

IMPLEMENTATION OF MARKER ASSISTED SELECTION INTO BREEDING PROGRAMS

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Summary

Advances in molecular genetics and in high-throughput technologies for genotyping now enable the incorporation of molecular data in genetic improvement programs for livestock on a more comprehensive basis. In addition, these methods enable a change from using markers on a within-family basis for detection and selection on quantitative trait loci (QTL) to using markers that are in linkage disequilibrium across the population. Methods for including alternate types of markers, based on the structure of their associations with phenotype, will be described and issues related to their incorporation into genetic evaluation and selection programs will be discussed.

Introduction

To date, most genetic progress for quantitative traits in livestock has been made by selection on phenotype or on estimates of breeding values (EBV) derived from phenotype, without knowledge of the number of genes that affect the trait or the effects of each gene. In this quantitative genetic approach to genetic improvement, the genetic architecture of traits of interest has essentially been treated as a 'black box'. Despite this, the substantial rates of genetic improvement that have been and continue to be achieved in the main livestock species, is clear evidence of the power of quantitative genetic approaches to selection. Their success does, however, not mean that genetic progress could not be enhanced if we *could* gain insight into the black box of quantitative traits. By being able to study the genetic make-up of individuals at the DNA level, molecular genetics has given us the tools to make those opportunities a reality. Molecular data is of interest for use in genetic selection because genotype information has heritability equal to 1 (assuming no genotyping errors), it can be obtained in both sexes and on all animals, it can be obtained early in life, and it may require the recording of less phenotypic information (Dekkers and Hospital 2001, Meuwissen and Goddard 1996). Objectives of this paper are to review strategies for the use of genes or markers in genetic improvement through marker-assisted selection (MAS). The focus will be on selection within lines or breeds, which is the main avenue for genetic improvement in poultry and other livestock species.

Types of molecular data and linkage disequilibrium

Methods to detect and use molecular data for genetic improvement rely on the ability to genotype individuals for genetic loci that are associated or correlated with phenotype. Such associations will only exist for loci that either are the quantitative trait locus (QTL) (direct markers) or that are linked to the QTL (indirect markers). The latter requires not only linkage between the marker locus and the QTL but also presence of linkage disequilibrium (LD). Marker-QTL LD represents the phenomenon that alleles at the marker and QTL are not independent and is central to the use of genetic markers for both QTL detection and their use in MAS. Thus, an understanding of LD and the factors that affect the presence, extent and type of LD (population-wide vs. within

family) is important. Briefly, consider a marker locus with alleles M and m and a linked QTL with alleles Q and q . An individual with genotype $MmQq$ could have haplotypes MQ/mq or Mq/mQ , where / separates the two homologous chromosomes, and reflect the marker-QTL *linkage phase* for the individual. Presence of LD relates to the relative frequencies of alternative haplotypes in a population. In a population that is in linkage *equilibrium* (LE), alleles at the two loci are randomly assorted into haplotypes, i.e. chromosomes or haplotypes that carry marker allele M are no more likely to carry QTL allele Q than chromosomes that carry marker allele m . Because of the recombination that takes place every generation, loci in a closed population are expected to be in LE. However, there are several factors that can create LD between loci, including mutation, selection, drift (inbreeding), and migration or crossing (see Goddard and Meuwissen 2005 for further details). Once LD is created, the rate of decay of LD between two loci then depends on their recombination rate. Thus, for tightly linked loci, any LD that has been created will persist over many generations. But for loosely linked loci ($r > 0.1$), LD will decline rapidly over generations. The amount and extent of LD that exists in the populations that are used for genetic improvement is the net result of all the forces that create and break-down LD and is, therefore, the result of the breeding and selection history of the population, along with random sampling. On this basis, populations that have been closed for many generations are expected to be in LE, except for closely linked loci. Thus, in those populations, only markers that are tightly linked to QTL may show an association with phenotype, and even then there is no guarantee because of the chance effects of random sampling.

Although a marker and a linked QTL may be in LE across the population, LD will always exist *within* a family, even between loosely linked loci. Consider a sire with haplotypes MQ/mq . This sire will produce four types of gametes: non-recombinants MQ and mq and recombinants Mq and mQ . Because the non-recombinants will have higher frequency, depending on the recombination rate between the marker and the QTL, this sire will produce gametes that will be in LD. This LD will extend over larger distances (up to 20 cM), because it has undergone only one generation of recombination. This specific type of LD, however, only exists within this family; progeny from another sire, e.g. an Mq/mQ sire, will also show LD, but their LD is in the opposite direction because of the different marker-QTL linkage phase in the sire. On the other hand, MQ/mQ and Mq/mq sire families will not be in LD because the QTL does not segregate in these families. When pooled across families, these four types of LD will cancel each other out, resulting in LE *across* the population. Nevertheless, the within-family LD can be used to detect QTL and for MAS, provided the differences in linkage phase are taken into account, as will be demonstrated later.

Based on the above, three types of observable genetic loci for use in QTL detection and MAS can be distinguished, as described by Dekkers (2004):

- 1) Direct markers: loci that genotype the functional polymorphism for a QTL.
- 2) LD-markers: loci that are in population-wide linkage disequilibrium with a QTL.
- 3) LE-markers: loci that are in population-wide linkage equilibrium with the functional mutation but in linkage disequilibrium on a within-family basis.

Because of the extensive LD that exists within families, LE-markers can show associations even if they are up to 20 cM from the QTL. Thus, the LE-marker approach requires only a limited number of markers (one every 10-20 cM) to detect and use QTL. This approach does, however, require a family structure (e.g. paternal half-sib families) because co-segregation of the markers and QTL must be followed within families. In addition, the use of LE-markers puts additional demands on their application in MAS, as will be explained later, in contrast to direct or LD markers.

Within closed outbred populations, the extent of population-wide LD will be limited and LD markers must by necessity be close to the functional mutation. Studies in human populations

have generally found that LD extends over less than 1 cM. Opportunities to utilize population-wide LD in livestock populations may be considerably greater because of the effects of selection, inbreeding and crossing. Indeed, Farnir et al. (2000) identified substantial LD in the Dutch Holstein population, which extended over 5 cM. Similar results have been observed in other livestock species (e.g. in poultry; Heifetz et al. 2005). Thus, in livestock, having markers within 1-2 cM from the QTL will have a reasonable chance to result in sufficient LD with the QTL to allow detection and enable MAS. There is, however, no guarantee that a given marker that is within 1 cM will be in LD with the QTL, because presence of LD is very random and can differ substantially within even a small region of the genome.

Three strategies can be used to find markers that are in population-wide LD with QTL (Anderson, 2001):

- 1) the candidate gene approach, which involves evaluating markers that are in or close to genes that are thought to be associated with the trait of interest (Rothschild and Plastow 1999)
- 2) QTL fine-mapping approaches, starting from a previously identified QTL region, e.g. based on a cross, by saturating the region with markers.
- 3) a genome scan using population-wide LD based on a high-density marker map, with a marker every 0.5 to 2 cM.

Application of in particular the latter approach has been enhanced by recent advances in genome technology, which has enabled sequencing of entire genomes, including of several livestock species; the genomes of the chicken and cattle have been sequenced and public sequencing of the genome of the pig is underway. In addition, sequencing has been used to identify large numbers of positions in the genome that include single nucleotide polymorphisms (SNPs), i.e. DNA base positions that show variation. For example, in the chicken, over 2.8 million SNPs have been identified by comparing the sequence of the Red Jungle Fowl to that of three domesticated breeds (International Chicken Polymorphism Map Consortium, 2004). This, combined with reducing costs of genotyping, now enables genome-wide detection of QTL using LD-mapping with high-density marker maps. These technologies, combined with functional genomics, will eventually lead to the identification of the functional mutations (i.e. direct markers), but to date only a limited number of direct markers have been identified (Andersson 2001, Andersson and Georges 2004, Dekkers 2004).

Incorporating molecular data in genetic evaluation procedures

The three types of molecular loci described previously differ not only in methods of detection but also in methods of their incorporation in genetic evaluation procedures; whereas direct and, to a lesser degree, LD-markers, allow selection on genotype across the population, use of LE-markers must allow for different linkage phases between markers and QTL from family to family. The main strategies for incorporating marker data in genetic evaluation programs will be described in the following.

Genetic evaluation using LE-markers based on within-family LD.

Use of within-family LD between a QTL and a linked marker requires marker effects or, at a minimum, marker-QTL linkage phases to be determined separately for each family. This requires marker genotypes and phenotypes on family members. If linkage between the marker and QTL is loose, phenotypic records must be from close relatives of the selection candidate because associations will erode through recombination. With progeny data, marker-QTL effects or linkage phases can be determined based on simple statistical tests that contrast the mean phenotype of

progeny that inherited alternate marker alleles from the common parent. Alternatively, marker-assisted animal models using Best Linear Unbiased Prediction (BLUP) have been developed to incorporate marker data in genetic evaluation for complex pedigrees (Fernando and Grossman 1989), as will be described in the following.

Starting with the standard animal model (Lynch and Walsh 1998 Chapter 26):

$$y_i = \mathbf{X}_i \mathbf{b} + u_i + e_i,$$

where y_i is the phenotype of individual i , $\mathbf{X}_i \mathbf{b}$ represent fixed effects, u_i is the individual's breeding value, and e_i is a residual. In this model, information from relatives is included in estimation of an individual's breeding value (\hat{u}_i) by modeling covariances between breeding values across individuals based on $\text{var}(\mathbf{u}) = \mathbf{A} \sigma_u^2$, where \mathbf{u} is a vector of individual breeding values u_i , \mathbf{A} is the additive genetic relationship matrix, and σ_u^2 is the additive genetic variance for the trait. Matrix \mathbf{A} is determined from pedigree and quantifies the similarity of breeding values of each pair of individuals. For example, the additive genetic relationship of two paternal halfsibs i and j is $1/4$, indicating that paternal halfsibs share 25% of their genes with each other.

This model can be expanded to model a QTL as follows (Fernando and Grossman 1989):

$$y_i = \mathbf{X}_i \mathbf{b} + v_i^{\text{pat}} + v_i^{\text{mat}} + u_i + e_i$$

where v_i^{pat} and v_i^{mat} are the effects of the QTL allele individual i received from its sire and dam, and now u_i is the individual's breeding value for genes other than the modeled QTL (i.e. the polygenic breeding value). In contrast to the polygenic breeding value, for which covariances are still determined based on pedigree: $\text{var}(\mathbf{u}) = \mathbf{A} \sigma_u^2$, for the QTL, marker data can be used to determine covariances between QTL allele effects among relatives: $\text{var}(\mathbf{v}) = \mathbf{G} \sigma_Q^2$, where \mathbf{v} is the vector of paternal and maternal QTL alleles across individuals, σ_Q^2 is the variance explained by the QTL, and \mathbf{G} is the identity by descent (IBD) matrix among QTL alleles. Matrix \mathbf{G} can be obtained using marker and pedigree data. For example, if two paternal half-sibs i and j have inherited the same alleles from their sire for markers that flank the QTL, they are likely identical by descent for the paternal QTL allele, i.e. they have the same $v_i^{\text{pat}} = v_j^{\text{pat}}$ and the element of \mathbf{G} that corresponds to v_i^{pat} and v_j^{pat} will be 1. Thus, matrix \mathbf{G} links QTL allele effects that are expected to be equal or similar based on pedigree and marker data and enables data from relatives to be used to estimate an individual's QTL effects.

These models result in estimated breeding values (EBV) for QTL effects (\hat{v}_i^{pat} and \hat{v}_i^{mat}) along with a polygenic EBV (\hat{u}_i). The total EBV is the simple sum of these estimates:

$$\text{EBV}_i = \hat{v}_i^{\text{pat}} + \hat{v}_i^{\text{mat}} + \hat{u}_i.$$

These models can be extended to multiple QTL as follows:

$$y_i = \mathbf{X}_i \mathbf{b} + \sum_k (v_i^{\text{pat},k} + v_i^{\text{mat},k}) + u_i + e_i$$

where the summation is over all QTL regions k . One of the main computational limitations of this method, however, is the large number of equations that must be solved, which increases by two per animal for each QTL that is fitted. Thus, the number of QTL regions that can be incorporated is limited.

Genetic evaluation using LD-markers based on population-wide LD.

The model of Fernando and Grossman (1989), using LE-markers, models similarity between QTL alleles only on a within family basis. Thus, QTL alleles from individuals that cannot be shown to be related based on the available pedigree (which usually only goes back a limited number of generations) are assumed to be independent, even if these two individuals have the same marker genotypes. This is because marker-QTL linkage phases may differ from family to family. Thus, markers and the QTL are assumed to be in LE among founders. As a result, only data from within the family is used to estimate QTL effects. When markers are tightly linked to the QTL, they can however be in substantial LD with the QTL across families, as explained previously, and data from (apparent) unrelated individuals that have similar marker genotypes or haplotypes can be used to estimate QTL effects.

There are different ways to incorporate such population-wide LD information in genetic evaluation. One, which is due to Meuwissen and Goddard (2000) and was developed for QTL fine mapping but can also be used for genetic evaluation, is based on an extension of the model of Fernando and Grossman (1989):

$$y_i = \mathbf{X}_i \mathbf{b} + v_i^{\text{pat}} + v_i^{\text{mat}} + u_i + e_i$$

but with the covariance among QTL alleles now derived based on marker-QTL LD *across* rather than within families. In this method, the variance-covariance structure among QTL effects is modeled using an IBD matrix that is based on population-wide LD, \mathbf{G}_{LD} . Elements of this matrix that correspond to QTL effects of individuals i and j are based on probabilities that the QTL alleles associated with the markers or marker haplotypes carried by i are IBD to the QTL alleles associated with the markers of marker haplotypes carried by j . These IBD probabilities can be derived based on similarity of the marker haplotypes that the individuals carry. For example, when haplotypes that are carried by individuals i and j are identical for a larger number of markers around the QTL position, they are more likely to trace back to a common ancestor and, therefore, be IBD, depending on the population history. Thus, although pedigree and marker data may not go back far enough into history to trace back to this common ancestral haplotype, IBD probabilities for these 'related' haplotypes can be derived when making assumptions about the historical population structure, such as effective population size and number of generations since the mutation that created the polymorphism at the QTL, along with QTL position. Simulation and analytical methods to compute these probabilities have developed by Meuwissen and Goddard (2001).

In practice, marker information may be available for several generations, enabling the use of both within-family LD (based on pedigree and marker data) and population-wide LD (based on population history and marker genotypes of founders). Combining the LE and LD approaches, the variance-covariance matrix for the QTL effects can be modeled as $\mathbf{G} = \mathbf{G}_{\text{LD}} + \mathbf{G}_{\text{CS}}$, where \mathbf{G}_{LD} is the IBD matrix among QTL alleles in the founders and \mathbf{G}_{CS} is the IBD matrix that is based on pedigree and marker data, computed as described for LE-markers (Meuwissen et al. 2002, Lee and van der Werf 2004).

An alternative to modeling population-wide LD as covariances between QTL using IBD is to simply include the marker genotype or haplotype as a fixed effect in the animal model evaluation, as suggested by Fernando and Totir (2004) and Fernando (2004). Thus, if only LD information is available:

$$y_i = \mathbf{X}_i \mathbf{b} + \mathbf{g}_i^k + u_i + e_i,$$

where g_i is the fixed effect of the marker genotype or marker haplotype. If marker data are available for several generations, fixed effects can also be added to the within-family LD model of Fernando and Grossman (1989) as:

$$y_i = \mathbf{X}_i \mathbf{b} + g_i^k + v_i^{\text{pat}} + v_i^{\text{mat}} + u_i + e_i,$$

with covariances among QTL effects modeled based on within-family LD alone.

An advantage of modeling population-wide LD effects as fixed rather than random is that fewer assumptions about population history are needed. A disadvantage is that estimates are not 'BLUP'ed', i.e. regressed toward a mean depending on the amount of information that is available to estimate their effects. This will be important if some of the genotype or haplotype effects cannot be estimated with substantial accuracy because the number of individuals with that genotype or haplotype is limited.

If only additive effects are of interest, g_i can be replaced by a regression on the number of copies of allele 1 at marker k that is carried by individual i (n_i^k):

$$y_i = \mathbf{X}_i \mathbf{b} + n_i^k h^k + u_i + e_i,$$

where h^k represents the average effect of one copy of allele 1 for QTL k , relative to some base (e.g. as a difference from the other allele). The total EBV of the animal can then be estimated as:

$$\text{EBV}_i = n_i^k \hat{h}^k + \hat{u}_i.$$

These models can be expanded to include multiple LD markers by adding substitution effects for each marker:

$$y_i = \mathbf{X}_i \mathbf{b} + \sum_k n_i^k h^k + u_i + e_i.$$

Models can also be expanded to include marker haplotypes for QTL region k by including a regression on the number of copies of each haplotype l that is carried by individual ($= n_i^{h,k}$):

$$y_i = \mathbf{X}_i \mathbf{b} + \sum_h n_i^{k,l} h^{k,l} + u_i + e_i$$

All these methods are in principle aimed to model QTL that have already been detected. This involves a two-step approach, in which first high-density marker maps are used to detect significant QTL regions based on population-wide LD, and in the second step these QTL regions are implemented for genetic evaluation. Meuwissen et al. (2001) suggested an alternative approach that eliminates the need for prior QTL detection. In this approach, marker haplotypes are fitted for each, e.g., 1 cM region of the genome using the following model:

$$y_i = \mathbf{X}_i \mathbf{b} + \sum_k \sum_l n_i^{k,l} h^{k,l} + e_i,$$

where the summation is over all QTL regions k and all haplotypes l that are present in the population for each region k . A difference from the previously described haplotype regression approach, is that in this approach haplotype effects are modeled as independent random effects, rather than fixed effects. The advantage of treating haplotype effects as random is that it allows a large number of regions to be fitted simultaneously (3000 or more), which would not be possible for fixed effects, and that the estimates are BLUPed. Implementing this procedure does require the variance associated with each 1 cM region. In the work of Meuwissen et al. (2001), these were

either assumed to be equal for each 1 cM region or estimated from the data using Bayesian procedures with alternate prior distributions. In essence, this procedure estimates breeding values $\hat{h}^{k,l}$ for each haplotype (l) and QTL region (k) and EBV of individuals are computed by simply summing EBV for the haplotypes that they contain:

$$EBV_i = \sum_k \sum_l n_i^{k,l} \hat{h}^{k,l} .$$

Using this procedure, Meuwissen *et al.* (2001) demonstrated through simulation that, for populations with an effective population size of 100 and a 1 or 2 cM spacing between markers across the genome, sufficient disequilibrium was present that genetic values could be predicted with substantial accuracy for several generations on the basis associations of marker haplotypes with phenotype on as few as 500 individuals. New high-throughput technologies now enable to conduct this for reasonable costs. In addition, opportunities may exist to utilize this approach on a limited scale by saturating previously identified QTL regions with markers.

Note that in the approach proposed by Meuwissen *et al.* (2001) no polygenic effect is included since all regions of the genome are included in the model. It may, however, be useful to include a polygenic effect because LD between markers and QTL will not be complete for all regions. In addition, this model assumes all haplotype effects are independent both within and across regions. Further improvements of the model could be made by incorporating IBD probabilities to model covariances between haplotypes within a region, as in Meuwissen and Goddard (2000) and by incorporating covariances between adjacent regions caused by LD between regions. These additions would, however, lead to increasing computational complexity of the model.

Genetic evaluation using direct markers.

When individuals can be genotyped for the functional mutation of the QTL, genotype can be included as a fixed effect in the genetic evaluation model, similar to including genotype or haplotype for LD-markers as fixed effects. For individuals that are not genotyped, genotype probabilities will need to be derived (Israel and Weller 1998).

Integration of MAS in breeding programs

The main principles and limitations of MAS in both livestock and plant breeding were described by Dekkers and Hospital (2001) and issues related to livestock were further described by Dekkers (2004). An important consideration in this regard whether work toward the use of LE-, LD-, or direct markers (Dekkers 2004). Opportunities for increases in genetic gain from a given QTL are lowest with LE-markers because of the limited information that is available to estimate effects on a within-family basis, while for both LD- and direct markers, effects are estimated from data across families. Accuracy of estimates may be slightly lower for LD-markers than for direct markers as a result of incomplete marker-QTL disequilibrium and a greater number of effects that must be fitted, i.e. marker haplotypes versus QTL genotypes (Hayes *et al.* 2002).

Although the use of LE-markers requires fewer markers to be genotyped because they can be some distance from the QTL, this strategy requires phenotyping and genotyping of relatives of each selection candidate, because QTL effects must be estimated for each family and updated frequently. In contrast, LD- and direct markers allows data from unrelated individuals that have the same marker genotype to contribute to the EBV of a selection candidate. Thus, estimates of effects

can be based on a random sample of individuals in the population that are genotyped and phenotyped and used for unrelated individuals. Thus, for the same number of individuals phenotyped and genotyped, LD- and direct markers are expected to result in greater accuracy of EBV. In either approach, however, it will be essential to estimate its effects within the population under selection to guard against potential interactions of the QTL with the background genome and the environment. This is more important for LD-markers than direct markers because the degree of LD between the markers and QTL may differ between populations and may change over time. Thus, it will also be prudent to re-estimate the effects on a regular basis.

Acknowledgements

The author acknowledges Rohan Fernando for stimulating discussions regarding the topics described herein.

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