LIVESTOCK IMPROVEMENT CORPORATION LECTURE

Perspectives for marker assisted selection in dairy cattle breeding

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INTRODUCTION

Animal breeders have been manipulating genes for centuries, in the sense that by selecting the best performing individuals as the parents for the next generation, they have increased the frequency of favourable alleles and concomitantly decreased the frequency of unfavourable alleles at genetic loci influencing the traits of interest. With the introduction, in the fifties, of complex biometrical methods to estimate breeding values from own performances and performances of relatives, so-called "mass selection" has matured into an extremely powerful methodology. Modern breeding programs are largely responsible for the dramatic increase in the efficiency of livestock production that society has witnessed during the second half of this century.

While these conventional breeding strategies directly affect individual loci, the exact molecular nature of the target genes remains essentially unknown. Indeed, quantitative geneticists refer to the pool of genes they are unconsciously manipulating as the "black box". Recent advances in molecular genetics, however, have made the isolation and characterisation of the molecular substrate of artificial selection a feasible objective. The identification of the genes underlying livestock production traits - commonly referred to as quantitative trait loci (QTL) because of the nature of the corresponding trait - would not only be a major scientific achievement, but is very likely to lead to more efficient breeding programs through so-called "Marker Assisted Selection".

Indeed, the benefits of using direct information on the genes underlying production traits in breeding programs are threefold. First they can *increase the accuracy of selection* by providing more information on an animal's breeding value than otherwise obtained using just phenotypic information (Smith and Simpson, 1986; Lande and Thompson, 1990; Zhang and Smith, 1993; Meuwissen and Van Arendonk 1992; Hoeschele and Romano 1994; Meuwissen and Goddard 1995). Second, markers can be used to *decrease generation interval* by allowing selection at earlier stages in life (e.g.Kinghorn *et al.*, 1991). Third, markers can be used to *increase the selection differential* by allowing screening and pre-selection among larger numbers of candidates for later selection (Kashi *et al.*, 1990; Mackinnon and Georges, 1996 unpublished results). Moreover, dissecting genetically complex traits into their Mendelian components might reveal unexpected genetic mechanisms requiring novel breeding schemes for their optimal exploitation. The identification of a novel form of *dominant negative imprinting* at the ovine *callipyge* locus perfectly illustrates this point. Results obtained so far indeed suggest that 100% hyper-muscled callipyge offspring can only be obtained by mating *CLPG/CLPG* homozygous rams (exhibiting a normal phenotype) with *clpg/clpg* conventional ewes (Cockett *et al.*, 1996 unpublished results).

Dairy cattle breeding offers a unique niche for the implementation of Marker Assisted Selection. Because of the extensive use of artificial insemination, genetic progress is mainly realized through the use of genetically superior sires. The identification of these elite sires, however, requires time-consuming and expensive progeny-testing, taking approximately 5-6 years and of the order of US$35,000 per bull. Marker-assisted preselection of young dairy bulls prior to progeny-testing has the potential to substantially increase the cost-effectiveness of dairy sire production (Soller and Beckmann, 1983).

POSITIONAL CLONING

While a number of strategies can be envisaged to pinpoint the genes underlying production traits, the most popular one to date follows the "positional cloning" avenue (for instance, Collins, 1995). The essence of this method is in the localisation of the genes of interest using linkage analysis aided by a battery of genetic markers covering the whole genome. This initial localization is followed by genetic and physical fine-mapping of the identified genomic region, that may ultimately culminate in the cloning of the target gene based on its map position. The success of the positional cloning route amongst animal geneticists mainly stems from the direct use they will likely be able to make of the initial mapping data in breeding programs using Marker Assisted Selection.

Implementing this positional cloning strategy however, first requires the development of mapping tools such as comprehensive marker maps, mapping software, large insert (YAC, PAC) libraries, etc. During the last five years, a number of groups have invested very diligently in the development of these tools for the major livestock species. What has primarily driven this field in recent years is the description of microsatellite sequences as an abundant source of highly polymorphic, well dispersed genetic markers. Comprehensive microsatellite based linkage maps are now available for the major livestock species, especially cattle (Beattie, 1994). Technology is available and continues to be improved for high throughput PCR-based genotyping at microsatellite loci, making the large scale experiments required for the detection of QTL now accessible.

MAPPING QTL CONTROLLING MILK PRODUCTION IN ELITE DAIRY CATTLE BY EXPLOITING PROGENY TESTING

Several studies are presently being performed around the world, aiming at the localisation of QTL in the different livestock species. The first study in which a whole genome scan was undertaken to map genes influencing milk production and composition (Georges *et al.*, 1995), exploited two specific features of dairy cattle populations, namely: 1. Due to the extensive use of artificial insemination it is easy to sample very large half-sib families. By concentrating on the segregation of the genes originating from the common founder sire in a within family analysis one can efficiently reduce the genetic heterogeneity for the traits of interest in a given family. 2. Rather than use individual production records as phenotypic measurements, one can use breeding values (BV) of males, estimated from the production records of their daughters. As bulls typically have 50-100 daughters, the accuracy of these BV estimates is such that their heritabilities are of the order of 80% compared to 30% for the original phenotype, leading to a considerable reduction in environmental noise. It was estimated in this specific case that the use of estimated BV rather than production records allowed for a 3.5 - 4-fold reduction in required sample size.

Fourteen paternal half-brother families totalling more than 1500 individuals were genotyped for a battery of 160 microsatellite markers covering approximately 1600 KcM of the bovine genome. Using a maximum likelihood multilocus linkage analysis accounting for variance heterogeneity of the phenotypes, five chromosome regions (on chromosomes 1, 6, 9, 10 and 20) were identified that gave very strong evidence for the presence of QTL affecting milk yield and composition. The magnitude of the identified average effects of a QTL allele substitution was of the order of 3/4 to one additive genetic standard deviation. While it was realized that these estimates were likely biased upwardly (given the limited power of the experimental design and the ML statistical method chosen), these results nevertheless indicate that alleles with substantial effects are still segregating in these populations despite the intense selection. As the analysed milk yield and composition phenotypes are known to be highly correlated, the observation that the identified QTL were affecting several of these traits was not surprising. Although the inherent imprecision in the estimation of the effects calls for prudence in the interpretation, the mapped genes seemed to have fairly distinct effects on the different traits. As an example, while the QTL on chromosome 9 increased milk yield without significantly altering its composition, the QTL identified on chromosome 6 obviously leads to an increase in milk volume accompanied by a dilution of its solid components. These results therefore illustrate how QTL mapping should allow for a dissection not only of individual traits but of the correlations between them as well.

Several independent studies are presently being performed which will allow testing of the validity of the identified loci and hopefully reveal additional candidates. One of the largest of these studies is presently conducted jointly by Livestock Improvement Corporation and Holland Genetics. Analysis of chromosome 6 in the Dutch and New Zealand black and white cattle population demonstrated the segregation of what is very likely to be the same QTL as the one identified in the first study, given the position and nature of the identified effect (Spelman *et al.*, 1996 unpublished results). A systematic scanning of the rest of the genome in this pedigree material is in progress.

FUTURE CHALLENGES

Determining the coarse localisation of segregating QTL is only the first step in the positional cloning procedure, to be followed by fine-mapping of the region and eventually cloning of the underlying genes. At present, fine mapping efforts are hampered by the paucity of markers when one intends to concentrate on a very specific region of the genome. Chromosomal microdissection of specific bands or the use of flow-sorted chromosomes seem to be the strategies of choice to target specific areas of the genome. Their efficacy, however, remains to be demonstrated. Moreover, the informativeness of the family material is likely to become a factor limiting fine-mapping efforts as well. Identity-by-descent mapping strategies either within breeds or exploiting breed admixture might overcome these limitations to some extent. Devising strategies to identify the actual underlying genes and causal mutations remains a major intellectual challenge. Obviously, positioning one-self in order to be able to efficiently take advantage of the wealth of information that is being generated as a result of the human and mouse genome initiatives is crucial. Indeed, the complete physical and transcript maps that will soon be available in both these species, have shifted the cloning strategy of choice from positional to positional candidate cloning (Collins, 1995). The remarkable conservation of synteny and to a lesser extent gene order amongst mammalian species will allow animal geneticists to exploit this information as long as high resolution bridging strategies are being developed.

These studies demonstrate that one can identify QTL underlying the genetic variation of milk production and composition in elite populations using the existing technology. Whether it will be possible to explain enough of this genetic variation with mapped genes in order to warrant MAS programs however, remains an open question. Moreover, devising MAS schemes that cost-effectively utilize information on mapped QTLs is far from a trivial issue. A number of schemes have been proposed and continue to be refined. Concerns have been raised with regard to the risk of compromising long term genetic response at the expense of an accelerated short term response using MAS, due to the negative linkage disequilibrium that selection establishes between favourable major QTL alleles and the residual polygenes.

There is no doubt that we will witness the harvest of a multitude of QTL affecting most economically relevant production traits in livestock during the coming years, particularly in dairy cattle. Development of mapping tools was viewed as the major limiting factor for QTL mapping

in animal genetics only a few years ago, followed by scepticism about the feasibility to apply these tools to map genes underlying quantitative traits. Today, the major challenge and source of scepticism has become the design of novel selection schemes taking advantage of QTL mapping results. We have very little doubt that the dairy industry, with its reputation to eagerly adopt modern technology that has the potential to improve its efficiency, will show the required inventiveness to advantageously exploit mapped QTL.

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This paper was also presented at an OECD – sponsored conference untitled "Biotechnology for Dairy Production" in Wellington, February 1996. The Proceedings will be published by CABI (Welch, R.A.S., Davis, S.R., Prosser, C.G. and Burns, D.J.W. Eds.) 1997.