Use of molecular markers and major genes in the genetic improvement of livestock

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Recent developments in molecular biology and statistics have opened the possibility of identifying and using genomic variation and major genes for the genetic improvement of livestock. Information concerning the basis of these techniques and their application to the genetic improvement of animals is reviewed. Main marker molecular marker systems in animals (RFPL and microsatellites), genome maps, methods for detecting marker-QTL linkages and use of marker assisted selection, genetic fingerprinting and mixture models based on segregation analysis are analyzed. The characteristics in where the application of marker assisted selection can be more effective are those that are expressed late in the life of the animal, or controlled by a few pairs of genes. The first example corresponds to the longevity and carcass characteristics in meat-producing animals, the second, to the resistance to certain diseases or defects of simple inheritance.

The detection of major genes using mixture models with segregation analysis can direct the work of identification of DNA marker genotypes towards populations and characteristics with greater probability of detecting a QTL. The present trend indicates that molecular, pedigree and phenotypic information will be integrated in the future through mixture models of segregation analysis that might contain QTL effects through the markers, polygenic inheritance and uses powerful and flexible methods of estimation such as Gibbs Sampling.

Molecular techniques allow detecting variation or polymorphisms exists among individuals in the population for specific regions of the DNA. These polymorphisms can be used to build up genetic maps and to evaluate differences between markers in the expression of particular traits in a family that might indicate a direct effect of these differences in terms of genetic determination on the trait. More probably, the can prove some degree of linkage of the QTL effecting the trait and the marker (Albert et al., 1994; Lewin, 1994; Stan et al., 1996).

Furthermore, methods have been developed to detect the presence of major genes from the analysis of pedigreed data in absence of molecular information (Bovenhuis et al., 1997). These methods, based on mixture models and segregation analysis, allow to direct the potentially expensive and time consuming genotyping activities towards populations and characteristics with a greater probability of being controlled by a QTL and to optimize the collection of molecular data (Kinghorn et al., 1994).

The objective of this paper is to review some advances on molecular genetics and statistical methodology to identify and estimate the effects of major gene effects in animal populations, and its potential use in the genetic improvement of livestock.

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Restriction fragment length polymorphisms

Sex (1923) showed that an observable gene with simple mendelian inheritance could act as a marker for the segregation of a gene involved in the expression of a quantitative trait. Early work on detection of genome variation of potential markers focused on the analysis of proteins and blood type variation, but this was found to be impractical to be used as genetic markers. Protein systems lack adequate polymorphism, genome coverage was low and there was a requirement for the gene to be expressed at the phenotypic level to make detection possible (Drinkwater and Hazel, 1991).

Beginning in 1980, an accelerated understanding of the structure and function of the genome has been obtained as animals (Schimenti, 1998). At first, techniques were developed to visualize the structure of the DNA based on the use of bacterial restriction enzymes that cut the DNA at sites with specific nucleotide sequences. With this basis, the technique denominated restriction fragment length polymorphisms (RFLPs) was developed. The identification of RFLPs requires the use of gel electrophoresis to separates the DNA fragments of differing sizes followed by transfer of the fragments to a nylon membrane (Southern blot) and visualization of specific DNA sequences using radioactive or chemiluminescent probes exposed to an X-ray film (Drinkwater and Hazel, 1991).

This methodology was the standard method in the identification of RFLPs before 1986, situation that limited the identification of the whole genome variation in animals. This technique was not very sensitive and could detect many RFLPs sites non-informative. Modification of the RFLPs technique is possible to identify multiple alleles, but still have practical drawbacks that make preferable the use of the PCR based microsatellite markers (Drinkwater and Hazel, 1991).

Polymerase Chain Reaction (PCR)

Mullis et al. (1986) developed the process known as polymerase chain reaction or PCR. This allows the amplification or reproduction in great amounts of particular regions of the DNA. In order to initiate the process of replication of the DNA, two informing sequence codes denominated primers are required which promote the beginning and reversion of the reaction of the polymerase (DNA-pol) at particular locations of the genome. A reproduction or amplification of thousands of copies of a chromosomal region or gene of interest is obtained by repeated cycles of synthesis and denaturalization (chain separation) of the DNA using temperature changes. Since the primers are specific sequences to bond to a determined region of the DNA, only the specific amplification of the desired sequence of DNA instead of amplifying the DNA in its totality is obtained (Buratowski, 1994; Koleske and Young, 1995; Stein et al., 1996).

Microsatellites

Microsatellites systems are composed of DNA repeats in tandem at each locus. The tandem repeats are usually simple dinucleotides (such as (TG)\_n, ) with each dinucleotide repeated about ten times. Its high degree of polymorphism in the number of repeats \(n\) allows its use as markers in genome mapping. The length of each allele is determined by PCR using specific oligonucleotides primers flanking the repeated sequence. The DNA products are visualized after electrophoresis. PCR based Microsatellites techniques facilitated the construction of genome maps in most livestock species because its abundance in the genome, the specificity of the primers, its high degree of polymorphism which several alleles and their easy detection (Albert et al., 1994; Lewin, 1994; Bishop et al., 1995; Smith and Smith, 1993; Stein et al., 1996).

Other marker systems

Other types of DNA genetic markers used mainly in plant breeding but with potential use in animals are randomly amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and amplification fragment length polymorphisms (AFLP) (Specht, J. personal communication, 1996).

New types of markers are single nucleotide polymorphic markers (SNP) based high density DNA arrays (Chee et al., 1996). In this technique of “gene chips”, DNA corresponding to thousand of genes are arranged on small matrices (“chips”) and probed with labeled cDNA from a tissue of choice. The information is then read by a device to be downloaded to a computer (Schimenti, 1998).

Sequence analysis

Highest resolution of DNA variation can be obtained using sequence analysis. Sequence analysis provides the fundamental structure of gene systems. DNA sequencing is generally not practical to identify variation between animals for the whole genome, but is a vital tool in the analysis of gene structure and expression (Drinkwater and Hazel, 1991).

Molecular genome maps

A step towards the use of genomic information in livestock improvement is the location of all markers and protein coding genes in the chromosomes. Thus the development of genetic maps of the species of interest is required for detecting QTL using genetic markers (Bovenhuis et al., 1997). Mapping based on recombination frequencies could be built up on the basis of genetic marker genotyping from DNA obtained from populations of reference, with several collaborating laboratories worldwide (Beadle, 1994). Development of PCR based techniques and microsatellite markers increased the efficiency of the mapping processes in all living organisms.

An aid in the development of livestock genome maps, has been the high level of conservation of gene sequences between humans, cattle, sheep, goat, pig and mice. By such reason, once loci of particular DNA sequences has been mapped in one species, the information is frequently of help in the genome mapping in another. Livestock species mapping had been greatly facilitated by the increasing availability of human and murine sequences (Beadle, 1994; Schimenti, 1998).
Additionally, the construction of physical maps has been favored by means of the development of techniques like the hybrids of somatic cells and fluorescence in situ hybridization (FISH) (Beattie, 1994). With the development and use of these techniques, genome mapping has had a spectacular advance in the last years. Whereas in 1990 150 positions in the bovine genome had been identified, for 1998 this number surpasses 1,500 positions.

Examples of current genome mapping information available in the Internet for several mammal species are:

- http://vega.crbm.cnrs-mop.fr/Vert-Genome_DBS.html

### Candidate gene approach

There are two main categories of genomic information that can be used in genetic improvement of livestock:

1. Genes with known on the expression of certain protein.
2. Genes with effects detected on the characteristic in statistical terms. These markers are based on polymorphic sequences of the DNA that is in the same chromosome (linked) to a gene of quantitative effect (QTL).

Examples of the first type (1) in livestock are:

1.1 The gene associated with porcine malignant hyperthermia. In this case, a (C-T) mutation at position 1843 of the gene calcium release channel ryanodine receptor locus (Ryr1) in recessive condition have a high probability of being the cause of the susceptibility to this disease (Huges and Lowden, 1996).

1.2 The myostatin (Mh) locus which contains a series of mutations which disrupt the myostatin function, detected in several European cattle breeds causing muscular hypertrophy (Georges et al., 1998).

1.3 A promising candidate gene for litter size in pigs is the estrogen receptor gene. Independent confirmation of such an effect in several populations might help to confirm the suspected QTL effect of this gene (Rothschild et al., 1996).

The approach for search genes of type (1), known as candidate gene, based on the physiological or biochemical affinity of gene with the nature of the characteristic, has not yet been useful in the identification of an important number of genes in quantitative traits. Its use has been considered with some skepticism in the case of diseases of the mouse (Frankel, 1995).

In the mouse, the number of identified potential markers type (1) is greater than in other species of mammals. In this species, the presence of inbred lines, short generation intervals and reduced costs by individual allow good conditions for detection.

Part of the limitations of the candidate gene approach seems to be the polygenic nature of most traits in animals, the appearance of mutations and the possibility that the supposed QTL is in fact a marker type (2) very next to gene, which allows some probability of recombination. Another possibility is the presence of interactions between genes in different loci (epistasis). These can modify the expression of genes in populations with different genotypes (races or crosses). As an illustration of these difficulties, there are animals detected as free of the porcine malignant hyperthermia mutation with the PCR based test that show the disease (Huges and Lowden, 1998).

### Marker-QTL linkage studies

The second type (2), corresponds to genes linked to the QTL with detectable variation by means of RFLP, microsatellites or other similar molecular systems (Beattie, 1994). Several studies have demonstrated relationships between molecular variants and the phenotypic expression in several characteristics in several animal species (Andersson et al., 1994; Ashwell et al., 1997; Beever et al., 1990; Georges et al., 1995; Haley, 1995). This has stimulated the idea to add the genomic to the phenotypic information to increase or speed up the selection response to the "traditional methods" in which it is known as marker-assisted selection or MAS.

In laboratory species, crosses between full inbred lines allow to produce F2 of segregating backcrosses suitable for efficient detection of QTL-marker linkages. In normal outbred livestock populations the linkage between the markers and the QTL (linkage disequilibrium) will disappear when is evaluated across families. This makes pointless to compare the average performance of animals with different molecular variants from the whole population to infer marker-QTL associations. Information from crosses of within family analysis is therefore required to detect associations or linkage disequilibrium between the marker and the QTL (Weller et al., 1990).

In livestock, there are basically four design possibilities for marker QTL linkage analysis.

1. Using F2 populations crossing two similar F1 populations, or a backcross between the F1 and one of the original populations.
2. Using a half sib sire design on which heterozygous sires for the markers are mated to a random sample of females and all the progeny is genotyped.
3. Using instead a granddaughter design on which a sire and their sons evaluated by progeny testing are genotyped.
4. Using crosses of individuals with extreme phenotypes for one trait or trait combination. Animals from divergently selected lines or from populations with wide variation for important traits are also used.

In outbred populations, only heterozygous sires or grandsires will be informative for the segregation of the marker for the marker and the QTL. In a family, where the marker and the QTL allele are in coupling phase, the effect detected for the substitution of allele A1 of the marker by allele A2 can be of for example -500 kg of milk. In another family, where the marker and the QTL allele are in repulsion, the result will be of +500 kg.

### Marker assisted selection (MAS)

It is currently possible to incorporate this information in the present systems of selection with BLUP, that is to say, to add the information corresponding to the markers to the systems of equations of the mixed model (Goddard, 1992; Kinghorn and Clarke, 1997).
The identification of the precise position of the QTL in the chromosome, avoids the ambiguity caused by the possibility of recombination between the marker and the QTL. In the later case we should strictly define this kind of selection as QTL assisted selection. For simplicity we treat this option as a special case of MAS without recombination among the marker and the QTL. Current marker use in MAS with the availability of relatively dense genome maps and multiple microsatellite markers available, will be more probably groups of allelic configurations of haplotypes, usually flanking the QTL, than a single marker. These haplotypes could in some cases have very low recombination rates with the QTL.

Several studies of simulation have evaluated the consequences of MAS in populations with a QTL segregating by comparing the use of a purely polygenic animal-BLUP model with a mixed model that incorporates both the polygenic effects and the haplotype identities. An extreme case is the result of Muewissen and Goddard (1996). They found possible increases until of 30 until a 64% in the genetic response to selection of different characteristics in the first five generations from selection when the QTL explains a 33% of the genetic variation in the base population. However, Ruane and Colleau (1995) found possible increases in selection response of only 0.2 to 1% in six generations using a single marker.

Pongpisantham (1994) found that the inclusion of markers could increase up to 15% the genetic response to selection for growth and for milk production in cattle nucleus that used multiple ovulation and embryo transfer (MOET) in the first six generations of selection.

In a review, Clarke (1997) concluded that substantial increases to the selection response are possible using MAS in several species. The range of the estimates of increase in selection response for all studies with parameters combinations giving the maximum responses were 2 to 38%. Similar range for parameters combinations giving minimum responses were -0.7 to 22.4%. She concluded that the increases in selection response from MAS could be overestimated by an inadequate modeling of the reduction in the QTL variance by selection.

The variation in the results are a consequence of differences in the assumed sizes of the QTL effects, allelic frequencies, recombination rates between the marker and the QTL, type of marker (single or haplotype), residual polygenic variances, environmental variances, number of generations of selection, population structures and selection procedures. This suggests to maintain an attitude of caution in absence of greater experimental evidence and a greater amount of simulation studies covering more specific situations, that allow to reach certain consensus on the value of the MAS in diverse characters and species.

Muewissen and Goddard (1996) concluded the following from their study on the use of the MAS:

1. MAS only can increase the rate of genetic gain in the long term, when there is a continuous advantage of new identified QTL (obviously, this has a biological limit determined by the maximum proportion of the genetic variance that can be explained by the segregation of QTL).
2. The extra genetic gain due to the MAS decreases very quickly with the number of generations of selection for a same QTL. The rate of identification of new QTL is difficult to predict.
3. The gain due to MAS for a certain QTL is higher when the characteristic is measured after the selection, as it happens with the fertility and carcass characteristics.

Some studies have shown possible economic advantages of the use of the MAS in dairy cattle (Muewissen and van Arendonk, 1992; Ruane and Colleau, 1996; Spelman and Garrick, 1997) when the benefits are evaluated for the industry as a whole. It is applied with progeny testing or multiple ovulation and embryo transfer (MOET) nuclei improvement schemes.

Other applications of genomic information in livestock improvement

The increase of the density of genome maps can have other potential beneficial effects in the genetic improvement. Through the identification of paternity in production systems, without controlled mating by means of the use of “genetic fingerprinting” or in the most precise estimation of the genetic relations between the animals, to calculate the additive numerator relationship matrix among animals (A), used in the mixed model equations for the genetic evaluations with BLUP (Nejati-javaremi et al., 1997).

Mayo (1996) analyzed the possible application of QTL technology to Merino sheep breeding in Australia. He concluded that introduction of new technology will be slow and suggests that the first technology to be used is cost-effective DNA parentage, due to the lack of pedigree information, which hinders the utilization of BLUP techniques.

Another application of genetic fingerprinting is the search of the maximization of dominance effects in crosses (to maximize heterosis) (Godshalk et al., 1990) and their use to control inbreeding. These possible applications are still preliminary ideas that require further evaluation. Current estimation methods of heterosis from crossing experiments are very expensive, thus, indirect prediction of heterosis from genetic diversity at the molecular level may be advantageous.

Marker assisted introgression

An application that has been mentioned in the literature is the introgression a major gene in another population by means of backcrosses assisted by molecular markers. In this case, it does not seem to exist advantage in using a single marker gene information, in comparison with the use of only phenotypic information when the characteristic is continuous and the considered genetic effects are additive (Groen and Smith, 1995). Nevertheless, it seems feasible that using a dense map that involves many chromosomal regions and with more than one allele of interest, the time for fixation of the major genes can be reduced.

Detection and use of major genes

In the last ten years statistical methodologies of detection of major genes based on pedigree and phenotypic information on populations have been developed for animal populations. These methods are based on the use of mixed models and segregation analysis to fit the data to a mixture genetic model that includes in addition to the polygenic effects, those of a bialalic major gene. Calculation is performed in two stages: firstly genotype probabilities are obtained, then major gene, fixed effects and polygenic effects are fitted and used to recalculate new parameters by regressing phenotypes on estimated probabilities. Calculation is iterated upon convergence (Kinghorn et al., 1989). Segregation analysis allows inferring the unknown genotypes from the probabilities of transmission of the gene given the phenotype of the individual and their relatives.
In mixture models, regression and Gibbs sampling estimation approaches have been implemented to obtain estimates of the major gene effects and allelic frequency (Bovenhuis et al., 1997).

Meuwissen and Goddard (1997) evaluated the effect of including different proportions of individuals genotyped for a QTL in a mixture model that is based on the analysis of segregation of Kerr and Kinghorn (1996) and a regression approach which uses the estimated genotype probabilities as weights in the estimation process. Unbiased estimates of QTL effect and frequency were obtained in absence of information on the genotype of the QTL, but some improvement in the precision of the estimates were observed as the proportion of genotyped individuals increased. The main limitation of this method is that the genetic hypothesis is generally limited (one biallelic locus), thus, the presence of more alleles could not be detected. Also, the location of the locus in the genome, in absence of markers, remains unknown.

Mixture models can be modified to include markers associated with the QTL, instead of the direct effect of the QTL in addition to the information of the pedigree and the phenotype. This is achieved by modifying the additive numerator relationship matrix (A), according to the conditional probabilities of transmission of the given QTL. The information of the markers (Kinghorn and Clarke, 1997).

These developments can make possible to evaluate the likelihood of the model or another fitting criterion, to prove the relation between the marker and the QTL in populations of animals with outbred mating structures. They also may increase the possibilities of making MAS in animals when incomplete information exists on the genotypes of the animals for the QTL or markers so that the use of the genomic information is optimized. Kinghorn (1997a) developed a method to evaluate the amount of genomic information that allows maximizing a function of economic utility for the analysis of QTL with mixture models.

Major genes have been detected using these methods for carcass characteristics in pigs based on a mixture model of inheritance and Gibbs Sampling (Janes et al., 1995). Also, important effects of major genes have been detected using Findgene software (Kinghorn, 1997b) for several carcass characteristics in cattle (Woodward et al., 1998) and for parasite resistance in sheep (McEwan and Kerr, 1998).

This methods that make use of information currently available in many animal populations, are an option for a preliminary screening for major genes that can contribute to rationalize the use of expensive QTL-marker linkage estimation experiments.

Discussion

A fundamental aspect for the efficient use of the markers for the MAS resides in the right detection of the location of the QTL in the chromosomes, as well as of the magnitude of its allelic effect and its allelic frequency. Most of the available studies for detection of QTL have limitations of power related to the design, structures of the pedigree and number of animals evaluated for the molecular variants. This causes that, in most of the cases, the estimations have relatively high statistical error. On the other hand, considering that in any study is necessary to accept a value for the error type I (it is to say to reject the hypothesis of existence of the QTL, when this reality it does not exist), it is possible that an important fraction of the QTL reported in the literature are a product of the expected number of null hypotheses incorrectly rejected. Low experimental power will reduce the detection rate of true QTL.

The accumulation of studies within the same chromosomal region can help to distinguish between the cases of real or false QTL detection. Meuwissen and Goddard (1996) demonstrated that the incorporation of a false QTL to MAS, can reduce the genetic gain to 14% in comparison with a usual polygenic BLUP model.

An alternative to determine the value of the MAS, is to use deterministic models of the selection response (Luo et al., 1997). The development of deterministic models that accurately predict the selection response using MAS, could simultaneously optimize selection schemes that involve the selection with BLUP, the use of reproductive techniques like the multiple ovulation and the production in vitro of embryos and the MAS in livestock populations. The use of stochastic simulation, although having advantages, presents as a main difficulty, the required time to obtain a suitable number of replicates. It is also difficult to maximize the response functions due to the number of combinations to study.

Most studies so far have dealt with the effect of MAS using rather simplified assumptions and a single trait affected by one QTL and polygenes. Studies using more realistic models such as multiple estimated QTL effects and multiple trait selection could help to make better decisions regarding the use of MAS in animal improvement.

While molecular techniques offer important options for the genetic improvement of livestock, the materialization of these expectations requires of the solution of a number important of technical problems to take advantage of all the information available an efficient way, to reduce the costs of generating genomic information and to obtain reliable estimations of the effects of the QTL and the application of the MAS and the genomic information in general for the improvement animal. A common problem related with QTL estimates is inconsistency, which means that a QTL effect is not expressed similarly in several years, or when is used in a different population (Mayo and Franklin, 1998).

Many epistatic effects have been found associated to QTL effects. In order to use molecular information in selection, reliable QTL effects should be incorporated in the models of analysis from population genetics in a way, which is consistent with observed variation. Evidence of widespread epistasis affecting QTL effects may restrict use and prediction of QTL effects outside the populations used for detection. This is particularly important when breed crosses instead of sire families in outbred populations are used as design to detecting the QTL effects (Mayo and Franklin, 1998).

Conclusions

A rational use of the molecular methodologies requires the simultaneous optimization of selection on all the genes affecting important traits in the population. The maximum benefit can be obtained when these techniques are used in conjunction with reproductive technologies like the artificial insemination, and collection and production in vitro of embryos to accelerate the genetic change (Bishop et al., 1995; Montaldo, H.H. and Meza-Herrera, C.A. 1993). There is a danger associated with a potentially inadequate use of QTL information, giving an excessively high emphasis to simple molecular information in detriment of the overall economic gain through all traits and their polygenic effects in the population. Dissemination of the information to the industry is therefore a complex issue concerning QTL effects and molecular markers.

The characteristics on which the application of the MAS can be effective are those that are expressed late in the life of the animal, or those that are controlled by a few pairs of alleles. The first example corresponds to the longevity and carcass characteristics in meat producing animals; the second, to the resistance to certain diseases or defects of simple inheritance.
Because of its high cost, the use of MAS could be justified, in animal nuclei that allow dilution of the costs when germplasm is extensively used towards the commercial population. Also in those characteristics in which the procedures of conventional selection have reached their limits in efficiency or the results have been not satisfactory.

The main advantage of including molecular information over pure mixture model and segregation analysis, is the possibility of evaluating the simultaneous effect of several QTL on the characteristcs of economic importance, and in the future increasing its precision and the complexity of the involved models of genetic action, for example QTL with multiple alleles. The present trend indicates that molecular, pedigreed and phenotypic information will be integrated in the future through mixture models of segregation analysis that might contain QTL effects through the markers, polygenetic inheritance and the use of powerful and flexible methods of estimation such as Gibbs Sampling.

Before the molecular information on the QTL which control the characteristics of economic interest is generated, the detection of major genes using segregation analysis could direct the work of identification of genotypes towards populations and characteristics with greater probability of detecting a QTL using molecular markers.

Many questions remain on the nature and action of the QTL involved on the variation of complex traits and about the nature and definition of QTL effects. The use of molecular techniques offers new opportunities and challenges for building and using more predictive and efficient statistical models for livestock improvement.

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