Does Marker-assisted Selection Make Dollars and Sense in a Fruit Breeding Program?

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Numerous authors have alluded to the potential benefits of marker-assisted selection (MAS) for fruit breeding programs (e.g., Baird et al., 1996; Conner et al., 1997; Dirlewanger et al., 1998; Foolad et al., 1995; Gianfranceschi et al., 1996; Gill et al., 1998; Gmitter et al., 1998; Hemmat et al., 1997; Lahogue et al., 1998; Maliepaard et al., 1998; Markussen et al., 1995; Wang et al., 1998). The cost–benefit relationships are presumed to be more favorable for many fruit crops than for annual crops because of their large size and long juvenile phase. Since the fruit is the item of economic value, many traits cannot be evaluated until seedlings mature. Thus, MAS during the juvenile phase has been proposed to speed the selection process or reduce the progeny sizes and the costs of carrying individuals to maturity in the field.

Despite the perceived benefits of MAS, efforts to develop maps and establish marker–trait linkages in fruit and nut crops lag behind those in many other crops (Paterson, 1996). In several fruit crops, linkages between markers and single genes affecting fruit traits, morphology, or disease or pest resistance have been established (e.g., Arus et al., 1999; Bartish and Weeden, 1999; Conner et al., 1997; Dirlewanger et al., 1998; Gardiner et al., 1999; Gianfranceschi et al., 1996; Gill et al., 1998; Gmitter et al., 1996; Lawson et al., 1995; Markussen et al., 1995; Quarta et al., 1999), but only in a few instances have inferred linkages with loci affecting metric or quantitative traits (QTL) been reported (Lahogue et al., 1998; Striem et al., 1996). Attempts to use MAS in fruit breeding programs are just beginning, using a few, simply inherited traits (Gardiner et al., 1999; Gill et al., 1998; Urbanietz et al., 1999).

Our objective in this paper is to compare the genetic efficiency and cost efficiency of MAS for simply inherited or polygenic traits vs. those of conventional fruit breeding schemes such as phenotypic selection among individuals and the less commonly used index selection on relatives for quantitative traits. We intend that this discussion provide fruit breeders with a framework in which to consider whether MAS will make genetic or fiscal sense in their cultivar development programs.

SPECIAL FEATURES OF FRUIT BREEDING PROGRAMS

From a plant breeder's viewpoint, fruit crops differ from most agronomic or forest crops because of a peculiar combination of features that also affect the prospect for successful use of MAS, viz., heterozygosity, asexual propagation, perennial nature, and perishability of product. Most fruit crops (with important exceptions in the genus *Prunus*, for example) have high heterozygosity in individuals and allelic richness in primary germplasm pools, which are maintained by various genetic or physical mechanisms that promote outcrossing. Propagation by asexual techniques, for testing and for cultivar release, is the norm and enables fruit breeders to conserve for horticultural exploitation all the genetic effects, additive and nonadditive, expressed in the phenotypes of superior individuals. The crops are perennials, with many featuring large plant size, long productive period, an extended juvenile phase for seedlings, and a marketable product that cannot be assessed until a seedling is physiologically mature. Their perishable product consists largely of water and, thus, is subject to numerous interactions of genetic effects with consumer preferences, as well as with biotic and abiotic factors during preharvest and postharvest periods.

The fruit breeder requires that selected individuals exceed a certain level of performance, a culling level, for each of a host of traits desired by producers, processors, and consumers. The culling levels for most traits are usually independent of one another. Each trait is also weighted relative to its importance for the commercial success of a cultivar. Most breeders also take advantage of multiple-stage selection by emphasizing a limited number of traits when first evaluating nonreplicated seedlings and then considering the full suite of traits in advanced testing of clonally replicated genotypes.

Inheritance of traits. A few traits may have demonstrably simple inheritance because the effects of one or two genes are large compared with environmental effects. Individuals in segregating populations can usually be assigned to discrete groups corresponding directly to their genotypes. For most traits of interest, however, individuals in a segregating population cannot be assigned to discrete classes, but are distributed continuously along a quantitative scale. Termed quantitative traits, their continuous distribution is presumably due to from several (oligogenic) to many (polygenic) gene loci with effects of varying magnitude that may be small relative to environmental effects. The simultaneous selection for multiple oligogenic or polygenic traits insures that only a small proportion of individuals will have favorable alleles at a large enough number of loci to be judged superior, and necessitates evaluation of large populations to increase the probability of obtaining and identifying them.

MAS FOR SIMPLY INHERITED TRAITS

In fruit crops, the best examples of simply inherited traits are resistances to certain diseases or pests, fruit color, and plant growth habit. In some crops, such as blueberry (*Vaccinium* sp.) or strawberry (*Fragaria ×ananassa* Duch.), simple inheritance has been established for only a few useful traits. In other crops, such as peach [*Prunus persica* (L.) Batsch] and apple (*Malus ×domestica* Borkh.), several important traits are controlled by a single locus. These traits are often the easiest for breeders to select for, as they can be assessed readily on a single-plant basis during initial evaluation of seedlings.

The most likely candidate traits for cost-efficient MAS in fruit breeding programs (Mehlenbacher, 1995) are: 1) fruit traits that can be evaluated only after a long juvenile phase; 2) certain types of disease or pest resistance that require difficult and expensive evaluation protocols, or may be impossible to perform because the pest or pathogen is absent; 3) traits for which progeny testing is the only conventional means to confirm the presence of an allele (e.g., the pyramiding of resistance genes); and 4) simply inherited traits in species such as peach or sour cherry (*Prunus cerasus* L.) in which true backcross introgression is not hampered by inbreeding depression or self-incompatibility.

The breeder contemplating MAS for a simply inherited trait should consider several genetic and economic issues of costs, logistics, marker–gene associations, breeding practices, population sizes, and constraints of two-stage selection.

Costs. A major issue is the costs of MAS, including the development costs for markers and methods, vs. those of conventional selec-

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tion techniques. For many simply inherited traits, inexpensive tests are available and markers will offer no savings.

Logistics. A mundane but critical issue is how MAS will be implemented. Are large populations to be screened? If so, is this technically feasible? To be timely, and to maximize the savings when seedlings are not carried through the juvenile phase, many breeding programs would need to evaluate as many as 10,000 to 50,000 genotypes in the 6 months before seedlings are transplanted to nurseries or orchards. If the objective is to test a much smaller set of potential parents, will any savings in cost or time that accrue to the program be substantial enough to justify implementation of the technology? Finally, if parent selection is the objective, marker selection must be at least as accurate as prediction based on phenotype, as any mistake at this stage will result in large carrying costs being incurred for undesirable plants.

Marker–gene associations. Unless the marker contains the gene itself, the success of MAS depends on marker–gene linkage disequilibrium in a population. For marker–gene associations to be useful, several criteria need to be considered. How many parents are used in a breeding program in a typical year and how are the parents mated? How robust are the marker–trait linkage relationships in the germplasm pools used by fruit breeders? Most studies of marker–gene disequilibrium in germplasm pools are based on self-pollinated species with extensive (sometimes thousands of years and generations) of domestication, breeding and selection. Once established in these crops, disequilibrium would be expected to break down very slowly because of mating scheme dynamics and intense selection.

Breeding practices. Breeding practices in self-pollinated species, or in inbred line development in outcrossing species, also facilitate the efficiency of MAS for simply inherited traits. Establishing and following linkages during inbreeding (e.g., recombinant inbred or backcross lines) are rather easy. Hence, the most practical and potentially powerful use of MAS is to facilitate introgression of simply inherited traits using backcrossing or recombinant inbreeding (Hospital et al., 1992; Tanksley and Nelson, 1996) by selecting for markers linked to the desired donor allele and against other markers in the donor parent genome.

Conversely, the mating schemes used by many breeders to foster optimum genetic advance and avoid inbreeding depression in fruit crops would tend to oppose the maintenance of marker–gene linkage relationships. Thus, an expense that is often ignored when MAS is considered in the context of a breeding program results from the need to establish and evaluate marker associations for each cross or each recombination cycle. The reasoning for this is simple. In the diverse germplasm pool of an outcrossing fruit crop there is no reason to expect a given marker genotype to be associated with a given useful gene in the germplasm at large.

Population size. Except in introgressive breeding, a breeder performing MAS for one or a few simply inherited traits will need to retain large populations with the desired marker genotype to maintain a reasonable probability of identifying one or more individuals of cultivar potential for all the other critical, quantitatively inherited traits. The number of superior individuals, with the potential to be cultivars, will be quite low in most populations because of the large number of traits for which an individual must have an outstanding genotype. The probabilities of identifying superior individuals depend on the breeder's ability to identify genetic superiority that is partially disguised by environmental effects (i.e., the concept of heritability), how genetic variation is distributed among and within families, and, not least, the number of crosses and family size (Knapp, 1998).

Constraints of two-stage selection. Two-stage independent culling (tandem selection) constrains breeding opportunities more than does single-stage selection. For example, if a breeder uses MAS in the juvenile stage to cull for one or more simply inherited disease resistance genes, the decision is made that these are absolutely necessary in every cultivar and every parent in the population. If the same initial population size is maintained prior to MAS in the juvenile stage (hoping to take a smaller set to the field and save money), then it is stochastically certain that the ultimate selection response for traits in the second stage of culling will be restricted because of genetic drift and linkage (Falconer and MacKay, 1996). Conversely, if the initial population is increased to insure adequate population size after MAS, the cost savings will diminish because more seedlings must be produced and carried through juvenile stage MAS. In a broader perspective, this paradox reflects the conflict between obtaining cultivars rapidly and improving the breeding population. Most fruit breeders use a single population for both purposes. Absolute two-stage culling is really most appropriate for a population dedicated to cultivar improvement.

MAS FOR POLYGENIC TRAITS

Theoretical considerations. Many economically important traits in fruit crops are considered to be polygenic and quantitative in their inheritance. The theoretical basis for the inheritance of quantitative traits and their response to selection has been developed over the past 80 years (Falconer and MacKay, 1996). The inheritance may be controlled by a few to many loci with genetic effects of varying size, but these effects are often small relative to environmental and genotype x environment interaction effects. The term heritability refers to the proportion of the phenotypic variance for a trait (Y) that can be ascribed to genetic components (broad sense heritability, H^2_Y) or to the portion of genetic variance due to additive allelic effects (narrow sense heritability, h^2_Y). Response to selection for a quantitative trait (R_Y) is a function of the intensity of selection (i), the heritability of the trait, and the amount of genetic variance for a trait ($\sigma^2_{g(Y)}$) expressed as:

$R_Y = i_Y h_Y \sigma_{g(Y)}$.

A trait of interest (Y) may also be selected for by selecting for another trait (X), provided that genetic factors controlling the traits are located close to one another on the chromosomes (linkage) or are the same (pleiotropy). The use of MAS as proposed for quantitative traits is essentially a form of indirect selection. Selection for the trait of interest is performed by indirectly selecting on the marker phenotype, or on an index that incorporates the marker phenotype, rather than on the phenotype for the trait itself.

The expected correlated response (CR_Y) of a trait of interest (Y) to indirect selection depends on the intensity of selection for X (i_X), the heritability of X (h^2_X), the genetic variance for the trait of interest ($\sigma^2_{g(Y)}$), and the strength of the genetic association, the genetic correlation ($r_{g(YX)}$), i.e., the existence of linkage and pleiotropy, and can be expressed as follows:

$CR_Y = i_X h_X r_{g(YX)} \sigma_{g(Y)}$

In comparing these equations, indirect selection on X is expected to improve Y more rapidly than direct selection on Y (i.e., $CR_Y > R_Y$), assuming the same selection intensity, when

$h_x r_{g(YX)} > h_y$.

Essentially, this relationship suggests that if the heritability of the trait of interest (h_y) is high, indirect selection using markers or any other trait is not likely to be more efficient because, in reality, $r_{g(YX)}$ is usually much <1. Considering MAS, the product of $h_x r_{g(YX)}$ will never be greater than h_y unless the proportion of variation explained by the markers exceeds h^2_y. This is a rare situation because as the value of h^2_y becomes less, the power to detect useful markers diminishes drastically (Lande and Thompson, 1990; Strauss et al., 1992). Thus, selection for polygenic traits using markers alone should only be considered when it confers savings in either program cost or time to release of a cultivar.

The expected genetic efficiency of MAS depends mainly on the heritability of the trait and the proportion of the additive genetic variance explained by the marker loci (p), which, in turn, depends on a genetic correlation existing between the marker genotype and the genetic value for the trait (Falconer and MacKay, 1996; Lande and Thompson, 1990). The genetic correlation depends on the actual linkage between the markers and the QTLs and the precision with which those linkages are estimated. The precision of estimation of marker–QTL linkages depends on the number of individuals in a family and the precision with which their phenotypes for the trait are estimated, i.e., the degree to which the genetic effects are not confounded by interactions of genetic and environmental effects.

Lande and Thompson (1990) proposed a modification of indirect selection that should be more efficient than selecting on markers alone. The effectiveness depends on the alternative genotypes for a marker

being in linkage disequilibrium with the alternative alleles at a QTL. If the value of $r_{g(YX)}$ is not zero, then indirect selection incorporating markers based on an index value (I) that includes both X (marker genotype) and Y (individual phenotype) can result in greater expected improvement in Y than selection based on Y alone. This index can be described as $I = b_1X + b_2Y$, where b_1 and b_2 are weighting coefficients based on genetic variances and covariance (Lande and Thompson, 1990). We will refer to this indirect selection incorporating markers as index-MAS.

Lande and Thompson (1990) compared the expected relative efficiency of index-MAS with that of other selection schemes. Their comparisons of index-MAS to phenotypic selection and selection based on information from relatives are the most pertinent to fruit breeding. Their analytical approach, assuming very large population sizes, suggested that index-MAS would be most useful for traits with low heritability. For traits with high heritability, an index incorporating markers provides little or no improvement in efficiency because the phenotype already provides considerable information about the genotype of an individual; the marker genotype provides very little additional information. We emphasize that Lande and Thompson's (1990) index-MAS requires that both the marker genotype and the trait phenotype be scored to assess the value of a QTL and to make selection decisions. In selecting for a trait expressed only in mature perennial plants, such as a fruit trait in a tree fruit crop, this means that cost or time savings due to elimination of plants early in the juvenile phase are not realized.

Since Lande and Thompson's (1990) publication, numerous researchers have conducted analytical and simulation studies of the efficiency of MAS schemes for response to selection. Most studies compared MAS or index-MAS with phenotypic selection under various genetic assumptions (e.g., Beavis, 1994; Dudley, 1993; Edwards and Page, 1994; Gimelfarb and Lande, 1994a, 1994b; Whittaker et al., 1995; Xie and Xu, 1998; Zhang and Smith, 1992, 1993). In general, these studies predict that MAS schemes may be more efficient than phenotypic selection for traits with low heritability when population size is very large. However, Moreau et al. (1998) compared analytical results with simulation studies for populations of realistically limited size and concluded that, for traits with heritability less than ≈0.2, the low power of QTL detection and the bias caused by the selection of markers reduce the efficiency. Over multiple generations of selection in a simulation study, Hospital et al. (1997) also reported the same observations, but, in addition, observed that the response to MAS was more variable than the response to phenotypic selection when heritability was low.

Nearly all the theoretical and analytical studies of MAS are constructed on the basis of additive genetic variance and narrow-sense heritability. Many consider selection for only one trait at a time. For cultivar selection with strictly asexual propagation, fruit breeders are more concerned with broad-sense heritability, which considers all genetic variance and is almost invariably greater than narrow-sense heritability. Since traits with very low heritability may be more rare when nonadditive genetic components are considered, the possibilities for using MAS in a breeding program may be more limited. Several issues become more complicated when MAS is performed simultaneously for multiple quantitative traits (Bernardo, 1999; De Koning and Weller, 1994; Xie and Xu, 1998). Marker–QTL associations are more uncertain and linkage disequilibrium in specific crosses may become more difficult to evaluate.

Knapp (1998) extended the analytical study of the relative efficiency of MAS to examine the probability of selecting superior individuals from a population—an important consideration for breeders developing cultivars in clonally propagated crops. He concluded that MAS is most cost-effective when the breeder is seeking individuals from only the top 1% to 2% of the population for a given trait. Even then, the relative efficiency of MAS depends greatly on the heritability of the trait and the selection intensity. Note that Knapp described selection for a single trait. In fruit breeding programs, breeders usually select for multiple polygenic traits simultaneously, and, although only 1% to 5% of the population might be retained overall, the selection intensity for any single trait is less stringent.

Empirical studies. Empirical evidence for the relative genetic efficiency of MAS for quantitative traits is scant at this time. Numerous studies have been published reporting putative marker–QTL associations in various populations but few associations have been confirmed in independent validation populations or tested in selection experiments. Studies by Beavis (1994) and Melchinger et al. (1998) in maize (*Zea mays* L.), Wilcox et al. (1997) in *Pinus radiata* D. Don, and Han et al. (1997) in barley (*Hordeum vulgare* L.) indicated that the number of QTLs and their estimated genetic effects may vary substantially in independent samples, even from the same populations. Furthermore, the cases where MAS has been most effectively employed for quantitative traits have usually been in wide crosses with QTLs of large effect (e.g., Bernacchi et al., 1998a). Even in these studies, the response to MAS has not been directly compared with the most efficient conventional technique(s) available to the breeder. Marker–QTL associations in wide crosses should be viewed cautiously, as such associations may be confounded with known problems in chromosome pairing or other violations of assumptions that prevent inference to elite breeding populations (Strauss et al., 1992).

Several studies have compared selection responses for MAS with phenotypic selection schemes. Unfortunately, their utility is limited because economic considerations are addressed only vaguely, if at all. Likewise, the best available conventional approaches that a breeder might use to improve response to selection are ignored in some studies. In selection studies for several traits in maize, Stuber (1995) found MAS to be similar to phenotypic selection in identifying superior individuals, though more predictable among years and locations. However, F_2:S_4 testcross yield performance in a maize pedigree did not differ between lines selected based on conventional F_2 testcross performance and those selected using a marker index based on F_2 testcross performance (Stromberg et al., 1994). Likewise, selection responses in barley for increased yield assessed in multiple locations were not substantially different among MAS at up to four loci, tandem MAS and phenotypic selection, and phenotypic selection alone (Romagosa et al., 1999). The effectiveness of MAS schemes (tandem genotypic followed by phenotypic and combined genotypic-phenotypic index selection), based on two QTL for four highly heritable (h^2 = 0.39 to 0.67) malting quality traits, was inconsistent compared with phenotypic selection depending on the trait, selection intensity, and which QTL was used (Han et al., 1997). In common bean (*Phaseolus vulgaris* L.), the effectiveness of MAS vs. phenotypic selection for tolerance to drought stress depended on the cross and testing environment (Schneider et al., 1997). In *Arabidopsis thaliana* (L.) Heynh., bidirectional response to selection for flowering time (average h^2 = 0.34) did not differ between MAS for six QTL vs. phenotypic selection (Van Berloo and Stam, 1999).

Alternatives to MAS for traits of low heritability. A major criticism of most comparative studies of the genetic efficiency is that MAS schemes are usually compared with phenotypic selection. This comparison greatly simplifies the assumptions necessary for analytical or simulation studies, but is less useful and unnecessary in empirical studies if phenotypic selection is not the conventional plant breeding technique with the greatest expected efficiency in a given crop or situation. For example, the most efficient practices, and the ones most commonly used, for cultivar development in annual, outcrossing crops usually involve half-sib or full-sib progeny testing. Phenotypic selection is usually reserved for improving base populations before incorporation into elite material.

In fruit crops, phenotypic selection is commonly used in the first stage of selection among seedlings, but more efficient alternatives exist for traits of low heritability. Considerable efficiency can be gained by selection based on an index of the performance of relatives (Falconer and MacKay, 1996). The simplest system is the evaluation of multiple ramets, or clonal testing, of each genotype (Shaw and Hood, 1985). The "relatives" are essentially copies of the genotype and the "index value" is the mean performance over all ramets. Although costly, clonal evaluation can make seedling stage selection much more efficient. In some cases the expected increases in efficiency are similar to those for MAS schemes (Kerr and Goddard, 1997). Because of resource limitations, the major problem with clonal testing is that increased clonal replication is usually performed at the expense of the number of genotypes evaluated. For this reason, most

fruit breeders select on genotypic value based on clonal replication at a later stage of selection.

Selection using an index on relatives is used widely in animal and forest tree breeding. The technique can take advantage of the considerable genetic structure in a given cycle of a fruit breeding program. Usually several families of full-siblings are planted, and, because one or more parents are used in multiple crosses, plants in one cross may also be half-siblings of plants in other crosses. The genetic relationships may be exploited by using an index value for each individual based on its performance *per se* and the performance of its relatives. The breeder can use the index for selection among and within families. The only additional requirement most breeders would have in using this procedure is that the performance of each individual must be assessed. However, this is also a requirement for MAS schemes in order to estimate the value of alternate QTL-marker states.

Lande and Thompson's (1990) analysis shows that use of MAS combined with an index on relatives is expected to be more genetically efficient than the index on relatives alone, although economic efficiency will likely be negative or, at best, marginal, when costs are considered. In using index-MAS as described by Lande and Thompson, there are no savings in time or maintenance costs compared with constructing an index on relatives. All progeny must be kept in both situations to obtain a complete set of data on individual phenotypes. Index-MAS entails the additional cost of obtaining each marker genotype. Index-MAS should be at least twice as effective as an index on relatives only when heritability is quite low and a substantial proportion of the phenotypic variance is due to dominance or genotype × environment interactions—precisely the conditions when QTL detection techniques are least powerful.

MAS for polygenic traits in fruit breeding. Quantitative genetic theory and the analytical, simulation, and empirical research available on MAS suggest that a fruit breeder considering MAS for polygenic traits be aware of five points.

First, the trait must be well understood. The effectiveness of MAS is limited by the measurement of the phenotype. The normal range of response of the trait must be known in the target population of environments. The trait must then be assessed in a way that measures the genetic component of this variation with the greatest possible precision. The nongenetic variance contributing to phenotypic variation must be minimized or accounted for in the experimental design so that the phenotype of each individual in a population is measured with the greatest possible precision. In fruit crops, perhaps more so than in many other crops, the breeder is plagued by higher order interactions of genetic × nongenetic effects; not only are there interactions with production environments, but also with rootstocks, training systems, postharvest environments, and consumer preferences. If a breeder knows that the genetic value of an individual for a trait can be satisfactorily estimated only after several years, or in multiple locations or under alternative postharvest regimes, then any evaluation that is less rigorous will diminish the expected effectiveness of MAS. Suboptimal evaluation may even lead to lower gains for MAS than would be expected for phenotypic selection with proper multiple environment evaluation. Using MAS cannot be viewed as a miraculous cure for inefficient breeding practices or imprecise evaluation procedures.

Second, large numbers of progeny are essential. Perhaps the major limitation to using MAS in a practical way is the low power of detection of marker–QTL linkages with the size of populations that fruit breeders typically use; models become overparameterized because there are too many predictors (markers) fitting too few observations (progeny phenotypes). Experiments with small numbers of progeny will underestimate the number of QTLs and the genetic effect per QTL will be biased upward (Melchinger et al., 1998; Openshaw, 1997). Analytical studies suggest that a sample size of 100 to 300 progeny, as commonly used in mapping or QTL discovery studies in fruits (Arus et al., 1999; Hadonou and Russell, 1999; King, 1996; Striem et al., 1996; Wang et al., 1998), will probably be useful for detecting only about five or fewer QTLs depending on the heritability and number of effective loci for the trait (Lande and Thompson, 1990; Strauss et al., 1992). Empirical QTL discovery studies for a variety of quantitative traits in forest tree species confirm these predictions (Wilcox et al., 1997). When the sample size for a population is small, and the number of QTLs with small effects is large, then the number of QTLs detected becomes a function of sample size. The power for detection of QTLs is so low that Knapp and Bridges (1990) demonstrated that, in many data sets where the number of possible marker genotypes is greater than the number of progeny (the usual situation), markers can be identified that account for all the additive genetic variance, but are in fact not even linked to a QTL.

Third, large numbers of markers may be needed (Lande and Thompson, 1990; Strauss et al., 1992). Screening individuals for marker genotype has become increasingly faster and simpler with improved technology. Nevertheless, the procedures are not yet trivial in expense or in the need for expertise. Screening large populations with a large number of markers may still be necessary in the absence of robust maps.

Fourth, in the context of a fruit breeding program, separate marker–QTL linkage analyses could be needed for *each* cross or population for which MAS is to be used. The reasons for this are simple. The loci that control the inheritance of a trait in one cross may not be the same ones that are important in another. Furthermore, with the high heterozygosity and allelic richness that characterize many fruit species, a breeder cannot assume that markers that are in disequilibrium with QTL in one cross will necessarily be in disequilibrium in another cross.

Fifth, juvenile stage-MAS for quantitative traits may not be practical. The greatest cost efficiency for MAS for quantitative traits in fruit crops is anticipated if initial culling for a mature trait can be performed during the juvenile stage based solely on markers, and then further selection is practiced, based on a marker phenotype index, when trees mature (Xie and Xu, 1998). Using an analytical approach, Xie and Xu (1998) determined that this two-stage selection scheme should provide a substantially improved cost efficiency only when $h^2 \geq 0.3$ and the cost of obtaining measurements based on phenotype is greater than the cost of developing and scoring marker loci. This situation could occur for traits expressed only at maturity in long-lived fruit trees, as carrying costs can be high (see Table 1) and as technological developments reduce marker discovery and scoring costs. Their analysis, however, assumes previous research to discover marker–QTL linkages. We believe that this is the key assumption highlighting the impracticality of MAS for quantitative traits for fruit crops in most instances. As described earlier, for reliable discovery of marker–QTL linkages, linkage analysis must be performed on a reasonably large number of individuals (preferably several hundred) for a given cross, carried to maturity for a mature-expressed trait. At this point the breeder could use index-MAS among several hundred progeny and have a reasonably good chance of selecting at least one individual within the top 5% for genotypic value (Knapp, 1998). Why would a breeder then repeat the cross, use marker selection of juveniles for initial culling, and then wait years until the selected progeny matured —all at the likely expense of sampling new combinations of parents?

POTENTIAL COSTS, BENEFITS, AND RISKS ASSOCIATED WITH USING MAS IN FRUIT BREEDING PROGRAMS

Fruit breeders are interested in new techniques that improve their genetic efficiency in selection and reduce their risk of failing to identify genetically superior individuals. New techniques must also be cost- or benefit-effective. They should not entail such large costs that the result is a lower gain per unit cost or an extravagant cost per individual selected relative to the program's budget or its ability to secure additional funds. Financial benefits related to MAS may be internal or external to the breeding program. Internally, financial benefits may be derived from reducing the length of the breeding cycle or the time to develop a product. Producers, marketers, consumers or the public at large (e.g., through environmental impact) may derive external benefits. If adoption of a new technique simply allows a program to develop the same type of cultivar product with no monetary or time savings, and no external recognition of product value, then additional investment is unlikely to be forthcoming.

Any cost/benefit analysis will be specific to the breeding program and trait(s) being considered. For most perennial fruit crops, the costs

of carrying seedlings over time in a breeding program are far more than for annual crops. Thus, selection during the early juvenile phase can produce the maximum cost savings for MAS or other indirect selection schemes. The main cost factors affecting the efficiency of MAS will be the relative costs of 1) maintaining plants before and after MAS and 2) juvenile MAS vs. mature evaluation.

Most fruit breeding programs can be represented as two-stage selection programs. In Stage 1, large populations of nonreplicated individuals are evaluated and a small proportion are selected for extensive asexual propagation for Stage 2 testing in replicated trials.

The large plant size and long life cycle, especially a long juvenile period, have the greatest negative impact on cost and time efficiency of fruit breeding programs during Stage 1. Much land, as well as labor for maintenance and evaluation, may be required for many years by the high proportion of inferior seedlings destined for culling. Stage 2, though usually more land- and labor- intensive per genotype evaluated, is focused on a drastically reduced number of elite genotypes because of intense selection in Stage 1.

Simply inherited traits. The selection for a simply inherited trait should have impact on the efficiency of Stage 1 of a breeding program when most highly heritable, simply inherited, traits can be evaluated on the basis of a single plant per genotype. If large population sizes must be retained after MAS for a simply inherited trait in order to maintain a high probability of selecting superior individuals, then any economic benefits from MAS depend on four factors: 1) the proportion of the population retained after MAS; 2) the genetic efficiency of the MAS or how the initial culling from MAS affects the probability of retaining superior individuals at the end of stage 1 seedling evaluation for all traits. (To the extent that undesirable genotypes for the simply inherited trait are not detected with MAS because of recombination or technical problems in marker genotyping, the program will incur useless costs for maintenance and later evaluation.); 3) the relative costs of growing, maintaining, and evaluating plants for other traits before and after MAS; and 4) the relative costs of evaluating the simply inherited trait by MAS vs. by other methods.

The relationship of these factors during Stage 1 of a breeding program can be expressed in terms of the cost of obtaining a selection ($/sel), a putative superior individual, in the following equation, which is similar to that of Namkoong (1970):

$$(\$/sel) = \frac{\left[N_{juv} * \left(\$_{juv} + \$_{MAS}\right)\right] + \left[N_{mat} * \left(\$_{mat} + \$_{msel}\right)\right]}{\left(N_{juv}\right)\left(s_{MAS}\right)\left(s_{mat}\right)}$$

in which the numerator describes all the costs incurred during Stage 1 of a breeding program and the denominator describes the culling process by which a small number of selections are made from a large number of seedling individuals. The individual terms of the equation are described below:

N_{juv} = no. of seedlings grown and subjected to MAS in the juvenile phase

N_{mat} (= N_{juv}*s_{MAS}) = no. of seedlings carried to maturity for evaluation after MAS

s_{MAS} = proportion of seedlings retained after MAS

s_{mat} = proportion of seedlings identified as superior individuals ("selections") after mature phase evaluation

$\$_{juv}$ = cost on a per individual basis of producing seed, growing seedlings to MAS and of any other non-MAS evaluations that must be conducted on juvenile plants

$\$_{mat}$ = the present value of the future cost of maintaining an individual through maturity and complete evaluation in Stage 1 of the program

$\$_{MAS}$ = the costs associated with marker evaluation of an individual for simply inherited trait(s)

$\$_{sel}$ = the present value of the future cost of evaluating an individual after MAS, including evaluation for the simply inherited trait(s) preselected with markers, as well as all other traits that are evaluated to identify superior individuals

As a demonstration, we estimated direct costs ($\$_{juv}$ $\$_{mat}$, $\$_{msel}$) on a per individual basis for maintenance and evaluation during Stage 1 of our strawberry, apple and grape (*Vitis* sp.) breeding programs at the Univ. of Minnesota (Table 1). The costs include labor (wage and fringe benefits) for handling and maintenance of plants and for standard seedling evaluation, as well as charges for land, greenhouse space, equipment depreciation, and consumables. Because costs for $\$_{mat}$ and $\$_{msel}$ are incurred in the future, sometimes several years after the expenditure for MAS, the values for $\$_{mat}$ and $\$_{msel}$ were discounted (3% per annum) to their present value in the first year when the decision would be made to expend some of those funds on MAS.

Using these cost estimates, we calculated the internal break-even cost for employing MAS ($\$_{MAS}$) in the first year of Stage 1 of our programs (Table 2). For a simply inherited trait(s), s_{MAS} will depend on the homozygosity or heterozygosity of the parents at loci controlling the trait(s) and the proportion of the population retained, which is related to the heterozygosity of the parents and number of loci under MAS. We examined four cases (the desired allele is represented in uppercase letters):

Case 1) s_{MAS} = 1.0 if no MAS is performed and undesirable individuals are eliminated at maturity.

Case 2) s_{MAS} = 0.5 for a monogenic testcross, Aa x aa, from which the Aa progeny will be desired

Case 3) s_{MAS} = 0.25 for a cross between heterozygotes, Aa x Aa when the homozygous progeny AA are desired, or a digenic testcross, AaBb x aabb or Aabb x aaBb, when AaBb are desired.

Case 4) s_{MAS} = 0.125 for a trigenic cross, AaBbCc x aabbcc, or AaBbcc x aabbCc, etc., when AaBbCc are desired.

In most breeding programs, a base value for s_{mat} can be estimated from historical records of the proportion of individuals selected in Stage 1. In our programs, and indeed in those of most breeders we have polled, s_{mat} ranges from 0.01 to 0.04; i.e., 1% to 4% of the individuals evaluated are selected for further testing in Stage 2 to confirm their cultivar potential. In a population segregating for a simply inherited trait(s) that is selected at maturity, juvenile phase MAS in Cases 2–4 should increase s_{mat} from the expected base value because individuals discarded at maturity that were undesirable for the simply inherited trait, but otherwise superior, will be discarded earlier in MAS.

We used a base s_{mat} value of 0.02 for Case 1 in our analyses. We also assumed that the loci under MAS had no genetic effects, positive or

Table 1. Estimated costs[z] and present values in year 1(PV)[y] to produce, maintain, and evaluate a seedling in grape and strawberry breeding programs at the Univ. of Minnesota on a per seedling basis.

Apple		Grape		Strawberry	
Year and operation	Cost (U.S.$)	Year and operation	Cost (U.S.$)	Year and operation	Cost (U.S.$)
Year 1 (produce seed and seedlings)	0.86	Year 1	0.89	Year 1	0.83
Year 2–3		Year 2		Year 2	
Nursery	1.69	Nursery	1.13	Plant to field	1.02
Year 4–8		Year 3–5		Year 3	
Orchard care	10.53	Vineyard care	6.27	Field care	0.39
Evaluation	2.40	Evaluation	3.36	Evaluation	0.28
Total	15.48		11.65		2.52
PV of yr 2–8 in yr 1		PV of yr 2–5 in yr 1		PV of yr 2–3 in yr 1	
Maintenance	10.78		6.86		1.36
Evaluation	1.97		3.02		0.25

[z]Estimates of labor costs were based on records from 1994 to 1998 in our programs. The costs were computed in using 1998 rates for labor, greenhouse space charges, and land rental, and based on the Univ. of Minnesota programs, which, in a given year, would produce 3000–5000 new seedlings in Stage 1 for each crop.

[y]Because costs for $\$_{mat}$ and $\$_{msel}$ are incurred in the future, sometimes several years after the expenditure for marker-assisted selection (MAS), the values for $\$_{mat}$ and $\$_{msel}$ were discounted (3% per annum) to their present value in the first year when the decision would be made to expend some of those funds on MAS.

Table 2. Maximum cost per seedling for juvenile phase (year 1) marker-assisted selection (MAS) in Cases 2, 3, and 4 (proportion of seedlings retained after MAS = 0.50, 0.25, and 0.125, respectively)[z] in order to break even with the conventional selection (Case 1, proportion of seedlings retained = 1.0) on a per selected seedling basis.[y]

Added conventional evaluation cost (U.S.$/seedling)	Break-even cost per seedling for MAS (U.S.$ per seedling)[y]								
	Apple			Grape			Strawberry		
	Case 2	Case 3	Case 4	Case 2	Case 3	Case 4	Case 2	Case 3	Case 4
0.00	5.70	8.25	9.22	4.42	6.36	7.11	0.69	0.99	1.08
0.50	5.92	8.57	9.59	4.64	6.70	7.48	0.92	1.32	1.44
1.00	6.16	8.90	9.96	4.87	7.02	7.85	1.15	1.64	1.81
2.00	6.60	9.55	10.68	5.31	7.66	8.58	1.60	2.29	2.54
3.00	7.05	10.21	11.42	5.77	8.32	9.32	2.05	2.94	3.27
4.00	7.51	10.86	12.15	6.21	8.98	10.05	2.50	3.60	4.00
5.00	7.96	11.50	12.89	6.66	9.64	10.78	2.95	4.25	4.74
6.00	8.40	12.16	13.62	7.11	10.28	11.51	3.40	4.90	5.47
7.00	8.86	12.82	14.35	7.56	10.94	12.24	3.85	5.56	6.20
8.00	9.31	13.47	15.09	8.01	11.58	12.97	4.30	6.20	6.94
9.00	9.75	14.12	15.82	8.46	12.24	13.71	4.75	6.86	7.66
10.00	10.20	14.77	16.55	8.91	12.90	14.44	5.20	7.51	8.40
20.00	14.70	21.30	23.87	13.41	19.42	21.76	9.70	14.03	15.73

[z]Case 1) $s_{MAS} = 1.0$ if no MAS is performed and undesirable individuals are eliminated at maturity.
Case 2) $s_{MAS} = 0.5$ for a monogenic testcross, Aa x aa, from which the putative Aa progeny will be retained.
Case 3) $s_{MAS} = 0.25$ for a cross between heterozygotes, Aa x Aa from which the putative homozygous progeny AA are desired, or a digenic testcross, AaBb x aabb or Aabb x aaBb, from which putative AaBb are retained.
Case 4) $s_{MAS} = 0.125$ for a trigenic cross, AaBbCc x aabbcc, or AaBbcc x aabbCc, etc., from which AaBbCc are retained.
[y]The break-even cost is related to the marginal added cost of conventional evaluation for the trait(s) under MAS. Break-even costs were calculated using the formula in the text and the estimated costs in Table 1. See text for further explanation of conditions and assumptions.

negative, through linkage or pleiotropy on other important traits, and that MAS was 95% effective on a per locus basis because of recombination or technical problems in determining marker genotype.

The internal break-even costs on a per seedling basis for MAS for the Univ. of Minnesota apple, grape, and strawberry breeding programs are presented in Table 2 in relation to the marginal additional cost for conventional evaluation of the traits under selection. Thus, if a trait is already evaluated in our program and would require no additional resources for phenotypic evaluation, then the break-even cost for us to screen for one locus controlling that trait would be $5.70 for apple, $4.42 for grape, and $0.69 for strawberry. If phenotypic evaluation of the single locus trait under selection required an additional $1.00 per seedling to implement, then the break-even cost for MAS would rise to $6.16 for apple, $4.87 for grape, and $1.15 for strawberry. The situation for MAS is more favorable in apple and grape, our longer cycle crops, than in strawberry. Nevertheless, using the current technologies it is problematic whether we could perform MAS unless the marginal increase in conventional evaluation costs for the trait(s) was large, i.e., greater than $5 to $10 per seedling.

The comparable break-even cost for MAS in this analysis should appropriately cover all the direct costs of marker screening, such as labor, consumables and laboratory equipment depreciation for sample collection, labeling of plants, DNA extraction, PCR reactions, and marker analysis and interpretation. In addition, it would include at least a portion of the marker development costs.

Polygenic traits. An economic analysis of MAS schemes for polygenic traits is more complicated than one for simply inherited traits, because the predicted genetic efficiency and, therefore, cost-benefit efficiency, depends on several parameters, such as heritability of the trait, the proportion of genetic variance explained by markers, selection intensity and the number of traits being selected using index-MAS or phenotypic selection, among others. However Knapp's (1998) analysis of the efficiency of selecting superior individuals from a population provides a framework for a simple model to estimate break-even costs when using index-MAS to select for a single trait.

In Table 3, we have estimated the break-even cost per seedling for index-MAS using the cost estimates for the University of Minnesota program in Table 1 and Knapp's (1998) predicted progeny sizes that would be required for testing if a breeder wanted to be 99 % confident of including at least one individual in the top 10%, 5%, or 1% for genotypic value among the individuals selected if the top 10%, 5% or 1% of individuals are selected for a trait evaluated in the mature phase. Even though index-MAS requires that all individuals would have to be grown to maturity to evaluate mature-phase traits (Lande and Thompson, 1990; and discussed above), Knapp's (1998) analysis predicts that fewer individuals should need to be tested for index-MAS than for phenotypic selection when $P > 0$. The "savings" from not growing, maintaining, and testing these individuals provides the internal funds to discover markers and screen the individuals for the markers. In this analysis, we have assumed there are no added costs of phenotypic evaluation beyond those accounted for in Table 1. If added evaluation costs for the trait were incurred, they would increase the break-even cost for marker screening as in Table 2.

The analysis in Table 3 suggests that the internal funds available per screening for marker discovery and screening would probably be substantial only for traits with heritability ≤ approximately 0.2 and when selection is very intense. As discussed above, however, discovery of QTL for traits with such low heritability lacks power, especially in this scenario with nonreplicated genotypes at only one location and with the relatively small population sizes screened in many cases in Table 3 (Knapp and Bridges, 1990; Lande and Thompson, 1990; Moreau et al., 1998). As with simply inherited traits, the internal funds available for MAS for a mature-phase trait are related to the length of the juvenile phase and the size of the plant. Thus, for apple and grape programs at the University of Minnesota, the available funds are far more substantial than for strawberry.

This analysis is obviously very simplified and accounts for few of the realistic choices and logistical considerations a fruit breeder encounters in selecting for polygenic traits. For example, this analysis considers only one trait and the selection intensities considered are probably more intense than those actually encountered for any single trait in a program in which selection is based on multiple traits. If the analysis were extended to a second trait, for example, only a slightly greater amount of funds saved from not performing phenotypic evaluation for the second trait would be available to fund discovery and screening for what would likely be a second complete set of markers. Nevertheless, this simple analysis illustrates the challenges a fruit breeder will face when justifying MAS based on internal cost-benefit considerations.

CONCLUSIONS

MAS schemes are most likely to make sense in a fruit breeding program under the following conditions: 1) the trait(s) is simply inherited; 2) the trait is expressed in the mature phase of long-lived species with long juvenile periods; 3) MAS is practiced very early in the juvenile phase; 4) the trait(s) requires high added costs for conventional screening in the target population of environments; 5) locus discovery and marker genotyping are economical due to inexpensive marker technology and highly robust marker–locus associations in a

Table 3. The predicted number of progeny from a family that a breeder needs to test for phenotypic selection or index marker-assisted selection (MAS)[z] for a normally distributed quantitative trait with a heritability of 0.1, 0.2, 0.5, or 0.9 if markers account for 20%, 40%, or 60% of the genetic variance (P = 0.2, 0.4, 0.6, respectively) to be 99% certain of selecting one or more progeny that are among the top 10%, 5%, or 1% for genotype value (target) when 10%, 5%, or 1% of the family is selected[y], and the maximum cost per seedling for screening a family in order to break even with phenotypic selection based on savings in number of progeny to be tested with index-MAS using cost data from Univ. of Minnesota breeding programs.[x]

	No. of progeny to be tested using:				Internal break-even cost per seedling for implementing MAS ($)								
	Phenotypic	Index-MAS when			Apple			Grape			Strawberry		
Heritability	selection	P = 0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6	0.2	0.4	0.6
					10% selected, 10% target								
0.1	203	68	55	50	30.73	41.66	47.37	23.13	31.35	35.65	4.80	6.51	7.41
0.2	155	77	61	53	15.68	23.85	29.79	11.80	17.95	22.42	2.45	3.73	4.66
0.5	95	79	67	58	3.14	6.47	9.88	2.36	4.87	7.43	0.49	1.01	1.54
0.9	57	57	56	55	0.00	0.28	0.56	0.00	0.21	0.42	0.00	0.04	0.09
					10% selected, 5% target								
0.1	359	109	93	90	35.50	44.28	46.27	26.72	33.32	34.82	5.55	6.92	7.23
0.2	264	124	100	91	17.48	25.39	29.43	13.15	19.11	22.15	2.73	3.97	4.60
0.5	154	128	109	97	3.14	6.39	9.10	2.37	4.81	6.85	0.49	1.00	1.42
0.9	95	95	94	93	0.00	0.16	0.33	0.00	0.12	0.25	0.00	0.03	0.05
					10% selected, 1% target								
0.1	1424	468	458	458	31.62	32.65	32.65	23.80	24.57	24.57	4.94	5.10	5.10
0.2	998	495	459	458	15.73	18.18	18.25	11.84	13.68	13.74	2.46	2.84	2.85
0.5	579	503	468	459	2.34	3.67	4.05	1.76	2.76	3.05	0.37	0.57	0.63
0.9	458	458	458	458	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
					5% selected, 5% target								
0.1	614	150	118	104	47.88	65.07	75.91	36.04	48.97	57.13	7.49	10.17	11.87
0.2	429	177	132	111	22.04	34.83	44.35	16.59	26.21	33.38	3.45	5.45	6.93
0.5	229	183	150	126	3.89	8.15	12.65	2.93	6.14	9.52	0.61	1.27	1.98
0.9	122	121	119	116	0.13	0.39	0.80	0.10	0.29	0.60	0.02	0.06	0.13
					5% se1ected, 1% target								
0.1	2298	507	460	458	54.68	61.85	62.19	41.15	46.55	46.80	8.55	9.67	9.72
0.2	1492	573	473	459	24.83	33.35	34.84	18.68	25.10	26.22	3.88	5.21	5.45
0.5	734	590	506	466	3.78	6.98	8.90	2.84	5.25	6.70	0.59	1.09	1.39
0.9	463	462	461	460	0.03	0.07	0.10	0.03	0.05	0.08	0.01	0.01	0.02
					1% selected, 1% target								
0.1	7677	916	655	554	114.26	165.96	199.03	85.99	124.90	149.79	17.86	25.94	31.11
0.2	4678	1151	762	605	47.44	79.55	104.21	35.70	59.87	78.43	7.42	12.44	16.29
0.5	1682	1208	914	714	6.07	13.01	20.99	4.57	9.79	15.79	0.95	2.03	3.28
0.9	689	681	668	645	0.18	0.49	1.06	0.14	0.37	0.79	0.03	0.08	0.17

[z]According to Lande and Thompson (1990).
[y]Based on analyses by Knapp (1998; Table 2).
[x]Cost data from Univ. of Minnesota breeding programs presented in Table 1.

germplasm pool; and, finally, 6) MAS will provide a substantially improved probability of selecting superior individuals compared with the best conventional breeding and evaluation practice.

If the costs for MAS are projected to exceed the internal break-even costs, then the additional investment must be justified based on benefits external to the breeding program. For certain market sectors, the scale of production may be large enough, and the competition for market share for new cultivars severe enough, that large additional expenditure for MAS can be justified even if genetic risk is reduced only marginally or time-to-market is improved fractionally. Such external economies of scale can easily be realized in the massive U.S. market for hybrid maize seed, for example, where even a few kilograms of yield advantage can translate into a market share advantage worth millions of dollars. The critical question for fruit breeders is which simply inherited traits, if any, have substantial external benefits that will translate into the increased public or private investment necessary to implement MAS?

Each case in each breeding program will be unique, but we predict that cases where MAS in fruit cultivar development programs will be justified based on genetic and economic efficiency will be few. Breeders will need to carefully evaluate specific program needs and alternatives to identify those most critical cases for which the substantial investment in markers and mapping technology represents the best opportunity for returns to stakeholders.

Literature Cited

Arus, P., J. Ballester, B. Jaurengue, T. Joobeur, M.J. Truco, and M.C. de Vicente. 1999. The European *Prunus* mapping project: Update on marker development in almond. Acta Hort. 484:331–336.

Baird, W.V., R.E. Ballard, S. Rajapakse, and A.G. Abbott. 1996. Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. HortScience 31:1099–1106.

Bartish, I.V. and N.F. Weeden. 1999. The use of interspecific crosses in *Malus* to map the genes of characters important for apple rootstock breeding. Acta Hort. 484:319–330.

Beavis, W.D. 1994. The power and deceit of QTL experiments: Lessons from comparative QTL studies, p. 250–266. In: 49th Annu. corn and sorghum industry research Conf. Amer. Seed Trade Assn., Washington, D.C.

Bernacchi, D., T. Beck-Bunn, D. Emmatty, Y. Eshed, S. Inai, J. Lopez, V. Petiard, H. Sayama, J. Uhlig, D. Zamir, and S. Tanksley. 1998a. Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions of desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium.* Theor. Appl. Genet. 97:170–180.

Bernacchi, D., T. Beck-Bunn, Y. Eshed, J. Lopez, V. Petiard, J. Uhlig, D. Zamir, and S. Tanksley. 1998b. Advanced backcross QTL analysis of tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum.* Theor. Appl. Genet. 97:381–397.

Bernardo, R. 1999. Two-trait selection response with marker-based assortative mating. Theor. Appl. Genet. 98:551–556.

Conner, P.J., S.K. Brown, and N.F. Weeden. 1997. Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. J. Amer. Soc. Hort. Sci. 122:350–359.

De Koning, G.J. and J.I. Weller. 1994. Efficiency of direct selection on quantitative trait loci for a two-trait breeding objective. Theor. Appl. Genet. 88:669–677.

Dirlewanger, E., V. Pronier, C. Parvery, C. Rothan, A. Guye, and R. Monet. 1998. Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. Theor. Appl. Genet. 97:888–895.

Dudley, J.W. 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. Crop Sci. 33:660–668.

Edwards, M.D. and N.J. Page. 1994. Evaluation of marker-assisted selection through computer simulation. Theor. Appl. Genet. 88:376–382.

Falconer, D.S. and T.F.C. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group, Essex, U.K.

Foolad, M.R., S. Arulsekar, V. Becerra, and F.A. Bliss. 1995. A genetic map of *Prunus* based on an interspecific cross between peach and almond. Theor. Appl. Genet. 91:262–269.

Gardiner, S., V. Bus, H. Bassett, A. White, C. Noiton, E. Rikkerink, R. Ball, and R. Forster. 1999. An updated genetic map around the V_f gene for resistance to apple scab and marker assisted selection for resistance. Acta Hort. 484:481–485.

Gianfranceschi, L., B. Koller, N. Seglias, M. Kellerhals, and C. Gessler. 1996. Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theor. Appl. Genet. 93:199–204.

Gill, G.P., C.F. Harvey, R.C. Gardner, and L.G. Fraser. 1998. Development of sex-linked PCR markers for gender identification in *Actinidia*. Theor. Appl. Genet. 97:439–445.

Gimelfarb, A. and R. Lande. 1994a. Simulation of marker assisted selection in hybrid populations. Genet. Res. Camb. 64:39–47.

Gimelfarb, A. and R. Lande. 1994b. Simulation of marker assisted selection for nonadditive traits. Genet. Res. Camb. 64:127–136.

Gmitter, F.G., Jr., Z. Deng, and G.A. Moore. 1998. Utilization of DNA markers in citrus breeding programs. Fruits 53:303–306.

Gmitter, F.G. Jr., S.Y. Xiao, S. Huang, X.L. Hu, S.M. Garnsey, and Z. Deng. 1996 A localized linkage map of the citrus tristeza virus resistance gene region. Theor. Appl. Genet. 92:688–695.

Hadonou, A.M. and K. Russell. 1999. Development of the cherry genome map. Acta Hort. 484:359–362.

Han, F., I. Romagosa, S.E. Ullrich, B.L. Jones, P.M. Hayes, and D.M. Wesenberg. 1997. Molecular marker-assisted selection for malting quality traits in barley. Mol. Breeding 3:427–437.

Hemmat, M., N.F. Weeden, P.J. Conner, and S.K. Brown. 1997. A DNA marker for columnar growth habit in apple contains a simple sequence repeat. J. Amer. Soc. Hort. Sci. 122:347–349.

Hospital, F., C. Chevalet, and P. Mulsant. 1992. Using markers in gene introgression breeding programs. Genetics 132:1199–1210.

Hospital, F., L. Moreau, F. Lacoudre, A. Charcosset, and A. Gallais. 1997. More on efficiency of marker assisted selection. Theor. Appl. Genet. 95:1181–1189.

Kerr, R.J. and M.E. Goddard. 1997. Comparison between the use of MAS and clonal tests in tree breeding programmes, p. 297–303. In: For. Res. Inst. of N.Z. Bul. No. 203.

King, G.J. 1996. Progress of apple genetic mapping in Europe. HortScience 31:1108–1111.

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci. 38:1164–1174.

Knapp, S.J. and W.C. Bridges. 1990. Using molecular markers to estimate quantitative trait locus parameters: Power and genetic variances for unreplicated and replicated progeny. Genetics 126:769–777.

Lahogue, F., P. This, and A. Bouquet. Identification of a codominant scar marker linked to the seedlessness character in grapevine. Theor. Appl. Genet. 97:950–959.

Lande, R. and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756.

Lawson, D.M., M. Hemmat, and N.F. Weeden. 1995. The use of molecular markers to analyze the inheritance of morphological and development traits in apple. J. Amer. Soc. Hort. Sci. 120:532–537.

Maliepard, C., F.H. Alston, G. van Arkel, L.M. Brown, E. Chevreau, F. Dunemann, K.M. Evans, S. Gardiner, P. Guilford, A.W. van Heusden, J. Janse, F. Laurens, J.R. Lynn, A.G. Manganaris, A.P. M. den Nijs, S. Sansavini, H. Schmidt, S. Tartarini, J.J. Verhaegh, M. Vrielinc-vanGinkel, and G.K. King. 1998. Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor. Appl. Genet. 97:60–73.

Markussen, T., J. Krüger, H. Schmidt, and F. Dunemann. 1995. Identification of PCR- based markers linked to the powdery-mildew-resistance gene Pl_1 from *Malus robusta* in cultivated apple. Plant Breeding 114:530–534.

Mehlenbacher, S.A. 1995. Classical and molecular approaches to breeding fruit and nut crops for disease resistance. HortScience 30:466–476.

Melchinger, A.E., H. F. Utz, and C.C. Schön. 1998. Quantitative trait locus (QTL) mapping using different testers and independent populations samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149:383–403.

Moreau, L., A. Charcosset, F. Hospital, and A. Galais. 1998. Marker-assisted selection efficiency in populations of finite size. Genetics 148:1353–1365.

Namkoong, G. 1970. Optimal allocation of selection intensity in two stages of truncation selections. Biometrics 26:465–476.

Openshaw, S. 1997. QTL identification and marker assisted selection in commercial maize breeding, p. 335. In: For. Res. Inst. of N.Z. Bul. No. 203.

Paterson, A.H. 1996. Genome mapping in plants. R.G. Landers Co., Austin, Texas.

Quarta, R., M.T. Dettori, I. Verde, A. Vantaggi, and R. Sciarroni. 1999. Progress in mapping the peach genome. Acta Hort. 484:377–381.

Romagosa, I., F. Han, S.E. Ullrich, P.M. Hayes, and D.M. Wesenberg. 1999. Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. Mol. Breeding 5:143–152.

Schneider, K.A. M.E. Brothers, and J.D. Kelly. 1997. Marker-assisted selection to improve drought resistance in common bean. Crop Sci. 37:51–60.

Shaw, D.V. and J.V. Hood. 1985. Maximizing gain per effort by using clonal replicates in genetic tests. Theor. Appl. Genet. 71:392–399.

Strauss, S.H., R. Lande, and G. Namkoong. 1992. Limitations of molecular-marker-aided selection in forest tree breeding. Can. J. For. Res. 22:1050–1061.

Striem, M.J., G. Ben-Hayyim, and P. Spiegel-Roy. 1996. Identifying molecular genetic markers associated with seedlessness in grape. J. Amer. Soc. Hort. Sci. 121:758–763.

Stromberg, L.D., J.W. Dudley, and G.K. Rufener. 1994. Comparing conventional early generation selection with molecular marker assisted selection in maize. Crop Sci. 34:1221–1225.

Stuber, C.W. 1995. Mapping and manipulating quantitative traits in maize. Trends Genet. 11:477–481.

Tanksley, S.D. and J.C. Nelson. 1996. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92:191–203.

Urbanietz, A., H. Schmidt, and F. Dunemann. 1999. Molecular markers in early seedling tests for scab and mildew in apples. Acta Hort. 484:429–434.

Van Berloo, R. and P. Stam. 1999. Comparison between marker-assisted selection and phenotypical selection in a set of *Arabidopsis thaliana* recombinant inbred lines. Theor. Appl. Genet. 98:113–118.

Wang, D., R. Karle, T.S. Brettin, and A.F. Iezzoni. 1998. Genetic linkage map in sour cherry using RFLP markers. Theor. Appl. Genet. 97:1217–1224.

Whittaker, J.C., R.N. Curnow, C.S. Haley, and R. Thompson. 1995. Using marker maps in marker-assisted selection. Genet. Res. 66:255–265.

Wilcox, P.L., T.E. Richardson, and S.D. Carson. 1997. Nature of quantitative trait variation in *Pinus radiata:* Insights from QTL detection experiments, p. 304–312. In: For. Res. Inst. Bul. No. 203.

Xie, C. and S. Xu. 1998. Efficiency of multistage marker-assisted selection in the improvement of multiple quantitative traits. Heredity 80: 489–498.

Zhang, W. and C. Smith. 1992. Computer simulation of marker-assisted selection utilizing linkage disequilibrium. Theor. Appl. Genet. 83:813–820.

Zhang, W. and C. Smith. 1993. Simulation of marker-assisted selection utilizing linkage disequilibrium: The effects of several additional factors. Theor. Appl. Genet. 86:492–496.