

# Marker-assisted Selection<sup>1)</sup>

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1) Based in part on Dekkers et al. (2001)

## 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 are clear evidence of the power of quantitative genetic approaches to selection. The success of quantitative genetic approaches 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.

Pork quality comprises a set of fresh meat, processing, and organoleptic characteristics that are important for the future profitability and competitiveness of the swine industry. These include intramuscular fat, cholesterol, ultimate pH, color, water holding capacity or drip loss, tenderness, cooking loss, and sensory traits involving taste (Sellier 1998). Genetics plays a key role in pork quality, as has been demonstrated by substantial breed differences and within-breed heritabilities. Heritabilities for most pork quality traits range from 0.15 to .5 (see Sellier, 1998 for a review). Improving meat quality genetically is difficult by conventional selection methods based on phenotype because most meat quality traits can only be measured after slaughter. In addition, only phenotypes of relatives can be used to estimate breeding values, which limits their accuracy. These limitations make meat quality traits ideal candidates for the use of MAS. Objectives of this paper are to review strategies for the use of genes or markers in genetic improvement, with specific emphasis on the application of MAS to genetic improvement of meat quality.

## Types of genetic markers

Application of molecular genetics for genetic improvement relies on the ability to genotype individuals for specific genetic loci. For these purposes, three types of observable genetic loci can be distinguished (Figure 1):

- 1) Direct markers: loci for which the functional polymorphism can be genotyped.
- 2) LD markers: loci in population-wide linkage disequilibrium with the functional mutation.
- 3) LE markers: loci in population-wide linkage equilibrium with the functional mutation.

Figure 1. Three types of observable molecular genetic loci.

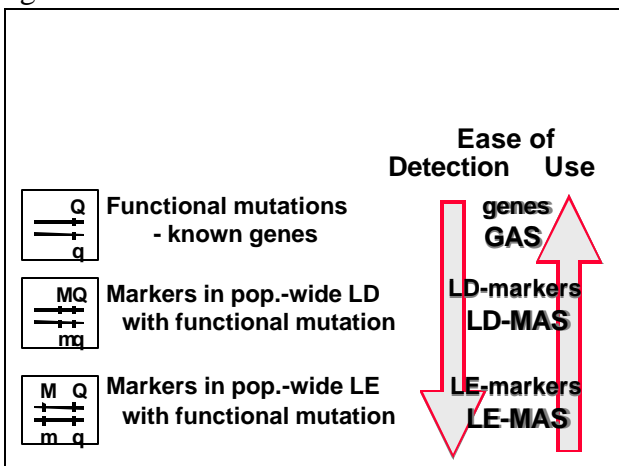
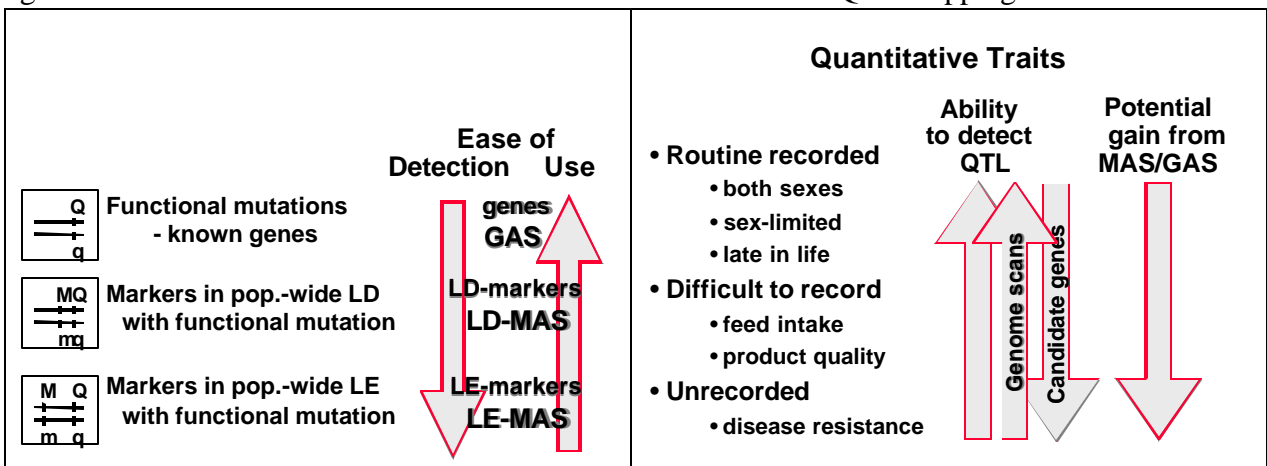


Figure 2. Types of quantitative traits and implications for QTL mapping and MAS.



Methods to detect these types of loci were described in Anderson (2001) and in previous papers in these proceedings. LE markers can be readily detected on a genome-wide basis using breed crosses or analysis of large half-sib families, requiring only sparse marker maps (20 cM spacing). Many examples of successful applications of this methodology for detection of QTL regions are available in the literature. Classification of a marker as an LD- versus LE marker depends on the population structure and the extent of LD that is present in that population. For breed crosses, LD is extensive and markers that are 10-20 cM from the QTL will still be in extensive population-wide LD. However, within closed outbred populations, the extent of LD will be limited and LD markers must by necessity be close to the functional mutation (within 1 to 5 cM depending on population history). In closed breeding populations, identification of LD markers, therefore, is more difficult and requires candidate gene approaches (Rothschild and Soller, 1997) or fine-mapping approaches (Anderson, 2001) with high-density marker maps. Functional mutations are most difficult to detect and few examples are available (Anderson, 2001).

## **Traits with MAS application**

Molecular markers have been used to identify loci or chromosomal regions that affect single gene traits and quantitative traits. Single gene traits include genetic defects or disorders and appearance. For the purposes of QTL detection and application, quantitative traits can be categorized into a) routinely recorded traits, which be further subdivided into traits that are recorded on both sexes, sex-limited traits, and traits that are recorded late in life, b) difficult to record traits (feed intake, product quality), and c) unrecorded traits (disease resistance) (Figure 2). The ability to detect QTL decreases in the order a), b), c) because of the availability of phenotypic data. For a similar reason, genome scans, which require more phenotypic data, are often used to detect QTL for traits in category a), whereas candidate gene approaches are more often used to identify QTL for traits that are not routinely recorded (b and c). Potential extra genetic gains from marker-assisted selection are greatest for traits in category c) and lowest for traits in category a), in particular for traits that are routinely recorded on both sexes prior to selection, in inverse proportion to the ability to make genetic progress using conventional methods (e.g. Meuwissen and Goddard, 1996).

## **General strategies for selection using molecular genetic information**

Once markers that are linked to QTL have been identified, their effects can be estimated based on the association between phenotype and genotype. The resulting estimates can be used to assign a 'molecular score' to each selection candidate, which can be used to predict the genetic value of the individual and used for selection. The constitution and method of quantification of the molecular score depends on type of LD that is used and on how the marker will be used in selection.

The three types of molecular loci identified in Figure 1 differ not only in methods of detection but also in methods of application in selection programs; whereas functional mutations 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. Thus, the ease and ability to utilize markers in selection is opposite to their ease of detection and increases from functional mutations to LD-markers and LE-markers (Figure 1). In what follows, selection on each of these three types of markers will be referred to as gene-assisted selection (GAS), LD-markers assisted selection (LD-MAS), and LE-marker assisted selection (LE-MAS).

In addition to a molecular score, individuals can also obtain a regular estimate of the breeding value for the collective effect of all the other genes (polygenes) on the trait. A summary of approaches and strategies for the use of molecular genetic information in genetic improvement is given in Table 1. Details of the various uses are provided in the remainder of this paper.

In general, at the time of selection, both molecular and phenotypic information is available for use in selection. The following three selection strategies can then be distinguished:

- 1) Select on the molecular score alone
- 2) Tandem selection, with selection on molecular score, followed by selection on phenotype-based EBV
- 3) Selection on an index of the molecular score and the regular EBV

Selection on molecular score alone ignores information that is available on all the other genes (polygenes) that affect the trait and is expected to result in the lowest response to selection, unless all genes that affect the trait are included in the molecular score. This strategy does, however, not require additional phenotypes, other than those that are needed to estimate marker-effects, and can be attractive when phenotype is difficult or expensive to record (e.g. disease traits, meat quality, etc.).

**Table 1. Strategies for the use of molecular data in genetic improvement programs**

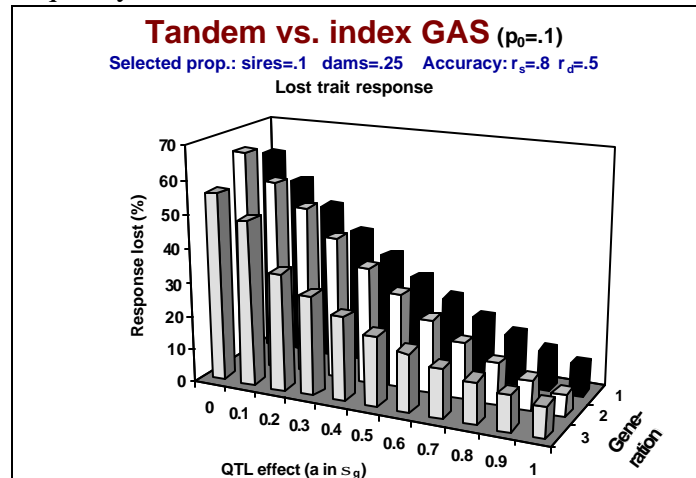
<b>Selection Program</b>	<b>Marker requirements</b>	<b>Information required to compute molecular score</b>	<b>Composition of molecular score</b>	<b>Selection criterion</b>
<b>BETWEEN BREED</b> ( <i>all LD-MAS, capitalizing on extensive LD in crosses</i> )				
<b><i>Introgression</i></b>				
• Foreground	<10 cM from QTL	Line origin of marker alleles at target loci	Presence/absence target alleles	Mol. score
• Background	~20 cM intervals genome-wide	Line origin of marker alleles	% recipient breed alleles <sup>1)</sup>	Mol. score (recipient trait phen.)
• Intercross	<5-10 cM from QTL	Line origin of marker alleles at target loci	# target alleles	Mol. score
<b><i>Synthetic line development</i></b>	<10 cM from QTL	Line origin of QTL/ marker alleles Estimates of QTL/marker effects	Sum of QTL/marker estimates	Mol. score Phen. EBV
<b>WITHIN BREED selection</b>				
<b><i>LD-MAS/GAS</i></b> Direct markers, Cand. genes, LD-markers	<1-2 cM from QTL	Genotype at QTL/markers Estimates of QTL/marker effects	Sum of QTL/marker estimates	Mol. score Phen. EBV
<b><i>LE-MAS</i></b>	<10 cM from QTL	Parental origin marker alleles Within-family estimates of QTL/marker effects	Sum of QTL/marker estimates	Mol. score Phen. EBV

- 1) Greater emphasis on markers linked to target loci to reduce linkage drag

If both phenotypic and molecular information is available on selection candidates, index selection is expected to result in greater response to selection than tandem selection (Figure 3). The reason is similar to why two-trait selection using independent culling

levels is expected to give lower multiple-trait response than index selection; two-stage selection does not select individuals for which a low molecular score may be compensated by a high phenotype-based EBV. The choice between these strategies (and other alternatives) also depends on other factors, such as market and cost considerations.

Figure 3. Response lost over 1, 2, and 3 generations from tandem versus index selection on a QTL with initial frequency of the favorable allele of 0.1, and additive effect  $a$ .



### Genetic improvement of meat quality using between-breed variation

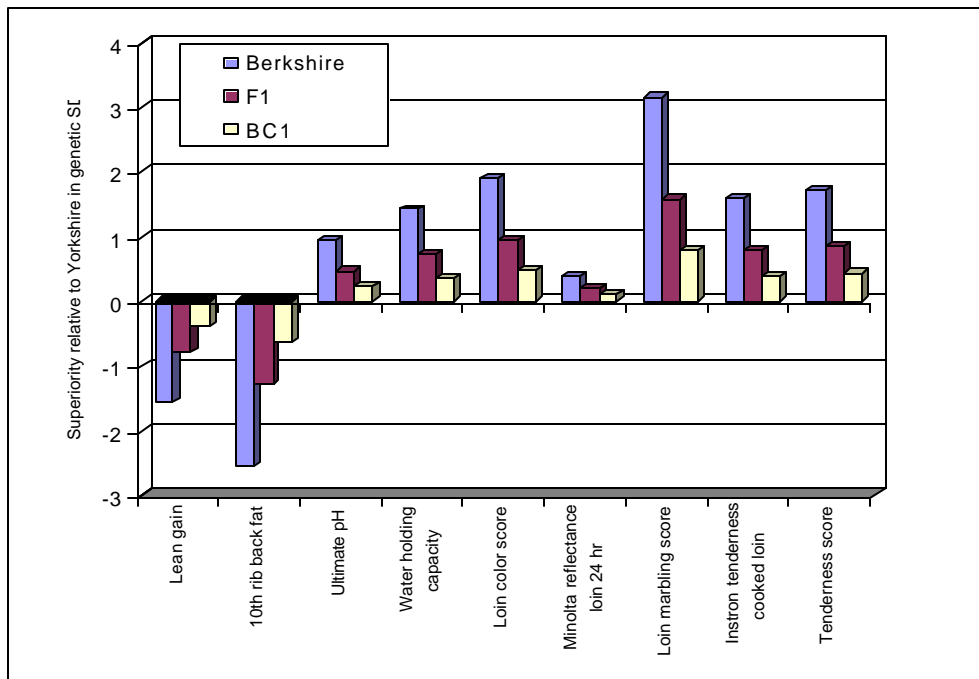
Genetic improvement of meat quality can be accomplished by within-breed selection and/or by capitalizing on between breed differences. Both can be achieved to some degree through conventional selection using phenotype but can be enhanced through the use of molecular information. In what follows, we will first discuss opportunities for utilizing between-breed differences and then evaluate strategies for within-breed selection.

Substantial differences in meat quality have been found between breeds (Sellier, 1998). For example, the Berkshire breed has been identified to have superior meat quality characteristics compared to other commercial breeds that are available in the US (Goodwin and Burroughs, 1995). Growth and backfat thickness of the Berkshire breed is, however, substantially inferior. An objective, therefore, could be to combine the superior meat qualities of the Berkshire breed with the superior growth performance characteristics of another breed. We will first discuss opportunities to achieve this using conventional selection strategies, followed by opportunities for the use of molecular genetic information.

#### *Conventional between-breed selection for meat quality*

Integrating the superior meat quality characteristics of the Berkshire into a purebred Yorkshire nucleus line by conventional means requires development of a synthetic line through an initial cross between the two breeds. This would be followed by selection within the synthetic on performance traits or, ideally, on a combination of performance and meat quality. The latter will depend on availability of records on meat quality (see later).

Figure 4. Genetic mean of the Berkshire breed and its crosses with Yorkshire, relative to the Yorkshire breed mean. Based on breed differences estimated in Goodwin and Burrows, (1995).



Assuming little or no heterosis, the initial cross would be intermediate to the parental breeds for both meat quality and performance. Depending on the relative importance of meat quality and performance, and breed differences for these traits, one or more backcrosses to one of the parental breeds could follow the initial cross. For example, assuming performance traits have greater economic importance than meat quality and that breed differences for performance traits are substantial, the F<sub>1</sub> could be backcrossed to the Yorkshire breed to reduce the genetic lag for performance characteristics. Other breeds could be introduced in such crosses also. Figure 4 shows the relative performance of the Berkshire and different crosses, expressed as a deviation from purebred Yorkshire in within-breed genetic standard deviations. This illustrates that through appropriate crosses a synthetic can be developed that has some of the superior meat quality characteristics of the Berkshire, although at some cost with regard to performance traits.

#### ***Marker-assisted improvement of meat quality using between-breed variation***

Molecular information can enhance the process of integrating superior qualities of different breeds in a number of ways. All of these rely on crosses which, as described in a previous paper in this proceedings (Dekkers, 2003), results in extensive LD, which can be capitalized on using MAS. If a large proportion of the breed difference in meat quality is due to a small number of genes, introgression strategies can be used. If a larger number of genes is involved, marker-assisted selection within a synthetic line is the preferred method of improvement. These strategies will be further described below.



### *Marker-assisted introgression*

Within the context of meat quality, the aim of an introgression program is to introduce one or more meat quality genes (target genes) from a breed that is superior for meat quality but inferior for performance (the donor breed) into a high performance line that lacks the target genes (the recipient breed). This is done through an initial  $F_1$  cross followed by multiple backcrosses to the recipient breed and one or more generations of intercrossing (Figure 5). The aim of the backcross generations is to generate individuals that carry one copy of the donor QTL allele but that are similar to the recipient breed for the rest of the genome. This is accomplished by successive backcrosses to the recipient breed to 'dilute' the donor genome, while maintaining the donor allele at the QTL by selecting only carriers as parents of the next generation. The aim of the intercrossing phase is to fix the donor allele at the QTL. The end result is a population that is similar to the recipient breed, except for carrying two copies of the donor allele at the QTL.

The effectiveness of introgression schemes is limited by the ability to identify backcross or intercross individuals that carry the target gene(s) and by the ability to identify backcross individuals that have a high proportion of the recipient genome, in particular in the region(s) around the target gene(s) (Figure 6). The latter affects the number of backcross generations required to recover the recipient genome. Molecular genetics can enhance the effectiveness of both phases of an introgression program. Effectiveness of the backcrossing phase can be increased in two ways: i) by identifying carriers of the target gene(s) (foreground selection), and ii) by enhancing recovery of the donor genetic background (background selection). Effectiveness of the intercrossing phase can also be enhanced through foreground selection on the target gene(s).

Figure 5. General outline of a QTL introgression program, designed to introgress favorable QTL allele (Q) from a donor breed into an otherwise superior recipient breed that lacks that QTL.

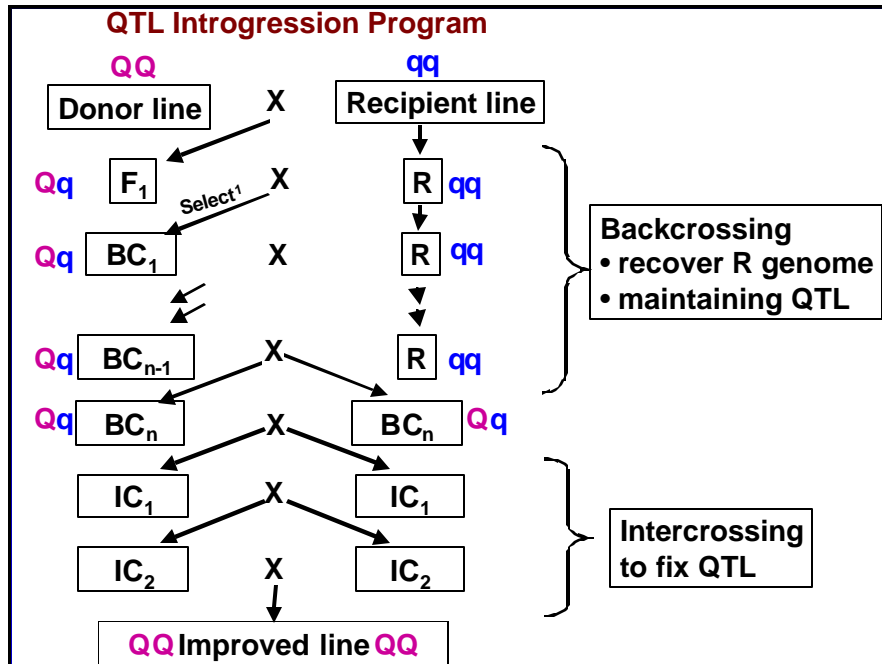


Figure 6. Opportunities to use markers in QTL introgression by fore- and background selection.

**Use of Markers in Introgression**

Cross	Progeny	Progeny genotypes	Freq. of Q	Genotype selected	% of R genome	Average	95% Range
D x R	F <sub>1</sub>	Qq	0.50	Qq	50	50	- 50
F <sub>1</sub> x R	BC <sub>1</sub>	Qq/qq	0.50	Qq	75	66.6 - 83.4	
BC <sub>1</sub> x R	BC <sub>2</sub>	Qq/qq	0.50	Qq	87.5	80.7 - 94.3	
BC <sub>2</sub> x R	BC <sub>3</sub>	Qq/qq	0.50	Qq	93.75	88.8 - 98.8	
BC <sub>3</sub> x R	BC <sub>4</sub>	Qq/qq	0.50	Qq	96.88	93.5 - 100	
BC <sub>n</sub> x BC <sub>n</sub>	IC <sub>1</sub>	QQ/Qq/qq	0.50	QQ (+Qq?)	99.?		
IC <sub>1</sub> x IC <sub>1</sub>	IC <sub>2</sub>	QQ/Qq/qq	>0.50	QQ (+Qq?)	99.?		
IC <sub>k</sub> x IC <sub>k</sub>	IC <sub>k+1</sub>	QQ/Qq/qq	>0.50	QQ	99.?		

**1) Identify Qq individuals in BC**

- single marker
- flanking marker

**2) Speed recovery of background genome during BC (and IC)**

- select on phenotype (among Qq BC animals) = traditional way
- select on markers spread over genome (among Qq BC's)

**3) Selection among juvenile animals (short gen. interval)**

**4) Type of environment not of concern**

Note that the use of markers in either fore- or background selection does not require estimation of the marker or QTL effects. Instead, their use relies on breed differences and the association of marker alleles with these breed differences as a result of extensive LD.

For foreground and intercross selection, selection is on a molecular score that is based on presence or absence of the target allele (only individuals that carry the allele are selected) (Table 1). If the target gene cannot be directly genotyped, carrier individuals can be identified based on markers that flank the QTL at <10 cM, because of the extensive LD in crosses. The markers must have breed-specific alleles, such that line origin can be identified. The effectiveness of foreground selection depends on the number of target genes and on the confidence interval for the position of those genes. The latter determines the size of the genomic region that must be introgressed. Both factors have a large impact on the number of individuals that is required to find individuals that are carriers for all target genes during the backcrossing phase and homozygous during the intercrossing phase. For the introgression of multiple target genes, gene pyramiding strategies can be used during the backcrossing phase to reduce the number of individuals required (Hospital and Charcosset, 1997; Koudandé et al. 2000).

For background selection, markers are used that are spread over the genome at < 20 cM intervals, such that most genes that affect the trait will be within 10 cM from a marker. Again, markers must have breed-specific alleles to allow the tracing of alleles back to their breed origin. Marker genotypes are then used to estimating the proportion of the recipient genome present in an individual, which is used as the molecular score (Table 1). Individuals with the highest proportion are selected. Combining foreground and background selection, selection will be for the donor breed segment around the target locus but for recipient breed segments in the rest of the genome. Foreground selection will result in selection for not only the target locus but also for donor breed loci that are linked to this locus, some of which could have an unfavorable effect on performance. To reduce this so-called linkage drag around the target locus, greater emphasis can be given in the molecular score used for background selection to markers that are in the neighborhood of the target locus (apart from the flanking markers, which are used in foreground selection).

Most studies have considered marker-assisted introgression (MAI) of single QTL (e.g., Hospital and Charcosset, 1997) but often several QTL must be introgressed simultaneously. Several studies (e.g. Koudandé et al., 2000) have shown that large population sizes are needed to obtain sufficient individuals that are heterozygous for all QTL in the backcrossing phase. This would make MAI not feasible in livestock breeding programs.

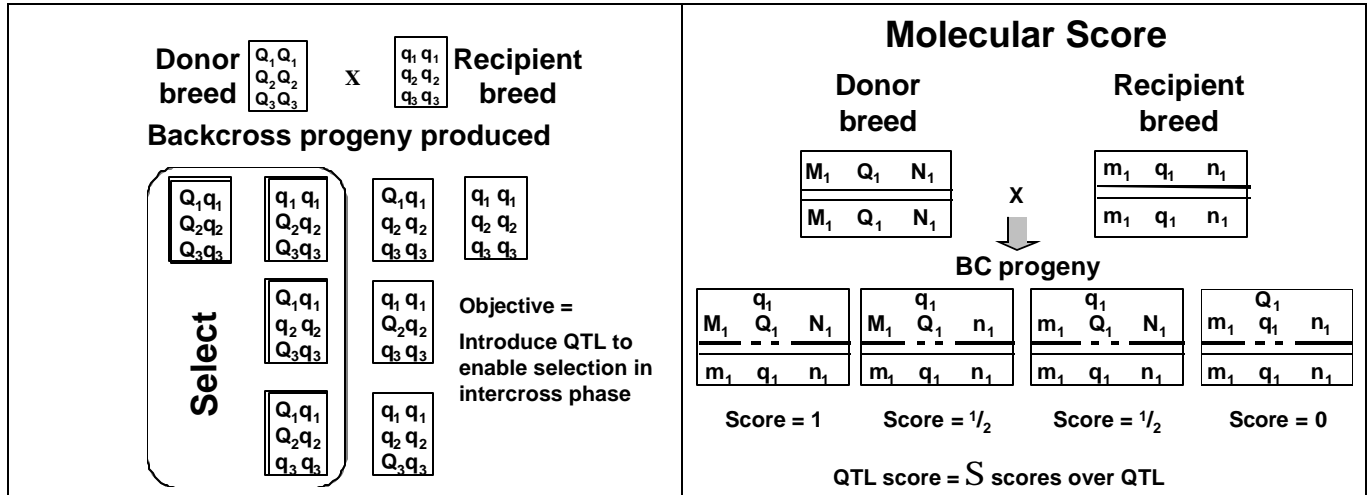
In many cases, however, immediate fixation of introgressed QTL alleles may not be required. Instead, the objective of the backcrossing phase can be to enrich the recipient breed with the favorable donor QTL alleles at high enough frequency such that they can be selected on following backcrossing (Figure 7).

In this case, individuals can be selected during the backcross phase based on a molecular score computed as the expected number of donor alleles at the  $n$  introgressed QTL, as determined from marker genotypes:  $MS = \sum_i^n P(Q_i)$ , where  $P(Q_i)$  is the probability that the individual carries the donor allele for QTL  $i$  (Figure 8). Probabilities  $P(Q_i)$  can be set

equal to 1, 1/2, and 0 if the individual carries 2, 1, and 0 donor alleles at the two markers that flank the QTL, ignoring double recombinants. Selection can be on a similar score during the intercrossing phase.

Figure 7. Introgression of multiple QTL. introgression

Figure 8. Molecular score for multiple QTL based on flanking markers.



Figures 9 and 10 shows the effectiveness on introgression using these strategies, as evaluated by the frequency of donor QTL alleles, averaged over QTL. Results are based on simulation for introgression of one, three, or five unlinked QTL, which are simulated at the center of marker intervals of 0, 5, or 20 cM (see Chaiwong et al., 2002 for further details). A total of 500 BC progeny were generated each generation by mating 2, 5, 10, or 20% of BC individuals to the recipient parental line.

Figure 9. Effectiveness of introgression of 3 QTL with known genotype (markers at 0 cM) apart.

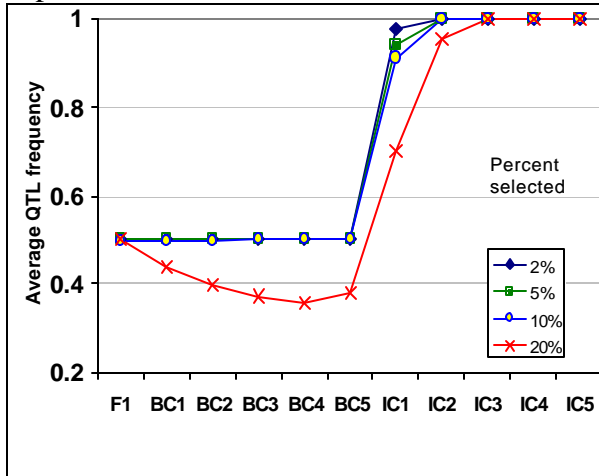
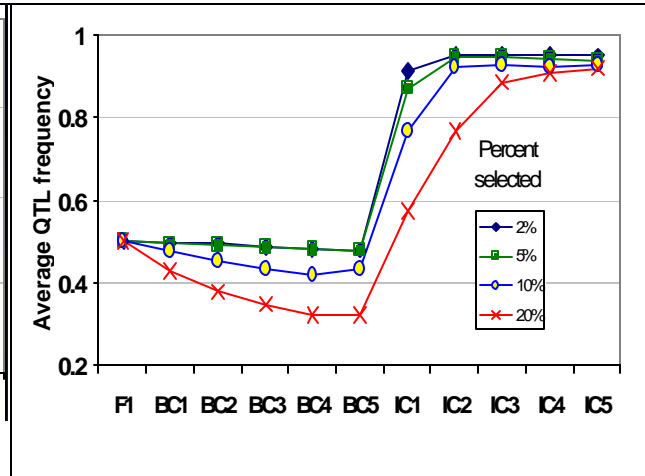


Figure 10. Effectiveness of introgression of 3 QTL using flanking markers 20 cM apart.



Results presented in Figures 9 and 10 show that, although it may not be possible to maintain a frequency of 50% during backcrossing in populations of limited size, MAI can introduce multiple QTL alleles at frequencies that will enable their selection following backcrossing. In this study, five generations of backcrossing were used and selection was on the QTL alone. Ultimately, the optimal selection strategy, including the number of generations of backcrossing, must be based on an economic analysis that involves the effects of the QTL, the difference in background genome effects, the opportunity cost of potential selection response that is lost for other genes, and the costs that are associated with an introgression program.

#### Marker-assisted synthetic line development

Lande and Thompson (1990) proposed a strategy for marker-assisted selection within a hybrid population created by crossing two inbred lines. The strategy capitalizes on population-wide LD that initially exists in crosses between lines or breeds. Thus, marker-QTL associations identified in the  $F_2$  generation can be selected on for several generations, until the QTL or markers are fixed or the disequilibrium disappears (Figure 11). Zhang and Smith (1992) evaluated the use of markers in such a situation with selection on BLUP EBV. They compared the following three selection strategies:

MAS: selection on a molecular score derived from marker effects

BLUP: selection on BLUP EBV derived from phenotype

COMB: combined selection on an index of the EBV based on markers and phenotype.

Data for a cross between inbred lines were simulated on the basis on 100 QTL and 100 markers in a genome of 2000 cM. Marker effects were estimated in the  $F_2$  generation using a two step procedure (Figure 11). In the first step, a separate  $F_2$  population from the same cross was used to identify markers with the largest effects. Then, to obtain unbiased estimates, the effects of those markers were re-estimated in the  $F_2$  population under

selection. The latter were used to obtain marker-based EBV, which were used as the molecular score throughout the selection process.

Figure 11. Marker-assisted selection on markers in synthetic lines.

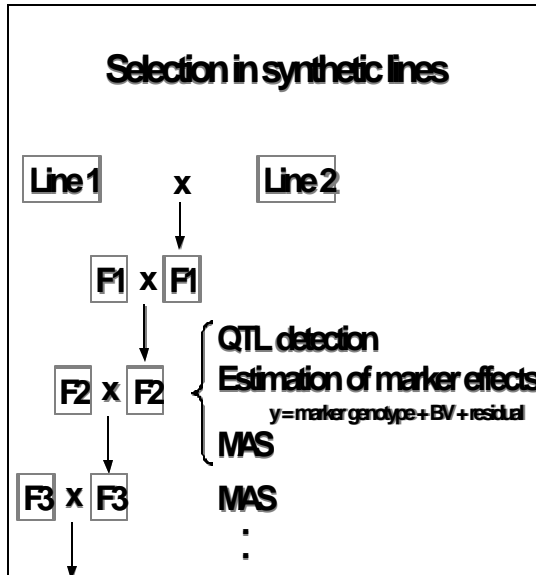
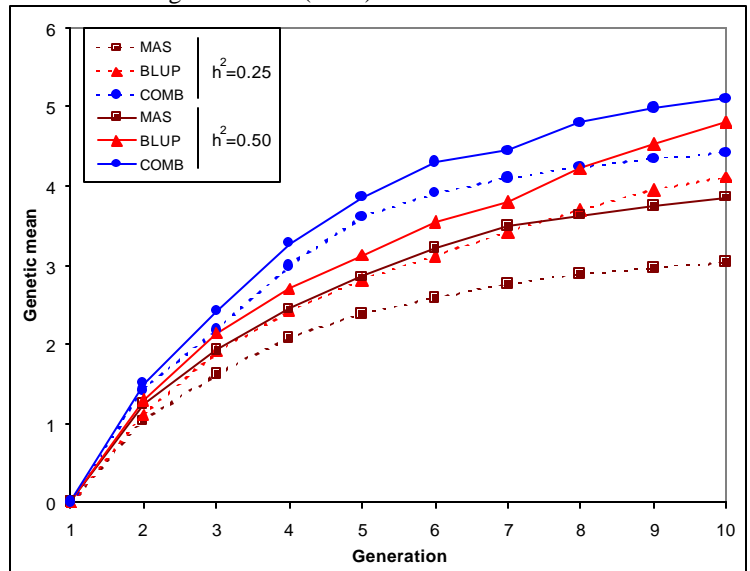


Figure 12. Genetic progress based on selection alone (MAS), phenotypic data alone (BLUP), or their combination (COMB) in a cross between inbred lines.

Based on Zhang and Smith (1992)



Results illustrated in Figure 12 show that index selection (COMB) resulted in greatest response, followed by selection on BLUP EBV and selection on markers alone. Rates of response declined over generations for all strategies because data were simulated using a finite number of loci, which were moved to fixation by selection. Rates of response declined faster for the MAS strategy because recombination eroded the disequilibrium between the markers and QTL. Nevertheless, substantial rates of response were obtained using selection on markers alone.

The MAS strategy of Zhang and Smith (1992) has potential for selection for meat quality traits because it does not require continuous phenotypic evaluation of meat quality traits, in contrast to the BLUP and COMB strategies. Although Gimelfarb and Lande (1994) showed that greater response could be obtained by re-estimating marker effects in subsequent generations, this would require the continuous recording of phenotypic data, the cost of which may not outweigh the benefits.

Zhang and Smith (1992) considered the ideal situation of a cross with inbred lines. Although the lines were not divergent for the trait of interest, they were homozygous at alternate alleles for all loci. Breeds used in a cross to enhance meat quality will typically have different means, which will increase the extent of linkage disequilibrium in the cross. However, both breeds will likely segregate for most QTL, which will reduce the disequilibrium. Nevertheless, even in crosses between commercial breeds of swine, substantial numbers of QTL have been found for which the breeds have sufficient

differences in frequency to allow their detection (Malek et al., 2001a,b, Grindflek et al., 2001). In addition, favorable effects have been found to originate from the breed with the lower mean for a number of QTL (Malek et al., 2001b).

A greater problem with the use of crosses between outbred instead of inbred lines is the limited ability to follow QTL past the  $F_2$  generation. In contrast to inbred lines, markers are not fully informative in crosses between outbred lines. Therefore, the ability to track breed origin of markers or marker haplotypes will decrease over generations, unless a substantial number of markers are genotyped within the QTL regions.

An important advantage of selection in a breed cross population is that it can capitalize on QTL identified in breed-cross studies. This could remove the first step in the estimation process used by Zhang and Smith (1992), i.e. that of identification of markers with large effects. Although this does entail the risk that different QTL may segregate in the population under selection, in particular if QTL studies were based on different breeds, there would be a substantial cost saving. It is crucial, however, that the second step of the estimation process be conducted in the population under selection, in order to obtain unbiased estimates of QTL effects that are relevant to the population under selection. This requires slaughter of a substantial number of  $F_2$  individuals to obtain phenotypic data on meat quality. Thus, the size of the  $F_2$  population must be sufficient to support both marker effect estimation and selection.

An alternative approach to QTL detection and estimation was suggested and evaluated by Whittaker et al. (1997). They used a cross-validation approach that allowed the same  $F_2$  population to be used for both selection of markers and estimation of marker effects, while maximizing power. This would remove the need for prior QTL information, although such information could still be useful for reducing the genotyping load by focusing only on the most promising genomic regions.

Genetic improvement within a synthetic should not only focus on meat quality but performance traits should also be considered. Thus, selection would be on an index of a marker-based EBV for meat quality and a BLUP EBV for performance traits. If available, marker-based EBV could also be included for performance traits. Instead of deriving the emphasis that is placed on meat quality versus performance traits on the basis of economic values, additional emphasis should be given to the meat quality traits in the initial generations, before the disequilibrium between markers and QTL erodes.

Instead of an  $F_2$  population, a backcross population could be used as the starting point for MAS selection for meat quality. This could be beneficial if the breed difference for performance is large and favorable effects for meat quality QTL originate from both breeds at alternate loci. Then, a backcross to the high performance breed would reduce the genetic lag for performance traits (Figure 4). The frequency of favorable QTL alleles from the other breed would, however, only be  $\frac{1}{4}$ . Thus considerable emphasis would need to be placed these QTL during the initial generations of selection. Use of a backcross for selection does not negate the use of an  $F_2$  cross or prior data on such a cross for marker selection or QTL identification.

## **Genetic improvement of meat quality using within-breed variation**

Most selection programs for swine focus on genetic improvement within a breed or line and the subsequent use of that line within a crossbreeding strategy. Within-breed selection requires information that captures differences between individuals within a breed, rather than the between-breed differences that were discussed previously. The purpose of this section is to describe opportunities for genetic improvement of meat quality based on within-breed selection programs, starting with conventional selection.

### ***Conventional within-breed improvement***

Consider selection in a terminal sire nucleus line within a multiple-tier breeding program. Since phenotypic evaluation of meat quality traits requires slaughter, selection for meat quality traits using conventional methods must be based on records on relatives of the selection candidates. The following two strategies could be considered

- 1) Slaughter one or more members of each litter for meat quality evaluation
- 2) Evaluate meat quality based on a progeny test

Strategy 1) would allow evaluation of selection candidates on the basis of data from full- and half-sibs. Although additional data would be available on full- and half-sibs of the parents of the selection candidates, which were evaluated in the previous generation, selection accuracies will be limited.

A further consideration for strategy 1) is its impact on selection intensities. In principle, the need to sacrifice littermates, which are themselves potential selection candidates, reduces selection intensities. This was the assumption made by Hovenier et al., (1994) and Meuwissen and Goddard (1996). In practice, the impact on selection intensities may, however, be limited because littermates selected for slaughter could be those that would not be selected for breeding based on growth performance. In addition, a limit is typically set on the number of individuals selected from a given litter, in particular on the boar side, to reduce rates of inbreeding.

In strategy 2) selected boars could be progeny-tested outside the nucleus. Resulting data could be available at the time of selection of the boar's nucleus progeny. This strategy would have no impact on selection intensities or generation interval, but accuracy of selection would be limited and data recording would be expensive. If the progeny test is based on crossbreds, this test would also provide data on crossbred performance for growth and backfat traits, which has become of substantial interest.

Although conventional selection for meat quality has been applied in a number of situations (Sellier, 1998), Hovenier et al. (1994) concluded that the benefit of including phenotypic data for meat quality traits in selection programs depends highly on the economic value of meat quality. Therefore, conventional selection for meat quality may

not be a viable option, unless pricing systems put significant emphasis on meat quality and the cost of routine collection of meat quality data is reduced.

### ***Within-breed improvement of meat quality using molecular data***

When considering within-breed improvement using molecular data, it is important to distinguish between the use of markers that are in population-wide LD with a QTL and LE-markers that are in population-wide equilibrium with the QTL. The latter require the use of the LD within families. The use of population-wide versus within-family LD has important consequences for the use markers in selection and for the phenotypic data that is required to support their use. Smith and Smith (1993) advocated the use of markers that are in population-wide LD with QTL because marker effects are easier to estimate and require smaller amounts of phenotypic data. This is important in particular for meat quality traits. Marker requirements are, however, greater for utilization of population-wide LD because they must be tightly linked to the QTL, whereas sufficient within-family LD will exist even for markers that are more distant from the QTL (within 10 cM). The use of population-wide versus within-family LD will be discussed further in what follows.

#### *Selection using LD-MAS based on population-wide LD*

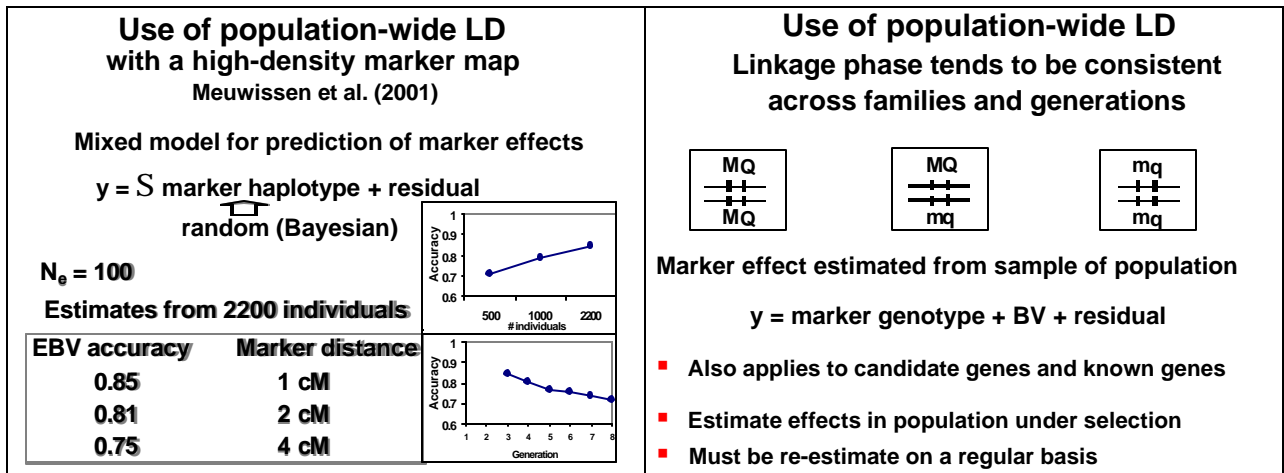
Although markers that are not within the functional gene are not expected to be in extensive LD with a QTL within a closed population, markers that are tightly linked to a QTL have a substantial probability to be in partial population-wide LD with that QTL because of the effects of drift, selection, mutation, and population admixture (Sved 1971, Goddard 1991, Meuwissen *et al.* 2000). This probability is higher in selected populations of small effective size, which is the case for livestock, as demonstrated by Farnir *et al.* (2000) for dairy cattle. The extent of LD can often be enhanced through the use of haplotypes of tightly linked markers. High-density marker maps with, e.g., a marker every 1 or 2 cM, will also include markers that are in tight linkage with the QTL and that have the potential to be in substantial population-wide LD, as was recently demonstrated by Meuwissen *et al.* (2001) through simulation. They showed 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 of associations of marker haplotypes with phenotype on as few as 500 individuals. Although genotyping costs would be high when applied to the entire genome, opportunities may exist to utilize this approach on a limited scale by saturating previously identified QTL regions with markers.

For markers that are in population-wide LD with the QTL, selection can be directly on marker genotype or on marker haplotype if multiple linked markers are used to track the QTL. It is, however, essential to estimate the effects of the markers within the population under selection to capture the degree of LD and linkage phases that are present in the population and to guard against potential interactions of the QTL with the background genome. For the same reason, it will also be prudent to re-estimate the effects on a

regular basis. Estimation requires marker genotypes and meat quality phenotypes on a random sample of individuals in the population and should be based on an animal model with marker genotypes or haplotypes included as fixed effects (Figure 14) (e.g. Short et al. 1997; Israel and Weller, 1998).

Figure 13. Genetic evaluation using a high-density marker map and population-wide LD in MAS.

Figure 14. Use of population-wide LD in MAS.



Because selection is on performance traits along with meat quality, selection should not exclusively be on marker effects for meat quality but must be in combination with EBV for performance traits. Ideally, estimates for inclusion in such an index are obtained from a multiple-trait animal model that includes the candidate gene markers as fixed effects. The model could also include any available phenotypic data on meat quality traits. Such a model could result in EBV for average daily gain ( $EBV_{ADG}$ ) and backfat ( $EBV_{BF}$ ), a polygenic EBV for meat quality ( $\hat{u}_{MQ}$ ), and an EBV for meat quality based on marker effects ( $\hat{g}_{MQ}$ ). These EBV can then be combined as follows:

$$I = v_{MQ}(\hat{g}_{MQ} + \hat{u}_{MQ}) + v_{ADG}EBV_{ADG} + v_{BF}EBV_{BF} \quad [1]$$

where  $v_{MQ}$ ,  $v_{ADG}$ , and  $v_{BF}$  are economic values. The breeding value for a marker can be derived using single locus quantitative genetic theory (Falconer and Mackay, 1996) from estimates of genotype effects ( $a$  and  $d$ ) and gene frequencies ( $p$  and  $q$ ) on the basis of the allele substitution effect  $a^2a + (p - q)d$ . Alternatively, the model of evaluation could include a regression on the number of favorable alleles, instead of genotype, in order to directly estimate  $a$  (Lande and Thompson, 1990). If the marker also has effects on performance traits, their EBV would also be separated into a marker-based EBV and a polygenic EBV. Index [1] can be expanded to include multiple markers by computing marker-based EBV as the sum of EBV for individual markers.

Selection on index [1] will in theory maximize the expected response in a breeding goal over one generation under an additive model. For markers that show dominance, the allele substitution effect  $a$  must be derived on the basis of gene frequencies among mates, rather than on frequencies among all selection candidates, in order to maximize single

generation selection response (Dekkers, 1999). However, unless the marker shows substantial dominance and selection pressure on the marker is large, such that changes in gene frequencies are substantial, optimization will have limited impact.

In theory, weights on the marker in index [1] must also be modified and optimized if the objective is to maximize response in the economic breeding goal over multiple generations, even under additivity (Dekkers and van Arendonk, 1998; Manfredi et al., 1998). The basic reason is that, although selection on index [1] maximizes response from the current to the next generation, it also changes frequencies at the marker and QTL. This affects opportunities for genetic progress in future generations, which is taken into account by the optimal strategies. The benefit of optimizing selection on markers compared to using index [1] will, however, be limited, unless the marker shows overdominance and explains a large proportion of the genetic variance in the breeding goal (Dekkers and Chakraborty, 2001).

Weights on the marker EBV in index [1] should also be modified if the selection objective includes other factors than, or in addition to, improvement of an economic breeding goal. For example, there may be benefit to rapidly fixing the favorable allele at the marker for marketing reasons or to reduce genotyping costs.

The previous considered simultaneous selection on the marker and phenotype-based EBV through an index. This is preferred over two-stage selection, in which selection is on markers in the first stage and on the basis of phenotype-based EBV in the second stage. Such a strategy eliminates individuals for which a high EBV for other traits or for polygenic effects for meat quality compensates for unfavorable genotypes at the markers, akin to the use of independent culling levels in multiple trait selection. There may, however, be benefit to have selection on the index that includes marker information follow a first stage of selection using phenotype-based EBV; only individuals that are selected in the first stage would need to be genotyped, which would save costs.

#### *Selection using LE-MAS 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 (Figure 15). 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 have been developed to incorporate marker data in genetic evaluation for complex pedigrees (Figure 16) (Fernando and Grossman, 1989; Goddard, 1992). These models result in BLUP EBV of QTL effects along with polygenic EBV. Because selection is on performance traits along with meat quality, these estimates should be combined with EBV for performance traits into an economic index. An index

similar to index [1] of the previous section can be used but with  $\hat{g}_{MQ}$  now representing the EBV for the marked QTL.

Figure 15. Use of within-family LD; inconsistency of linkage phase between sires.

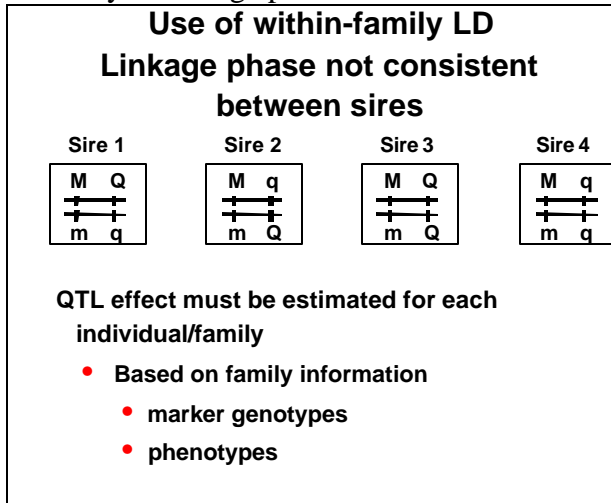
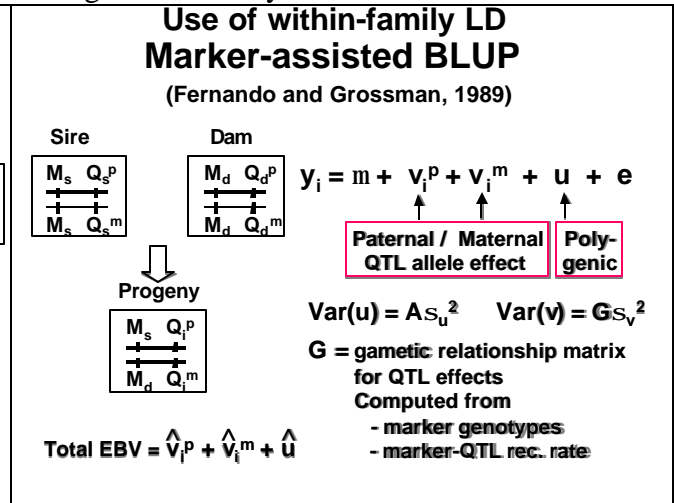


Figure 16. Marker-assisted genetic evaluation using within-family LD.



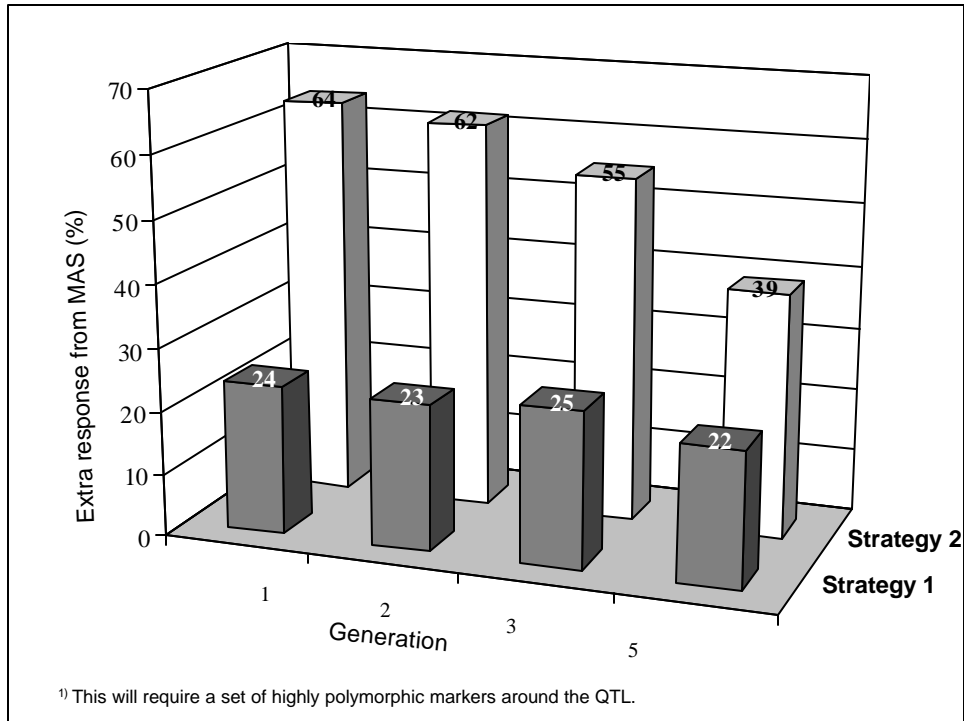
To evaluate the benefit of within-family MAS for meat quality traits, Meuwissen and Goddard (1996) considered two implementation strategies:

- I) A random two of four members of each full sib family is slaughtered to record meat quality data. The remaining individuals are selected on the basis of a marker-assisted EBV for meat quality, once data on their sibs is recorded.
- II) Animals are selected on the basis a marker-assisted EBV and non-selected animals are slaughtered to provide data for the next generation of selection.

For both strategies, all individuals were genotyped for a set of markers around a previously identified QTL. Marker-assisted EBV for meat quality were evaluated using the marker-assisted genetic evaluation model of Goddard (1992) by including the QTL as a random effect. Selection was on the sum of EBV for the QTL and polygenes, similar to the COMB strategy of Zhang and Smith (1992). Comparisons were to genetic gain from a conventional selection based on strategy I) but without availability of genetic markers.

Results illustrated in Figure 17 show that strategy I) gave 24% greater response than conventional selection. The benefit of strategy II) was substantially greater but declined over generations as favorable alleles at the QTL were fixed. The greater response from strategy II) compared to I) was in large part the result of the greater selection intensity that was achieved with strategy II) because half of the selection candidates were not slaughtered prior to selection. However, as discussed in previously, it is questionable whether this increased selection intensity can be realized in practice due to inbreeding considerations. Thus, the extra response of 24% appears more realistic.

Figure 17. Potential extra gains from MAS for meat quality traits based on within family linkage disequilibrium. Based on Meuwissen and Goddard (1996). QTL with multiple alleles that explains 1/3 of the genetic variance for a trait with 0.27 heritability. Marker haplotypes were informative such that transmission of QTL alleles could be followed from parent to offspring for 90% of offspring<sup>1</sup>. Marker and phenotypic data were available for five generations prior to the initiation of MAS.



Implementation of either strategy for selection on within-family LD requires extensive phenotyping and genotyping, which calls the economic feasibility of such programs into question. In addition, data should be available for several generations prior to initiating MAS to accurately estimate QTL effects. For example, results in Figure 17 assumed phenotypic and genotypic data for five generations prior to initiation of MAS and responses dropped substantially without the buildup of such data (Meuwissen and Goddard 1996). Although the same genotypic data can also be applied to performance traits, the benefit of MAS for these traits will be less than for meat quality traits (Meuwissen and Goddard 1996), in particular if markers are in QTL regions for meat quality rather than for performance traits. Nevertheless, correlated effects on other traits should be carefully considered and monitored when applying MAS.

Another obstacle for the use of within-family LD is that it requires knowledge of QTL regions that segregate within the population. Since most QTL mapping studies in pigs are based on the breed cross model, information about within-breed segregation of QTL is limited. Thus, within-breed QTL mapping studies must be conducted prior to implementation of MAS. Although such studies could concentrate on QTL regions previously identified in breed cross studies, substantial population sizes will be required to detect or confirm their segregation within a breed. Such a study was recently

conducted by Evans et al. (2002). They found that QTL regions identified in a cross between divergent breeds could indeed be confirmed to segregate within commercial lines. Related issues were discussed by Spelman and Bovenhuis (1998) in the context of implementing QTL knowledge in dairy cattle breeding programs.

### *LD-MAS versus LE-MAS*

An important consideration for the use of molecular genetics in breeding programs is whether to work toward the application of LE-MAS, LD-MAS, or GAS. Table 2 summarizes the relative requirements and opportunities for these three strategies. Requirements for detection are least for LE-markers and greatest for identification of functional mutations. However, once a functional mutation is identified, requirements for estimation and confirmation of effects in other populations are much lower than for LE-markers because the latter requires phenotypes and genotypes on pedigreed populations versus a random sample. Requirements for integration of genotype data in routine genetic evaluation procedures are also much greater for LE-MAS than for LD-MAS and GAS, both with regard to requirements of individuals that must be phenotyped and genotyped and with regard to methods of analysis. Genetic evaluation requirements are slightly greater for LD-MAS than GAS because LD-MAS requires identification and analysis of marker haplotypes and confirmation of marker-QTL linkage phases.

Table 2. Requirements and opportunities for implementation of LE-MAS vs. LD-MAS vs. GAS.

QTL detection requirements	LE-MAS < LD-MAS << GAS
Within-line confirmation requirements	LE-MAS >> LD-MAS > GAS
Routine genetic evaluation requirements	LE-MAS >> LD-MAS > GAS
Phenotyping – relatives (LE) vs. sample (LD/GAS)	LE-MAS >> LD-MAS > GAS
Genotyping – candidate + relatives (LE) vs. candidate only	LE-MAS >> LD-MAS > GAS
Analysis – MA-BLUP (LE) vs. fixed effect (LD/GAS)	LE-MAS >> LD-MAS > GAS
Genome-wide analysis opportunities	LE-MAS >> LD-MAS >> GAS
Implementation logistics	LE-MAS >> LD-MAS > GAS
Genetic gain opportunities (for given QTL)	LE-MAS < LD-MAS ~< GAS
Marketing opportunities (patents, product differentiation)	LE-MAS << LD-MAS < GAS

Whereas the requirements discussed above refer to a given QTL, LE-MAS allows for genome-wide analysis and evaluation of QTL with a limited number of markers. This is also possible for LD-MAS with high-density genotyping. Meuwissen et al. (2001) demonstrated that EBV of high accuracy could be obtained from a Bayesian mixed model analysis of marker haplotypes with high-density genotyping (Figure 13).

Opportunities for increases in genetic gain from a given QTL are lowest from LE-MAS because of the limited information that is available to estimate effects on a within-family basis (Villanueva et al., 2002), while for both LD-MAS and GAS, effects are estimated from data across families. Accuracy of estimates may be slightly lower for LD-MAS than GAS as a result of incomplete marker-QTL disequilibrium and a greater number of

effects (marker haplotypes versus QTL genotypes) (Hayes et al., 2002). Opportunities for intellectual property protection and product differentiation are greatest for GAS but limited for LE-MAS.

### Integration of MAS in breeding programs

It is clear that successful implementation of a MAS program requires a comprehensive integrated approach that is closely aligned with business goals and markets. Components of such an approach are illustrated in Figures 18 and 19. In practice, all three types of markers are available for the categories of traits described previously and a comprehensive approach is needed to collect, integrate, and analyze data on phenotypes for multiple traits, LE markers, LD markers, and genes and to develop selection strategies that meet business goals.

Figure 18. Key components for implementation of MAS.

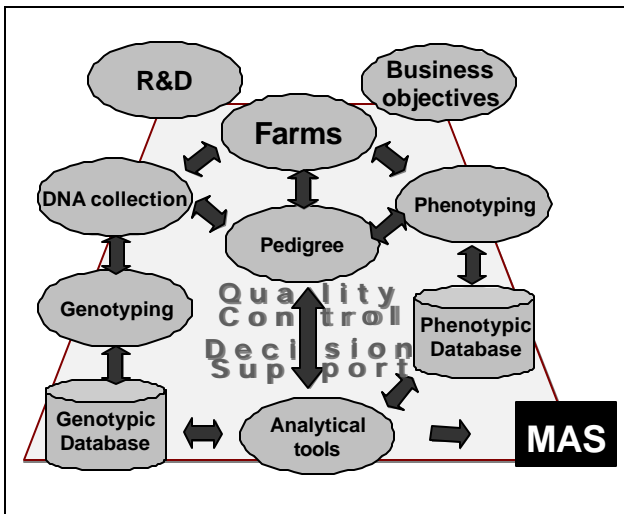
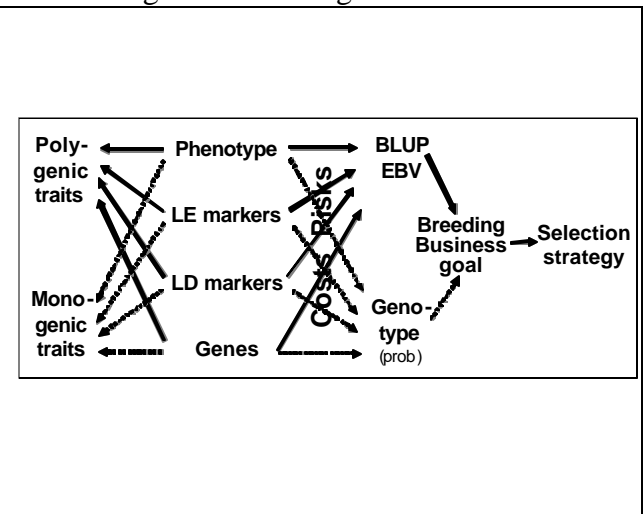


Figure 19. Integration of molecular in breeding and business goals.



Commercial application of MAS also requires careful consideration of economic aspects and business risks. Economic analysis of MAS requires a comprehensive approach that aims to evaluate the economic feasibility and optimal implementation of MAS. An excellent example of such an analysis is in Hayes and Goddard (2003), who conducted a comprehensive economic analysis of the implementation of LE-MAS in the nucleus breeding program of an integrated pig production enterprise. QTL detection and MAS on identified QTL regions for a multi-trait breeding goal and associated genotyping costs and extra returns from the production phase of the integrated enterprise were considered in the economic assessment. They concluded that implementation of LE-MAS was feasible for the assumed cost and price parameters. They also found that, in particular, if QTL detection was based on small sample sizes, stringent thresholds should be set during the QTL detection phase such that genotyping costs during the implementation phase are reduced and selection of false positives is minimized.

Whereas Hayes and Goddard (2003) evaluated economic returns from MAS from increased profit at the production level, which is proportional to extra genetic gain, most commercial breeding programs derive profit from increased market share of breeding stock or germ plasm. In general, implementation of MAS will have a greater impact on market share than on genetic gain. An example is in Figure 20, which evaluates the impact of pre-selection of young dairy bulls in a competitive market.

Figure 20. Genetic gain versus market share from pre-selection of young dairy bulls. programs.

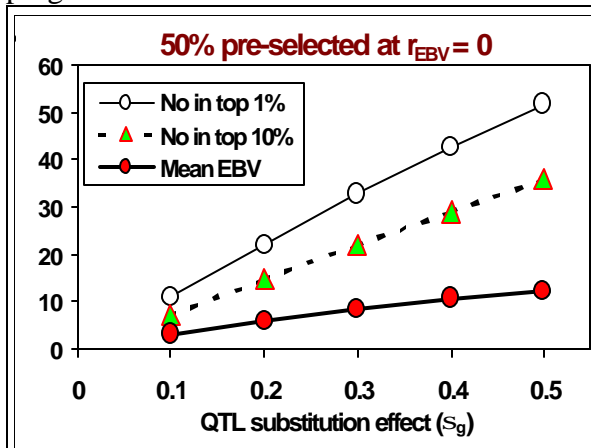
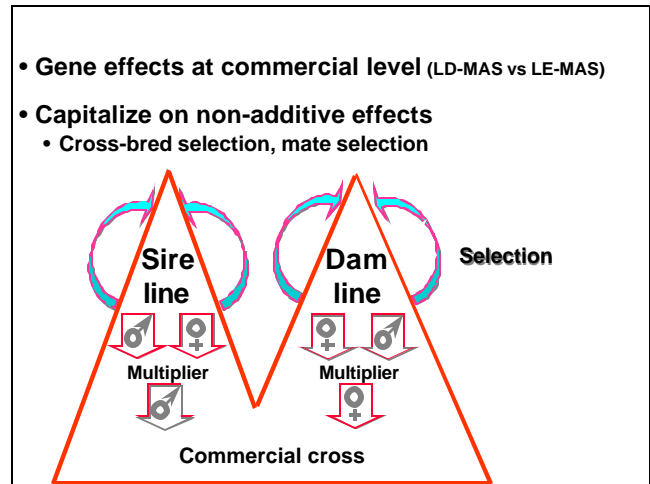


Figure 21. MAS in crossbreeding programs.



For a QTL with a substitution effect of 0.3 genetic standard deviations, pre-selection increased genetic gain of selected (top 10%) progeny-tested bulls increased by 7% but the number of bulls in the top 10 and 1% increased by 20 and 30%. This does not imply that the economic feasibility of MAS is greater in a competitive market because that also depends on absolute returns associated with a % increase in genetic gain versus market share; Brascamp et al. (1993) showed, in fact, that economic returns from increased market share were less than from increased production for a pre-selection situation similar to that considered here. Nevertheless, it is important that economic analysis is conducted in relation to business and market realities and goals.

## Discussion

Substantial progress has been made over the past decade in the identification of genes and genomic regions that affect meat quality. This includes identification of functional genes, candidate genes, and QTL regions. Whereas most initial studies used divergent breed crosses that included one exotic breed, recently several QTL mapping studies have been published on crosses of commercial lines (Malek et al., 2001b, Grindflek et al., 2001). In addition, a substantial number of QTL mapping studies are ongoing and will produce results over the next years.

An important observation that can be gleaned from these QTL studies is that there are substantial differences between commercial breeds in QTL for meat quality and that some of the favorable breed QTL effects originate from the breed with the lower mean meat quality. On the one hand, this offers opportunities for selection in synthetic lines to capitalize on the best of all breeds. On the other hand, this suggests that favorable alleles for QTL for meat quality may already be segregating in the breeds that have superior performance characteristics but poorer meat quality. Although there is some evidence for within-breed segregation of QTL (De Koning et al. 2001), the breed cross designs that are predominantly used for QTL mapping in swine have limited power to detect within-breed segregation of QTL.

If most QTL for meat quality are indeed segregating within commercially important breeds and lines, then within-breed selection for meat quality would allow superior meat quality to be attained through selection within those breeds. Opportunities for within-breed selection for meat quality are, however, limited by the inherent requirements for phenotypic recording, which is expensive and requires the sacrifice of potential selection candidates. This is the case for conventional selection on meat quality and also holds for MAS, unless markers that are in tight population-wide LD with the functional genes can be found, such that marker effects can be estimated and used across the population, instead of only within families.

Several procedures are available to find markers that are in population-wide LD with QTL. This includes fine mapping, candidate gene searches using human genome sequence, and use of high-density marker maps. Development of SNPs (single nucleotide polymorphisms) maps in livestock is a possible next step. Any of these approaches, however, requires substantial molecular genetic work, along with further development of statistical methods to detect and capitalize on population-wide LD with limited phenotypic data. From a genetic improvement perspective it is not essential that the functional polymorphism be identified. Such knowledge would, however, enable a better understanding of the physiological effects of the QTL, which in turn would allow better prediction of the effects of the QTL in different genetic backgrounds and environmental conditions.

Although MAI is used extensively in plants (Hospital, 2002), only few examples are available for livestock (Dekkers and Hospital, 2001). Hanset et al. (1995) reported on the successful introgression of the halothane normal allele into a Piétrain line that had a high frequency of the halothane positive allele. They used foreground selection on a marker that is closely linked to RYR. In general, however, the application of introgression programs to meat quality genes appears limited for several reasons:

- i) apart from some major genes, QTL studies show that meat quality is affected by a substantial number of genes with moderate effects. This makes the number of QTL to introgress more than can feasibly be handled within an introgression program.
- ii) Most QTL may already be segregating within the recipient breed, such that within-breed selection may be more effective than introgression.
- iii) Meat quality QTL are not very precisely mapped, which increases the size of the genome region(s) that must be introgressed and the population size required .

- iv) The economic benefit of the improved meat quality may not be large enough to compensate for the extra costs and reduced genetic gain in other traits that is associated with an introgression program.
- v) The introgressed gene(s) may have a different effect in the new genetic background, as has been observed in several plant introgression programs (Dekkers and Hospital, 2001).

Plant and mouse (Koudandé et al., 2000) studies on the introgression of QTL regions show that foreground selection based on markers was effective in moving the targeted region into the recipient genome. However, the improvement in performance of the recipient breed was generally less than expected based on the initial QTL effect estimates (Dekkers and Hospital, 2001). Apart from false positives or overestimation of effects in the initial population, reasons suggested for the lower response include presence of epistatic interactions among QTL, and between QTL and the genetic background, and genotype by environment interactions. Similar factors could reduce the realized gain from MAS in synthetic or purebred populations.

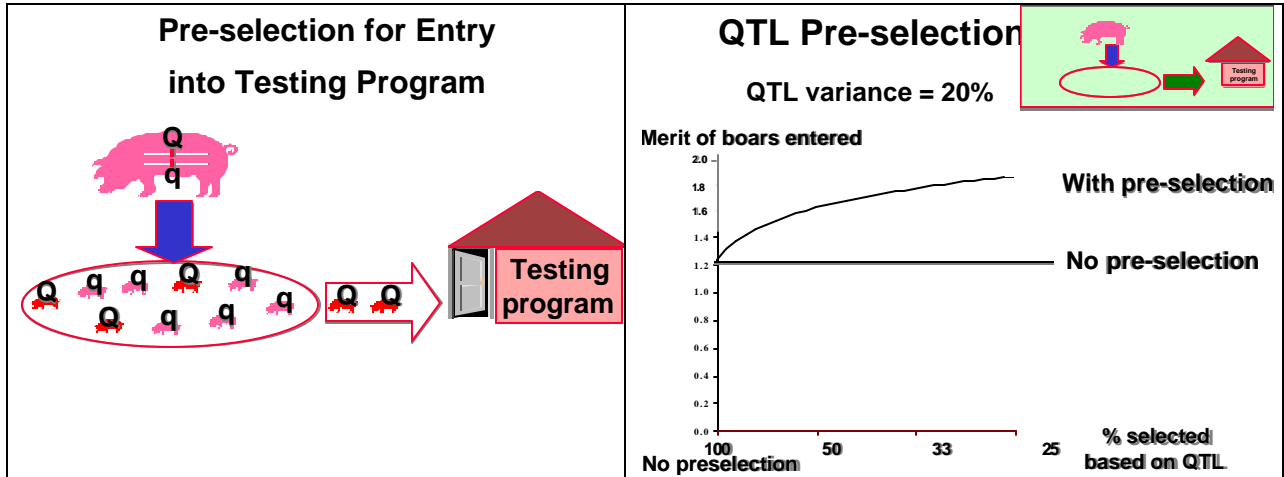
Success of MAS also depends on the consistency of QTL effects across populations and environments. Results from introgression programs in plants have found that effects tend to be consistent for major genes for simple traits but not for QTL for complex traits (e.g. yield) (Hospital, 2002). Inconsistent effects have also been observed for some well-studied genes in livestock. For example, for the ESR gene for litter size in pigs some studies have found no effect and interactions with line and environment have been identified (Rothschild and Plastow, 2002). Reasons for inconsistent results across studies and populations include statistical anomalies such as false positive or negative results (small sample sizes) and overestimation of significant QTL effects, as well as true effects, such as inconsistent marker-QTL linkage phases across populations for LD-markers, genotype by environment interactions, and epistatic effects. This points to the need to continuously evaluate and monitor gene or QTL effects in the target population and environment, which requires continuous emphasis on phenotypic recording. In addition, strategies must be developed to estimate gene effects at the commercial level for nucleus breeding programs, in particular if they involve crossbreeding (Figure 21). This also opens opportunities to use markers to capitalize on non-additive effects and assignment of specific matings.

Given the uncertainties about the sustainability of marker effects, it appears prudent to use molecular genetic information in a manner that does not prevent progress toward the overall breeding goal that can be achieved through conventional selection. When considering MAS on meat quality traits, this includes conventional selection for performance traits. A crucial concept in this regard is to apply MAS in selection space that is not or under-utilized by conventional selection (Soller and Medjugorac, 1999). A prime example is pre-selection on the basis of markers among members of a full-sib family for further testing, prior to availability of individual or progeny records. In such situations conventional selection has no basis for selection because EBV are derived from pedigree information, which is the same for all members of a full-sib family (Figure 22, 23). Family members can, however, differ for the markers they inherited, which then

provides a basis for selection, instead of having to make a random choice. Selection space for MAS can be increased with technologies that enhance the reproductive rate of, in particular, the female. Such strategies were evaluated by Kashi *et al.* (1990) for dairy cattle.

Figure 22. Marker-assisted pre-selection.

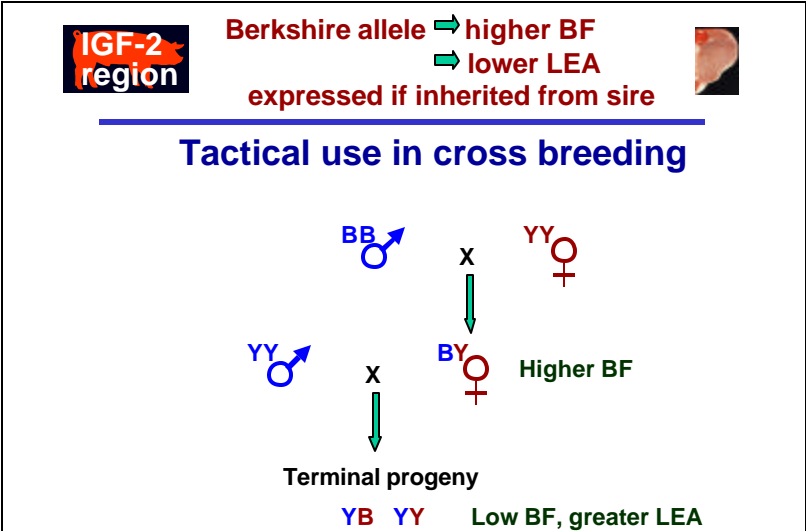
Figure 23. Extra genetic gain from pre-selection.



In addition to increasing selection space within a generation by increasing full-sib family size, space for MAS can also be created across generations by introducing several rapid generations of selection based on markers alone. Such programs were proposed by Georges and Massey (1991) for dairy cattle and subsequently by Visscher et al. (2000) for pigs. In such programs of ‘velogenetics’, the short generations for marker-assisted selection are facilitated by the use of reproductive technologies such as the recovery of oocytes from the unborn foetus, *in-vitro* maturation of oocytes, and *in-vitro* fertilization. These technologies are then combined with the selection of embryos for implantation based exclusively on the inheritance of markers that were previously estimated to have favorable effects. Enhancements to further reduce the generation interval in these programs were suggested by Haley and Visscher (1998) and Visscher et al. (2000). Although further advances in reproductive technologies are required for velogenetic programs to become feasible, they offer potential to improve meat quality through marker-assisted introgression, synthetic line development, and within-breed selection based on population-wide LD.

Recent gene and QTL mapping studies have also revealed that QTL may not be expressed in a Mendelian fashion. In particular, several studies have detected genes and QTL in pigs that are subject to gametic imprinting (Jeon et al., 1999, De Koning et al., 2000). Future studies will undoubtedly identify other epigenic phenomena that affect the inheritance and expression of QTL. These effects will need to be taken into account when designing selection programs. Although they may on the one hand complicate selection programs, they may also provide opportunities. For example, De Koning (2001) suggested that utilization of a combination of imprinted and sex-linked QTL would allow a diverse set of markets to be targeted through strategic crosses between a single set of breeds (e.g. Figure 24).

Figure 24. Tactical use of paternally expressed IGF-2 QTL in crossbreeding.



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