects in excess of 0.3 σ_P (phenotypic standard deviation) and thus are of sufficient size we might expect to be able to detect them. Is this a bad result? Not necessarily when you remember that such detectable QTL are by definition the largest

and in total will account for at least 22% of the variance in pigs (Hayes and Goddard, 2000). A similar analysis for dairy cattle performed by Hayes and Goddard, indicated that about 60% of the variance is created by such QTL. Since there is no reason for expecting a huge difference between the distribution of gene effects between pigs and cattle and since the shapes of the distributions were not accurately determined for either species, it is probably reasonable to think that about half of the variance for a trait will be explained if we can identify all QTL with effects greater than 0.3 σ_P. We conclude that it is definitely worthwhile to proceed.

Best Methods of exploiting QTLs

We identify QTL initially by finding an effect on the phenotype of a chromosomal region whose inheritance we can track using marker loci. If the region contains a QTL and a sire is heterozygous for that QTL, we will be able to recognise two groups of progeny in the sire family differing in expression of the trait. The problem is that the marker loci are not necessarily very tightly linked with the QTL. This means that a specific marker allele may be associated with a favourable QTL allele in one family and an unfavourable QTL allele in another. If we are going to exploit such markers in marker assisted selection, we have to work out which marker allele is associated with which QTL allele in each family. We must also be aware that these associations can change over time due to recombination. In genetic jargon, we must continually assess the phase relation between the marker and QTL alleles. Wouldn't it be nice if we could find a consistent relationship between marker alleles and the QTL alleles?

In fact, it is possible to find just such markers having "universal" value across families and across time in predicting the presence of favourable or unfavourable QTL alleles. For very closely linked loci, if there is high linkage disequilibrium, then a consistent pattern of relationship between marker and QTL will exist in all animals in the population. Linkage disequilibrium is a difficult genetic concept, hard to explain to students, and rediscovered at regular intervals by medical researchers working on the human major histocompatibility complex. For our purpose, we simply need to know that if linkage disequilibrium is strong between a marker and a QTL (and this is more likely the more closely together two genes lie on the same chromosome), the more widely useful the marker will be. It will be easier and more accurate to apply the markers as an aid to selection.

Hayes and Goddard as part of this program have systematically evaluated the application of marker assisted selection (MAS) in pigs using linked versus "linkage disequilibrium" markers and have concluded that MAS will only be truly valuable if we can identify markers in linkage disequilibrium with the QTL. Just identifying QTL is not enough. Another phase of investigation is required where very tightly linked markers in linkage disequilibrium with the QTL are found, exploitable immediately at the population as well as the family level. Tightly linked markers in linkage disequilibrium with the QTL are almost as good as identifying the gene causally responsible for the QTL effect (Meuwissen, Hayes and Goddard, 2001).

Identifying QTLs

Confidentiality requirements constrain what can be said about this. However we have identified a number of potentially valuable QTL for numerous traits, especially for our second project using eight sire families in which approximately 50

phenotypes have been measured. In fact, we are now faced, well before completion of the full genome scan on this resource, with the dilemma of which QTL leads to follow-up intensively and which to set aside. We are particularly interested in following up several QTL for food conversion ratio (FCR). This is a trait of considerable economic importance and for which conventional performance based selection is difficult. Many more markers will have to be identified and evaluated in the vicinity of our QTL (which like all QTL are relatively imprecisely mapped). We will also have to evaluate these markers in a large new resource being bred at Bunge Meat Industries.

We are also interested in QTL for boar taint and have some interesting leads to follow in this area, including candidate loci for which we have developed polymorphic markers now under evaluation to supplement the information from the genome scan.

Future Directions

Search for Markers in Linkage Disequilibrium with QTL

The search for markers in strong linkage disequilibrium with QTL is increasingly a goal for all marker assisted selection programs. In general, it will involve the identification of a set of marker loci bracketing the QTL region. Most specifically, it will require the identification of haplotypes of marker alleles and their association with favourable or unfavourable QTL alleles. For example, if there are three loci, A, B and C, each with alleles A/a, B/b and C/c, the existence of linkage disequilibrium might mean that we only ever see the combinations (haplotypes) ABC and abc, and never see AbC or any of the other possible combinations. The existence of linkage disequilibrium and the use of the term haplotype presupposes that some of the combinations of alleles are never found. If we can then establish that the favourable QTL allele is associated with say ABC and the unfavourable with abc, we will have a useful tool for selection. To reliably find sufficiently close markers to enable identification of such haplotypes, we will require many more markers than are currently available. Fortunately many laboratories throughout the world are contributing valuable resources to this task. The University of Minnesota and the INRA laboratories from France have developed a freely available radiation hybrid panel for very fine scale physical mapping in the pig. Many laboratories have developed large insert clone libraries, mainly BAC (bacterial artificial chromosome) libraries, as an adjunct to mapping and gene identification projects. The INRA group will actually will screen their gridded BAC library using PCR primers supplied by external parties and will provide the clones identified for the cost only of the postage. These resources will provide a vital source of genetic markers required for the high resolution phase of porcine genomics now upon us.

Novel Marker Methodology

Most domestic animal QTL mapping is currently done using hypervariable microsatellite markers. These markers are particularly useful as the number of marker loci is effectively unlimited (likely to be hundreds of thousands) and the markers are individually highly informative. However due to developments in large scale genotyping methodology, a new type of marker is set to take over in the future, as soon as the best of the many competing genotpying platforms establishes market dominance

and proves economically efficient. This new type of marker called, a single nucleotide polymorphism (SNP), is actually an old type of marker being reinvented due to the new genotyping systems. We used to detect these variants one at a time using restriction enzyme digestion and electrophoretic separation of fragments of DNA. Now we may be able to genotype them hundreds or possibly even thousands at a time using fully automated systems. Individually SNPs are much less informative than microsatellites, since they only have two alleles rather than many alleles like microsatellites, but there are so many of them in the genome and they will be so cheap to genotype (or so we are assured) that this will not matter.

SNP genotyping will not only impact on gene discovery in the pig but will also assist in the implementation of marker assisted selection, assuming that a reliable and economical SNP genotyping platform can be developed. Given the investment in this methodology in human genetics, this must be considered inevitable. For example, a non-profit group of 14 larger corporations and other organisations established *The SNP Consortium Ltd* in 1999 to develop up to 300,000 SNPs distributed evenly throughout the human genome and to make the information related to these SNPs available to the public without intellectual property restrictions. The project was anticipated to continue until the end of 2001 but by May 2001, 1,034,034 SNPA had already been identified and other groups were also involved in identifying human SNPs (The International SNP Map Working Group, 2001). How long it takes for this methodology to filter to animal genetics remains to be seen.

Delivery to Industry

Our immediate objective is to develop a haplotype of markers in strong linkage disequilibrium with the QTL for food conversion ratio mentioned earlier and provide these to industry as tools for selection. The time frame for achieving this objective is about three years. During this period, we will analyse approximately 3,000 boars going through the Bunge boar test facility on which FCR will be measured. Analysis of such a large number of animals will be required to identify useful marker loci in linkage disequilibrium with the QTL and to accurately estimate the effects of the QTL and the benefits of the selection using these markers. Implementation in industry will be straightforward given that the marker and QTL associations will hold throughout the population.

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