Limitations of molecular-marker-aided selection in forest tree breeding[1]

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The advances to date with quantitative trait locus identification in agronomic crops, which have mostly been with studies of inter- and intra-specific hybrids, are of little relevance to assessing the potential for marker-aided selection in nonhybrid forest tree populations. Although molecular markers provide great opportunities for dissection of quantitative traits in experimental populations, we expect that their near-term usefulness in most operational tree breeding programs will be limited. In addition to cost, this limitation results from quantitative trait locus – marker associations being limited to specific genetic backgrounds as a result of linkage equilibrium, interactions of quantitative trait locus effects with genetic backgrounds, genotype by environment interaction, and changes of quantitative trait locus allele frequencies among generations. Marker-aided selection within individually mapped full-sib families can substantially aid phenotypic selection, but only where large restrictions of genetic base are tolerated, trait heritabilities are low, markers are able to explain much of the additive variance, selection intensities within families are high compared with that among families, and very large numbers of progeny are examined. Broad use of marker-aided selection in the longer term will require substantial technical advances in a number of areas, including means for precise quantitative trait locus identification; reduction of large-scale mapping and genotyping costs; and changes in breeding and propagation systems. Consideration of trait characteristics suggests that marker-aided selection will be most efficient in direct selection with high-value, low-heritability traits such as height and diameter growth. These traits, however, often show genotype by environment interaction and unfavorable genetic correlations with other desirable traits, and are likely to be controlled by a large number of minor genes rather than relatively few major ones. Traits with the most potential for marker-aided selection in nonhybrid tree populations will therefore be strongly inherited ones for which phenotypic assay is difficult; examples might include wood quality, resistance to biotrophic pathogens, and resistance to air pollutants. Because of the large disequilibrium generated during hybridization and the great phenotypic variance that segregates in F_2 and backcross generations, interspecific hybrid programs lend themselves much more readily to marker-aided selection. Segregation distortion and related meiotic aberrations, however, may substantially hamper precise estimation of quantitative trait locus locations and phenotypic effects. Nonadditive quantitative trait locus effects will likely be greater in hybrid populations than in intraspecific populations. Rapid decay of disequilibrium due to recombination, and allele frequency shifts due to selective breeding and natural selection during early generations after hybridization, are likely to cause instability for quantitative trait locus – marker associations and quantitative trait locus phenotypic effects. Finally, interspecific hybridization of highly heterozygous individuals from species in linkage equilibrium will impede marker-aided selection.

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À ce jour, les progrès dans l'identification de loci contrôlant des caractères quantitatifs chez les plantes agricoles de valeur commerciale (dérivés principalement d'études impliquant des hybrides interspécifiques et intraspécifiques) sont peu pertinents pour évaluer le potentiel de la sélection assistée de marqueurs chez les populations non hybrides d'arbres forestiers. Même si les marqueurs moléculaires offrent des perspectives intéressantes pour disséquer les caractères quantitatifs chez des populations expérimentales, nous anticipons à court terme une utilité limitée pour ces stratégies dans la plupart des programmes opérationnels d'amélioration des arbres. En plus de prendre en considération les coûts élevés engendrés par l'utilisation de ces stratégies, cette constation découle du fait que les associations entre caractères quantitatifs et marqueurs sont limitées à certaines environnements génétiques à cause de l'équilibre de liaison, des interactions entre les effets découlant des loci contrôlant les caractères quantitatifs et les environnements génétiques, des interactions entre les génotypes et les milieux, et des changements d'une génération à l'autre au niveau des fréquences d'allèles se retrouvant à ces loci. La sélection assistée de marqueurs au sein de descendances biparentales cartographiées individuellement peut faciliter substantiellement la sélection phénotypique, mais seulement lorsque des restrictions importantes sont tolérées au niveau de la base génétique, que les héritabilités sont faibles, que les marqueurs sont capables d'expliquer la majeure partie de la variance additive, que les intensités de sélection à l'intérieur des descendances sont élevées comparativement à celles retrouvées parmi les descendances, et lorsqu'un nombre élevé de descendances sont évaluées. L'usage élargi de la sélection assistée de marqueurs à long terme nécessitera des développements techniques substantiels dans un certain nombre de domaines, incluant les moyens d'identifier précisément les loci contrôlant les caractères quantitatifs, la réduction des coûts associés à la détermination des génotypes et à la cartographie à grande échelle, et des changements dans les systèmes de croisement et de multiplication. La considération des attributs des caractères quantitatifs suggère que la sélection assistée de marqueurs sera la plus efficace lors de la sélection

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directe à partir de caractères de haute valeur montrant une faible héritabilité, tels la hauteur et le diamètre. Toutefois, ces caractères démontrent souvent des interactions entre les génotypes et les milieux, des corrélations génétiques défavorables avec d'autres caractères souhaitables, et ils sont probablement contrôlés par un nombre important de gènes mineurs plutôt qu'un nombre relativement petit de gènes majeurs. Conséquemment, les caractères montrant le plus de potentiel pour la sélection assistée de marqueurs chez les populations non hybrides d'arbres seront ceux démontrant une forte héritabilité et pour lesquels l'évaluation phénotypique est difficile; par exemple, la qualité du bois, la résistance aux pathogènes biotrophes et la résistance aux polluants atmosphériques. En raison de l'important déséquilibre de liaison produit par l'hybridation et de l'importante variance phénotypique montrant une ségrégation au sein de la F_2 et des générations subséquentes issues de rétrocroisements, les programmes d'hybridation interspécifique se prêtent mieux à la sélection assistée de marqueurs. Toutefois, les distortions de ségrégation et les aberrations méiotiques qui leurs sont associées peuvent gêner substantiellement la précision d'estimation de la localisation des loci contrôlant les caractères quantitatifs et de leurs effets sur le phénotype. Les effets non additifs dérivés de ces caractères seront probablement plus importants au sein des populations hybrides qu'au sein des populations intraspécifiques. Une érosion rapide du déséquilibre de liaison due à la recombinaison, et des changements abrupts de fréquences alléliques dus aux croisements sélectifs et à la sélection naturelle durant les premières générations suivant l'hybridation, causeront probablement une instabilité des associations entre les loci contrôlant les caractères quantitatifs et les marqueurs, ainsi qu'une instabilité des effets phénotypiques. Finalement, l'hybridation interspécifique d'individus fortement hétérozygotes dérivés d'espèces en équilibre de liaison diminuera l'efficacité de la sélection assistée de marqueurs.
[Traduit par la rédaction]

Introduction

Molecular genetic markers, specifically restriction fragment length polymorphisms (RFLPs) and those generated via the polymerase chain reaction (PCR), have for the first time made it possible to map entire genomes of virtually any species. Because the markers are effectively unlimited in number and genomic distribution, they have renewed interest in use of chromosomal markers to assist in phenotypic selection during breeding. They have also stimulated a great deal of basic research directed at dissecting the number, distribution, and gene action of loci controlling quantitative traits. In this paper, we review studies of quantitative trait dissection only in so far as they help us to understand the potential for marker aided selection (MAS) in near- to medium-term operational forest tree breeding programs. Their importance for understanding of quantitative trait architecture, an immense basic research opportunity, is largely ignored.

We will emphasize the limitations and shortcomings of MAS; other papers in this volume explore its potential for accelerating improvement in a variety of traits and taxa. We concentrate on the theoretical limits to using MAS, rather than on technical obstacles to its practice, though there are some substantial practical impediments (e.g., selection with dominant molecular markers such as the popular randomly amplified polymorphic DNAs (RAPDs) (Williams *et al.* 1990)). Our focus will be primarily on use of MAS in "conifer-type" breeding, as exemplified by most programs with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the northwestern United States and loblolly pine (*Pinus taeda* L.) in the southeastern United States. These programs are characterized by testing and selecting large numbers of genotypes derived from multiple genetic backgrounds; maintenance of high genetic diversity in production forests; low to modest operating budgets; highly outbred materials not derived from recent hybridization events; sexual (seed) rather than clonal propagation; and emphasis on capture of additive genetic variation. Other programs, such as those for interspecific hybrids and deployment of clonal propagules, will be considered briefly and separately.

We proceed by (*i*) reviewing the nature of DNA markers, especially in comparison to the common biochemical markers they replace; (*ii*) discussing briefly what has been accomplished with quantitative trait locus (QTL) studies of agronomic plant species, and its relevance for forest trees; (*iii*) considering the characteristics of forest trees and tree breeding programs into which MAS must fit; (*iv*) identifying the major factors that govern usefulness of MAS in tree breeding programs; (*v*) exploring how breeding programs might be altered to take advantage of MAS; and (*vi*) discussing the unique opportunities and problems for MAS provided by interspecific hybrid breeding programs. We conclude by briefly considering the value of QTL methods in relation to alternative approaches to forest breeding and biotechnology.

DNA maps and markers

The terms QTL and genetic marker have been used in various ways. By QTL we refer to polymorphic loci containing alleles that differentially affect the expression of a continuously distributed phenotypic trait such as height. Such loci are rarely observed directly, but are instead inferred to exist via the correlation of linked genetic markers with phenotypic trait values. By genetic markers we refer to effectively neutral, nuclear genome markers, usually RFLPs or RAPDs, that may be linked to, but are not themselves, QTL. By MAS we refer to use of genetic markers to aid, and only rarely to replace, phenotypic selection.

Despite the existence of polymorphic genetic markers for more than 2 decades, only recently has interest in nuclear genome mapping and QTL analysis become widespread. This interest arises from the abundance of genetic markers that DNA methodology provides, which are effectively limitless in number and genomic distribution. Efforts to relate allozyme polymorphisms to yield and fitness components of trees were largely unsuccessful or ambiguous (Bush and Smouse 1992). By approaching the same problems with an order of magnitude more markers, full chromosome coverage, and carefully designed pedigrees, researchers can now determine the architecture of quantitative traits with unprecedented precision.

Given this power, why do only a minority of forest geneticists appear to be adopting DNA markers? The main impediment is cost. Despite numerous improvements in the last decade, RFLPs are more expensive to employ than allozymes in terms of equipment needed, consumable materials, salaries, and required technical sophistication. However, PCR-based methods such as RAPDs (Williams *et al.* 1990), which do not require development of probe banks or gel-blot hybridizations, may reduce costs and allow molecular genetic markers to be more widely adopted.

Experience with agronomic plants

The use of allozymes and DNA markers to facilitate selection has been studied far more intensely in agronomic crops than in trees. Development of molecular-marker maps is well advanced in a variety of crops (reviewed in Neale and Williams 1991; Stuber 1991), and QTL have also been analyzed in several taxa, particularly tomato, maize, lettuce, and soybean. Fruit quality and interspecific hybrids of tomato have received the most attention. These studies are sometimes cited as evidence of the promise of MAS for forest trees.

We believe, however, that the work completed to date with QTL identification in crops is of limited relevance to assessing the likelihood of success of MAS with forest trees. First, the majority of the results reported (tomato and soybean) have been conducted on interspecific hybrids and derived generations (Nienhuis *et al.* 1987; Osborn *et al.* 1987; Paterson *et al.* 1988, 1990, 1991; Weller *et al.* 1988; Keim *et al.* 1990*a*, 1990*b*; Kinzer *et al.* 1990). Hybridization produces great linkage disequilibrium (D) and results in segregation of huge amounts of phenotypic variation. It would have only been surprising had such studies *not* found many major QTL. Although locally important, interspecific hybridization is not a widespread form of tree breeding. Second, the results from studies within species are of segregating populations derived from hybridization among inbred lines of maize (reviewed by Stuber 1991). Inbreeding and hybridization produces D, enhancing QTL effects; to our knowledge, however, inbreeding has never been adopted in an operational breeding program in a forest tree species. Third, the traits studied, particularly in the interspecific cases, were largely of a morphological and chemical nature: fruit pH, ripening, and soluble solids in tomato; hard seededness in soybean. With the possible exception of wood anatomical and chemical features, there are few traits of a parallel nature that are important in trees. The yield-related traits of emphasis in tree breeding programs are likely to be less strongly inherited and more subject to interactions with genetic background and environment. Fourth, there have been some strong indications that heterozygosity of markers is useful for predicting heterosis in maize (Edwards *et al.* 1987; Lee *et al.* 1989; Smith *et al.* 1990; Stuber 1991). This heterosis may result from the D and dominance variance expressed in association with inbreeding and hybridization; studies in outbred trees have shown much weaker (Strauss 1986; Strauss and Libby 1987), or undetectable, heterosis associated with genetic markers (reviewed by Mitton and Jeffers 1989; Bush and Smouse 1992). Fifth, a major use of MAS in crop breeding will be to minimize linkage drag (the "hitchhiking" of undesirable chromosome segments derived from the nonrecurrent parent due to linkage) during introgression and backcrossing (Tanksley *et al.* 1989). However, because of the long generation times in forest trees and their limited degree of domestication, this form of breeding is unlikely to be of consequence for a long time to come. Finally, nearly all studies to date have confined their analyses to single crosses or pedigrees. In maize, when more than one cross was studied, there was little effort to compare or cross validate results between crosses (Edwards *et al.* 1987; Stuber *et al.* 1987). In the tomato hybrids studied by Paterson *et al.* (1991), broad chromosomal regions were identified that contained QTL common to different kinds of hybrids; however, many QTL were also unique to specific hybrids. Thus, to date there is limited evidence that important loci in one genetic background will be important in others. Given the diversity of germ plasm typically employed in tree breeding programs, such information is crucial to predict the potential of MAS.

Tree culture in relation to MAS

Culture of trees is distinct from that of most agronomic crops in several respects, and these impinge on the potential for MAS. First, long rotation ages require that genotypes be evaluated in field tests for many years. If MAS can effectively shorten the evaluation cycle, then its impacts on forest breeding may be relatively greater than its impacts on annual crop breeding. Conventional methods of early testing and evaluation, however, can already significantly shorten the testing cycle (Lambeth 1980; Menzies and Carson 1991), thereby limiting the additional impact of MAS. Another dimension of the long rotations for trees is that substantial genetic diversity within forests is usually maintained as a buffer against environmental variability (Kleinschmidt 1979). Application of MAS to forestry will therefore in most instances have to address a wide range of genetic backgrounds.

Clonal propagation is possible in many forest species and could allow the amplification of highly valuable genotypes identified by MAS. Despite many genetic advantages, however, clonal propagules usually cost far more to use operationally than seedlings do (Libby and Rauter 1984). MAS would have to markedly enhance seedling value to overcome this obstacle. Nonetheless, clonal propagules are finding their way into operational use in some intensive conifer programs, where they provide a means for both amplifying elite full-sib families for planting and taking advantage of advanced maturation state, as well as other factors (Miller 1991). Continuation of this trend would brighten prospects for MAS considerably. Finally, forest management is generally extensive rather than intensive. Because initial investments must be carried for many years and wood crops are less valuable than many agronomic crops, forest culture has typically received only modest funding in comparison with agriculture. The cost of an MAS program, however, is likely to be high. For example, Walton (1990) calculated that the cost of setting up an RFLP analysis station to work in conjunction with an operational crop breeding program would roughly equal the cost of setting up another breeding station. The problem of cost may be exacerbated if we accept Lande and Thompson's (1990) concept that MAS is substantially better than phenotypic selection only when applied to traits of low heritability. Analyzing such traits to identify statistically significant QTL will require very large sample sizes (discussed below).

Key genetic factors influencing success of MAS

Linkage equilibrium will result in weak and variable marker–QTL associations

Forest trees are predominantly outcrossing in their system of mating; mean outcrossing rates for wind-pollinated conifers typically exceed 80 to 90%, though variability among trees and environments is substantial. Insect-pollinated trees show more selfing, but are still predominantly outbred (reviewed in Muona 1990).

Outcrossers tend to show little or no D, whereas highly selfing species show strong D (Brown 1979). Forest trees demonstrate the same trend. In yellow-poplar (*Liriodendron tulipifera* L.), high D was found among six allozyme loci in

seedlings, but not in adult trees. The D appears to result largely from mating system effects that disappear as trees age (Roberds and Brotschol 1985). D among 14 loci in lodgepole pine (*Pinus contorta* Dougl.) was significant for gametes, but not for adult trees, and was found only among rare alleles at tightly linked loci (Epperson and Allard 1987). A significant number of cases of D among 10 loci in Douglas-fir was likewise found only among gametes, not mature trees, and appears to be associated with mating system effects on rare allele sampling and viability (Yeh and Morgan 1987). Thus, apart from transient D found among gametes or juvenile populations, forest trees appear to be in linkage equilibrium. This is not surprising given the large effective population sizes (N_e) in forest trees, which, as suggested for lodgepole pine (Epperson and Allard 1989), probably well exceed 1000 individuals for large numbers of generations. At this level, D would be expected only at recombination frequencies (r) below 0.025 centimorgans ($r < 0.25N_e$: Lande and Thompson 1990). This recombination frequency is well below the level of saturation anticipated for even the densest molecular maps (ca. 1–10 centimorgans).

Because populations of forest trees are weakly differentiated, only very modest D can be generated by hybridization among them. On average, 92% of the total gene diversity resides within populations of long-lived, woody, perennial plant species (Hamrick and Godt 1990). To our knowledge inbred lines have not been developed for any operational tree breeding program; crossing among lines is therefore also not a potential source of D in forest tree breeding.

The above considerations indicate that D is naturally very low in forest trees, and breeding programs do not appear likely to alter this situation in the near future. The consequence for MAS is inconsistent coupling–repulsion relationships between markers and QTL in different families and populations (Mackay 1990). For MAS to be successful, it must take advantage of the natural D within full-sib families, and correlations among QTL alleles and marker alleles must be determined for each pedigree of interest. Moreover, recombination distances are likely to differ among genetic backgrounds (Strauss and Conkle 1986) and sexes (Moran *et al.* 1983), affecting the confidence with which a marker can be used to identify QTL. For example, Moran *et al.* (1983) found that recombination between two loci in Monterey pine (*Pinus radiata* D. Don) was 43% higher in males than in females. Thus, coupling–repulsion problems aside, unless linkage is tight, useful markers of QTL in females may be inadequate in males, substantially complicating MAS.

One possible means for reducing the work required to map and perform MAS on many families would be to focus on extreme phenotypes (Lander and Botstein 1989). A variant of this option would be mapping a few families in detail to allow QTL to be identified, and then genotyping only extreme phenotypes in other families to determine coupling–repulsion phases between the marker alleles and QTL alleles. Whether this will suffice to make MAS feasible will depend on many genetic and economic factors, including whether the same loci will indeed be associated with major QTL in different families. Moreover, because the extreme individuals and families of interest to genotype will differ among traits, such a strategy would be useful in only those cases where one or a very few traits are under selection (Stuber 1991). Even with such shortcuts, MAS would still be likely to impose some constraints on the number of families that can be tested, with a consequent reduction of genetic base and opportunity for among-family selection.

Genotype × environment associated with present environments and anticipated anthropogenic changes will narrow the scope of MAS

In comparison with agronomic crops, forest trees are grown under conditions of great environmental heterogeneity. Lands are less intensively cultivated and fertilized, trees must survive both winters and growing seasons for many years before harvest, and occurrence in remote or topographically difficult landscapes limits accessibility for chemical and cultural manipulations. Environmental heterogeneity is particularly acute in the western United States, where great climatic variability associated with montane topography can occur over short distances. Environmental variability requires large samples for estimating QTL effects on environmentally sensitive traits (Michelemore and Shaw 1988) and potentiates the expression of genotype × environment (G × E).

G × E is commonly observed for a wide variety of traits in forest trees, including rate of volume growth (Bridgewater and Stonecypher 1978), disease resistance (Powers and Matthews 1980), and wood quality (Loo *et al.* 1982; McKimmy and Campbell 1982; McKinley *et al.* 1982). Its significance depends on whether it causes changes in genetic ranks rather than merely in magnitude of superiority, and how many genotypes and environments contribute to it (Bridgewater and Stonecypher 1978). When G × E is important, QTL may need to be verified or newly identified in many environments, adding substantially to the expense of MAS. Stuber (1991) cited only a few cases of QTL–environment interactions. Paterson *et al.* (1991), however, reported that of 29 QTL identified in tomato hybrids grown in three environments, only 4 were common to all environments, and 15 were unique to single environments.

The problem of G × E is particularly troublesome for MAS in that QTL must be identified during one rotation or testing cycle and then applied to another if selection is to be accelerated. For example, QTL might be determined in a 10- to 20-year test and then used to help rogue a seed orchard producing seedlings planted one to several decades after the start of the first test. Apart from genetic changes in populations between rotations (discussed below), environments also often change substantially among rotations. Changes in regeneration and silvicultural practices are likely to influence genetic performance (e.g., nursery practice, site preparation, pesticide application, fertilization, thinning) (Bridgewater and Stonecypher 1978). Moreover, major anthropogenic changes in environment are expected, for example, in ambient CO_2, temperature, precipitation, and atmospheric pollutants. They are likely to present major new stresses on tree populations and to affect genotypes differentially (Houston and Stairs 1973; Perry and Maghembe 1989). G × E is not a problem unique to MAS; it complicates and adds expense to conventional breeding as well. Nonetheless, the added cost of mapping the expression of QTL in several environments does represent a substantial obstacle to adoption of MAS.

Genetic correlations are often important in tree populations, complicating the use of QTL effects

Many of the traits of most importance to tree breeders (such as rate of growth, adaptation to stress, and wood strength)

show antagonistic relationships with one another. For example, selection for more rapid growth is often associated with reduced tolerance of frost damage (Rehfeldt 1989) and reduced wood density (Zobel and van Buijtenen 1989). Higher order interactions, such as a genetic correlation that varies among environments, are also probably common, e.g., an unfavorable genetic correlation between growth and wood density in one plantation but not in another (Megraw 1985). As with G × E, the problems posed by unfavorable genetic correlations are not unique to MAS, but render questionable the worth of MAS' additional expense. A potentially large and distinct contribution from MAS, however, might be the ability to separate QTL that give rise to antagonistic pleiotropy from those due to linkage. By allowing breeders to emphasize the latter kinds of genetic effects, unfavorable correlated responses to selection could be minimized.

MAS may require large sample sizes in breeding programs in which multiple traits are being selected. It is well known that genetic correlations require large samples for accurate estimation (Falconer 1981). Sample size requirements are not likely to be substantially larger for estimation of QTL–trait correlation effects than for genetic correlations (Knapp and Bridges 1990; S. Knapp, personal communication, 1991). But, when many traits and markers are to be included in an MAS index, very large sample sizes appear to be necessary for accurate estimation of index weights (discussion in Lande and Thompson 1990). This is a significant concern because breeders are usually interested in improvement of multiple traits, and the costs of large samples may be beyond the means of operational programs.

MAS is likely to be most effective in those traits for which indirect selection is least important

Lande and Thompson (1990) showed that in comparison with phenotypic selection, the theoretical efficiency of MAS can only be large with traits of low heritability (h^2). For example, with traits of relatively high heritability (h_2 = 0.2–0.5), under a variety of selection schemes the efficiency of MAS compared with that of phenotypic selection rarely exceeds 1.5, depending on the proportion of additive variance explained by the markers (Fig. 1 in Lande and Thompson 1990). We believe, however, that for forest trees the likelihood of successful application of MAS will be greater for high than for low heritability traits.

Traits of higher heritability are often ontogenetically and physiologically simpler than those of low heritability, i.e., most phenotypic variance in these traits can be directly traced to a limited number of biochemical processes. It is therefore reasonable to expect them to be affected by fewer genes, which can be more readily identified by QTL analysis, than for complex traits. We refer to them here as oligogenic traits. Examples include resistance to biotrophic pathogens such as rusts (Kinloch and Stonecypher 1969), most of the variance for which probably arises from specific genes for pathogen recognition; secondary compound concentration (Strauss and Critchfield 1982), most of the variance for which arises from activities of specific enzymes in biosynthesis of terpenoids and phenolics; wood quality (Zobel and Talbert 1984), which depends on specific aspects of xylem cell dimensions such as wall thickness, length, and orientation; and shoot phenology (Campbell 1979; Skroppa 1982; Ekberg *et al.* 1985), which depends on specific sensory molecules such as phytochrome, and on agents such as growth regulators that transmit their signals throughout the plant. Although these traits are also undoubtedly influenced by minor genes, we expect that because of the limited number of processes that separate genes from phenotypes, most of their variance will be attributable to relatively few major genes. Control of some of these traits by single Mendelian genes has, in fact, been demonstrated in some genetic backgrounds (Kinloch and Littlefield 1977; Strauss and Critchfield 1982).

In contrast, traits of a more physiologically complex nature include height growth, diameter growth, reproductive output, resistance to necrotrophic pathogens, and tolerance of certain abiotic stresses such as moisture and temperature extremes. Because they are likely to be influenced by a larger number of underlying physiological processes, they are probably influenced by a very large number of genes. We refer to them here as polygenic traits. Regardless of whether this dichotomy will be proven to be correct, we believe that it is useful for thinking about how different traits are likely to respond to MAS.

In addition to the lower heritability and more complex genetic basis of polygenic traits in comparison with oligogenic traits, the former also frequently show stronger interaction with environment and substantial antagonistic pleiotropy. These traits will therefore be more difficult objects of MAS than oligogenic traits. Thus, despite the theoretically greater efficiency of applying MAS to low-heritability polygenic traits than to high-heritability oligogenic traits, it is primarily oligogenic traits that appear to have a high likelihood of success in forest trees. This relation reduces substantially the potential economic superiority of MAS over phenotypic selection alone. Of course, were MAS to be less expensive or faster than phenotypic selection, as might be the case for some kinds of difficult to assay oligogenic traits (e.g., air pollution tolerance), it could be useful even with little or no enhanced genetic efficiency.

The broad genetic base over which to extrapolate, and temporal changes in gene frequencies and environments among rotations, will limit consistency of QTL effects

We argued above that linkage equilibrium will cause inconsistent coupling–repulsion relationships among marker loci and QTL in different genetic backgrounds. Here we address a related but distinct point, that the important QTL, and alleles at the QTL, will themselves frequently differ among genetic backgrounds (Mackay 1990).

QTL may differ among genetic backgrounds for a number of reasons. First, both a QTL and its genetic marker will not be polymorphic in all backgrounds. If we consider loci with a heterozygosity of 50% in linkage equilibrium with one another, the probability of both the QTL and marker being heterozygous in a random parent tree is only 25%. For loci with heterozygosities of 20%, only 4% of the parents would segregate for both. In cases where flanking polymorphic loci are necessary for confident scoring of a QTL, the frequency of parents with the three loci heterozygous will be 12 and 0.8%, respectively. The problem of inadequate marker polymorphisms can be mitigated by use of a dense map; however, the QTL itself must nonetheless be polymorphic for the markers to be useful.

Second, depending on the effects of other genes, the expression of QTL alleles may differ greatly. Tanksley and Hewitt (1988) reported that QTL for tomato fruit characters differed

among the genetic backgrounds into which genes were introgressed, and showed unfavorable pleiotropic effects. For trees, it is easy to imagine many such effects. As a simple illustration, QTL for shoot phenology may have large effects on diameter growth in a genotype with a late onset of reproduction but small effects in a genotype that reproduces early and heavily, i.e., in a genotype in which most of the advantage of the extended season of growth would be shunted into reproductive parts. Thus, particularly in breeding programs that utilize germ plasm from a wide variety of sources, the physiological and therefore the genic nature of superior yield characteristics may vary widely. While different loci and different kinds of gene action can be used in multiple breeding populations, the use of QTL in each population would require developing independent QTL–marker associations. Alternatively, if QTL are found for only some of the potentially useful loci, but are intensively selected in single-population breeding, then the genetic variance at other loci will be diminished because of reduced effective population size.

Third, phenotypic selection during the first few generations of breeding is likely to alter the frequencies of alleles for major QTL. For example, selection for more permissive growth, such as by extension of the growing season, may be a major source of genetic variance for yield during the initial round(s) of selection. Such QTL, however, may not be important past the first generation(s), both because of their decreased polymorphism in breeding populations, and because of an increasing probability of encountering antagonistic pleiotropy as the trait is moved further from the mean (for example, as a result of damage from frost and drought associated with extending the growing season). Thus, not only might the importance of QTL effects change over time, they might also change in direction.

Interspecific hybridization will greatly facilitate identification of QTL, but MAS will still encounter substantial problems

Interspecific hybridization will generate great D, providing much stronger associations between QTL and markers than within species. Moreover, by combining species that differ substantially in a number of traits, it potentiates the segregation of great amounts of phenotypic variance. As with most of the agronomic QTL studies conducted to date (reviewed above), these factors should make it possible to detect major QTL in F_2 and backcross progenies rather easily.

MAS in hybrid populations, however, will be limited by four factors:

(1) Meiosis in hybrids and derived generations is rarely normal and is expected be more abnormal in the more highly differentiated species combinations. Such abnormalities have been best studied in *Drosophila*, where they have been shown to include reduced recombination, distorted segregation, incomplete disjunction, production of chromosome aberrations, and enhanced transposon activity (Thompson and Woodruff 1978). High levels of segregation distortion may preclude statistical testing of linkages (Bailey 1961). Although these problems do not to date appear to have precluded map construction in hybrids, they will at least cause estimation of marker and QTL maps to be imprecise and, for some chromosome sections, perhaps impossible.

(2) Nonadditive genetic interactions among QTL alleles, particularly for yield and fitness-related traits, are likely to be substantially greater in hybrid than in intraspecific progenies. Within species, alleles have been selected to function within a common developmental program; such coevolved alleles have been referred to as co-adapted gene complexes. The breakup of such epistatic gene complexes has been invoked to explain the falloff in performance of *Drosophila* hybrids in the F_2 generation (Falconer 1981). In poplar hybrids, although morphological characteristics in the F_1 typically show additive inheritance for morphological traits, for yield, F_2 progeny apparently show a similar breakdown to that observed in *Drosophila*. Backcross progeny, however, that maintain a full set of alleles from one parent do not (R. Stettler, personal communication, 1991). In addition to occasional hybrid vigor, phenotypic characteristics of hybrids commonly transgress those of the parental species in unpredictable ways; for example, despite the generally dominant nature of resistance to biotrophic pathogens within species, the loblolly × slash pine (*Pinus elliottii* Engelm.) hybrid has less resistance to fusiform rust (*Cronartium fusiforme* Hedgc. & Hunt ex Cumm.) than either of the parental species (Kinloch and Stonecypher 1969). Evidence for nonadditive gene effects also comes from anecdotal guidelines for hybrid breeding; exceptional hybrid individuals are usually sought and, where possible, clonally propagated to maintain their unique genotypes (Zobel and Talbert 1984).

(3) Interspecific hybrid performance therefore appears to be significantly dependent on allelic interactions. In contrast, most studies of genetic variance within species, at least for conifers, suggest that additive genetic variance is predominant (e.g., Carson 1986). Phenotypic effects of QTL alleles in hybrids will thus be more likely to differ according to genetic background and change as genetic composition undergoes substantial alterations during early generations following initial hybridization. This change will tend to make QTL effects more unstable than those within species, with obvious complications for MAS. Note, however, that this conclusion is based largely on indirect biological evidence, rather than on experiments intended to estimate nonadditive variances in hybrid populations and compare them to intraspecific estimates. Contrasting the nature of gene action in conspecific versus hybrid cellular environments would be a valuable contribution from QTL studies of trait architecture.

(4) Unlike hybridization in maize and other inbred crop or wild plant species, interspecific hybridization of forest trees involves the mating of highly heterozygous individuals. This means that polymorphism will exist at both marker loci and QTL. Despite the generation of much D by hybridization, marker–QTL associations produced by hybridization will be diluted by the lack of D within species. Thus, as is the case within species, different alleles may be associated with QTL in different crosses. This may not be a large problem if sets of marker alleles are fixed in different species; however, at least for allozymes, apparently homologous alleles frequently appear to have remained polymorphic in closely related tree species (Wheeler *et al.* 1983; Manos and Fairbrothers 1987; Millar *et al.* 1988; Rajora 1990).

When might MAS be powerful enough to justify reductions of genetic base and major changes in tree breeding?

We foresee three circumstances in which adoption of MAS might make a major contribution in conifer-type breeding programs. The first is in cases where a severe threat to forest health exists and the need to speed genetic adaptation is urgent. Such urgency may dictate that all available means are utilized almost without regard to cost. Examples include stresses imposed by airborne pollutants and rapid global environmental changes. In these cases, making and using QTL maps in a large number of families and environments would be conceivable.

Second, where extremely high value families are known and limited numbers of clones or genotypes are to be identified for extensive use, mapping and MAS may be justified even if they will produce only a modest increment in value. Examples would be where they are used to identify elite embryogenic lines for massive propagation from within an elite family or where elite clones are to be identified for insertion of new genes and eventual wide deployment. Clonal propagation may not, however, be a necessity. Large gains for additive variance could be made if single genotypes were selected with MAS from within several elite families and control-pollinated with one another, or placed individually in seed orchards with MAS-selected representatives of other families (to avoid inbreeding). The advantage here would be that the same progeny trees used to map QTL could be grafted into seed orchards, whereas clonal propagation usually requires that new genotypes be selected from juvenile materials (usually seedlings for rooted cutting or organogenic propagation, and immature or ungerminated embryos for embryogenic propagation).

Third, in some cases conducting MAS within mapped families may add significantly to gain under phenotypic selection among and within families. Since relatively few families could likely be analyzed, such an approach could proceed only where maintenance of high levels of genetic diversity in production forests is not a necessity, e.g., on some industrial rather than public lands. To compare these alternatives we examined how different values of trait heritabilities and fractions of additive variance explained by markers affect relative efficiency.

We modified eq. 4 of Lande and Thompson (1990) to obtain an expression for efficiency (E). The expression is based on an optimal selection index that includes information from both marker loci and phenotypic traits. It assumes stability of marker–QTL associations and a two-stage selection process where families are first selected on the basis of their mean phenotypes and then individuals within families are selected on the basis of either phenotypic information in combination with marker information (MAS) or phenotypic information alone:

$$[1]\quad E = \frac{h_f + \left(\frac{i_{wf}}{i_f}\right)\sqrt{p + \frac{(1-p)^2\, h_{wf}^2}{1 - (h_{wf}^2\, p)}}}{h_f + \left(\frac{i_{wf}}{i_f}\right) h_{wf}}$$

where h_f is the square root of among-family heritability, h_{wf} is the square root of within-family heritability, i_{wf} is the selection intensity within families, i_f is the selection intensity among families, and p is the fraction of additive genetic variance explained by markers. When only within-family gain is to be considered, the appropriate ratio for relative efficiency is the same as that shown in eq. 1, except that the terms for the square root of among-family heritability and among-family selection intensities are deleted.

When only within-family selection is practiced and selection intensities are equal for both MAS and phenotypic selection, MAS will bolster phenotypic selection by more than 50% only when within-family heritabilities are low ($h_{wf}^2 \leq 0.30$) and markers explain the majority of the additive variance ($p \geq 0.7$) (Fig. 1). With highly heritable traits ($h_{wf}^2 \geq 0.4$), MAS can bolster efficiency only modestly even when markers explain a large majority of the additive variance.

When phenotypic selection among families is also practiced and selection intensities among and within families are in both cases equal, the relative contribution of MAS is decreased. Under a variety of among- and within-family heritabilities, MAS will bolster phenotypic selection by more than 50% only when both within- and among-family heritabilities are very low and markers explain at least 50% of the additive variance (Fig. 2).

If selection intensity within families were up to twofold higher than that among families (but still equal for phenotypic and marker-based selection within families), the contribution of MAS would be increased, but to a substantial degree only for traits of very low heritibility. With such traits (e.g., $h_f^2 = 0.5$ and $h_{wf}^2 = 0.1$) (Fig. 3), a selection intensity within families that is twofold greater than that among families results in MAS enhancing efficiency more than 50% when markers explain at least half of the additive variance. With a highly heritable trait ($h_f^2 = 0.9$, $h_{wf}^2 = 0.5$), doubling of selection intensity has little effect on efficiency.

In practice, if MAS were to be carried out, we might expect the application of large selection intensities within families. This is because the fixed expense of mapping individual families and starting a genotyping program (laboratory setup, DNA extractions, probe or primer bank development, etc.) will be far greater than the variable costs of adding additional trees. Moreover, for difficult to assay traits, selection intensities within families might actually be higher for markers, particularly PCR-based markers, than for phenotypic selection. Although we did not estimate efficiencies for this scenario, this would also be likely to significantly elevate the efficiency of MAS only with weakly inherited traits and where most of the additive variance is accounted for by markers. Nonetheless, problems related to sample size might limit the success of this option.

Increasing the selection intensity substantially is likely to require that very large numbers of progeny be examined and few retained for breeding. For example, for common selection intensities such as 1.4 (20% of individuals retained), a doubling of selection intensity would require that about 0.6% of the progeny be retained, or 167 progeny would need to be genotyped for each one that is selected (Falconer 1981). Multiple-trait selection would exacerbate this problem. Imposing high selection intensities on several uncorrelated traits would require that many hundreds of individuals be examined for each individual to be selected. While this is not beyond the means of PCR-based molecular markers, it nonetheless implies a very substantial additional effort, especially because it will likely have to be applied to several families. It also would result in a drastic reduction of genetic diversity, which may be unacceptable in many programs.

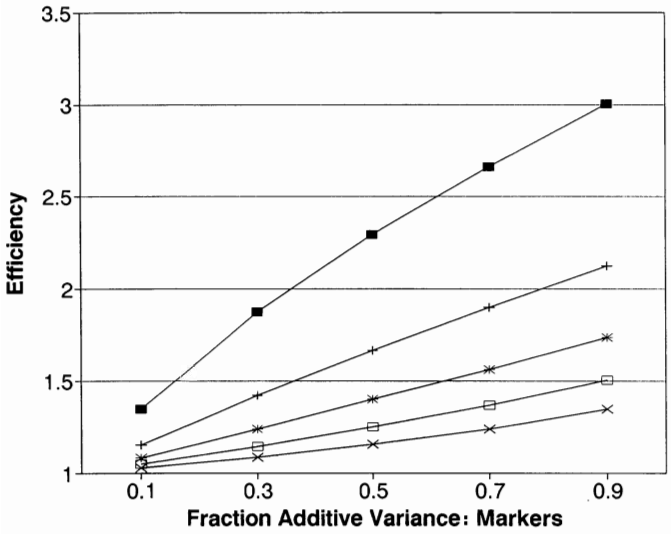

FIG. 1. Efficiency of combined MAS and phenotypic selection within full-sib families versus phenotypic selection within full-sib families alone, where selection intensities are assumed to be equal. Efficiency values are shown for a range of proportions of additive variance in phenotypes explained by markers and within-family heritabilities (h^2_{wf}) ($h^2_{wf} = 0.1$ (■), 0.2 (+), 0.3 (∗), 0.4 (□), and 0.5 (×)).

Regardless of selection intensity, MAS can be worthwhile only if markers explain much of the additive variance within families, and it must be applied to weakly heritable traits. Explaining much of the additive variance requires that sample sizes be large to enable detection of all important QTL. Weakly heritable traits, and those controlled by large effective numbers of QTL, require sample sizes on the order of a few hundred to a few thousand individuals so that most of the additive variance will be detected (Lande and Thompson 1990). For example, detecting half of the additive variance explained by markers at the 5% confidence level would require approximately 98 individuals when the trait heritability is 0.5 and 5 effective QTL control the trait. Detecting half of the additive variance at the 1% confidence level would require approximately 2300 individuals when the trait heritability is 0.1 and 11 effective QTL control the trait (Table 1). Thus, in addition to requirements for very large samples during within-family selection, very large samples also will be necessary for initial QTL identification.

In maize, QTL have successfully accounted for a great deal of phenotypic variance within families. With 17–20 allozyme markers and sample sizes of 1800–1900 plants, Edwards *et al.* (1987) were able to explain 8–40% of the phenotypic variance within F_2 full-sib families. Although crossing of heterozygous parents in forest trees will limit efficiency compared with that with the wide crossing of inbred lines of maize studied here, substantial D will exist within full-sib families, aiding QTL identification. With full genome coverage by markers and large samples, QTL should be identified that account for a large fraction of the additive variance within families.

The requirement for very large samples to detect QTL, and to impose high selection intensities within families, is likely to limit operational adoption of MAS in forest trees. As discussed above, low-heritability traits (*i*) are of most importance in tree breeding, (*ii*) are likely to be controlled by more effective QTL than high-heritability traits, and (*iii*) from the viewpoint of efficiency, stand to gain the most from MAS. The several hundred to several thousand progeny samples required for efficient detection and use of MAS on such traits are likely to present a substantial obstacle to practical implementation. For highly heritable traits controlled by few effective QTL, the efficiency of MAS is low unless nearly all of the additive variance is accounted for; such traits will therefore also require very large samples for QTL detection and use.

TABLE 1. Sample sizes needed to detect additive genetic variance

		n_E				
h^2_{wf}	α	1	2	5	11	23
0.5	0.05	22	33	98	230	480
	0.01	43	65	200	450	960
0.1	0.05	110	160	490	1100	2400
	0.01	220	330	1000	2300	4800

NOTE: Data represent the approximate sample sizes (number of individuals) needed to explain 50% of the additive genetic variance in a quantitative trait within a full-sib family as a function of the within-family heritability (h^2_{wf}), the effective number of QTL (n_E), and the confidence level (α) for detection of a QTL from a very large number of molecular marker loci. (Adapted from Fig. 3 of Lande and Thompson (1990)).

This unfavorable outlook for MAS, however, might be brightened if there is substantial nonadditive variance and if the necessary mating or clonal propagation system is available to capitalize on it. A great deal of apparent dominant and overdominant gene action has been associated with markers in maize (Edwards *et al.* 1987; Lee *et al.* 1989; Smith *et al.* 1990). In the most striking case to date, Smith *et al.* (1990) reported a very strong correlation ($r^2 = 0.87$) between RFLP-derived Nei's genetic distances among inbred parents and grain yield when using 257 restriction enzyme–probe combinations. Although these results are interesting, a number of factors must temper the inferences that can be drawn from them. First, the correlation was magnified by the inclusion of a number of closely related pairs of parents that gave rise to hybrids with little potential as commercially elite lines. Second, as discussed earlier, it is unclear whether results comparable to those found with crossing of inbred lines could be obtained within populations or families of outbreeding trees. Third, it remains to be established whether use of nonadditive trait – marker associations would prove to substantially enhance efficiency compared with that with phenotypic selections of full-sib, hybrid, or clonal materials.

It should be noted that we have ignored both economics and potential time savings due to MAS in our calculations. Were we to factor in the cost of MAS, it is clear that the efficiency of MAS relative to that of pure phenotypic selection would be substantially lower. For example, were the funds spent on mapping markers and QTL, and on genotyping numerous progeny for within-family selection, instead used to increase sample size (and thus selection intensity) under pure phenotypic selection, many of the potential benefits of MAS might well be negated. On the other hand, if MAS is able to shorten the testing cycle substantially below that already possible with short-term phenotypic testing, or to reduce the expenditures necessary for conventional phenotypic selection and testing, our efficiency calculations would

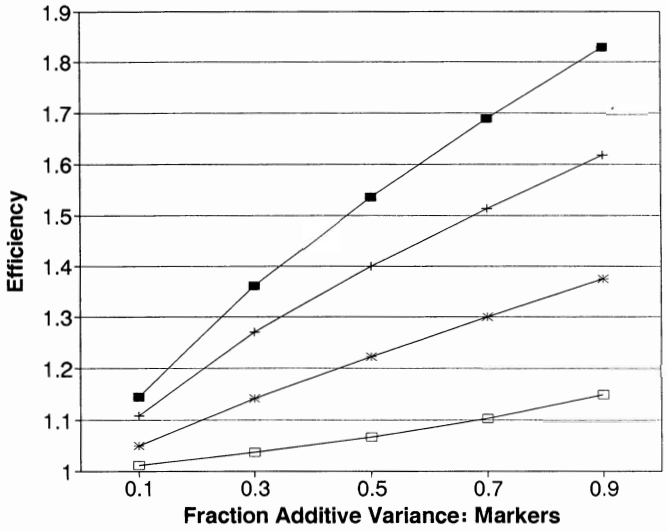

FIG. 2. Relative efficiency of phenotypic selection versus MAS among and within full-sib families. Phenotypic selection among families is followed by either combined MAS and phenotypic selection within full-sib families or pure phenotypic selection within full-sib families. Within- and among-family selection intensities are assumed to be equal in both cases. Efficiency values are shown for a range of proportions of additive variance in phenotypes explained by markers and among-family (h_f^2) and within-family (h_{wf}^2) heritabilities ($h_f^2 = 0.2$, $h_{wf}^2 = 0.1$ (■); $h_f^2 = 0.5$, $h_{wf}^2 = 0.1$ (+); $h_f^2 = 0.8$, $h_{wf}^2 = 0.2$ (*); $h_f^2 = 0.9$, $h_{wf}^2 = 0.5$ (□)).

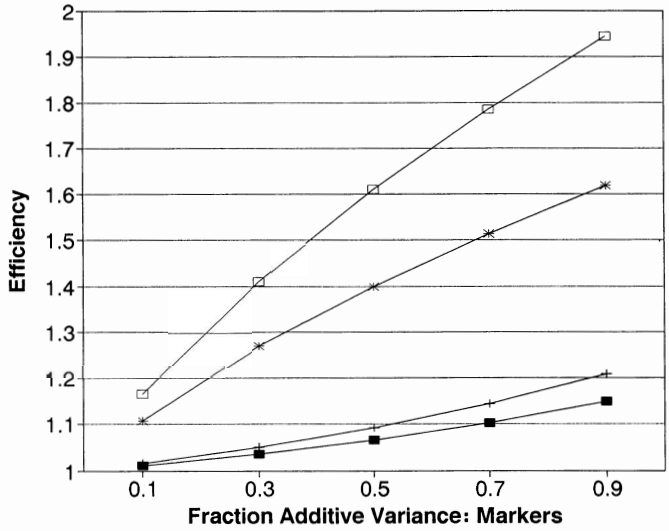

FIG. 3. Efficiency of MAS when selection intensity within families varies independently of that among families. Conditions were identical with those in Fig. 2 except that the ratio of within-family (i_{wf}) to among-family (i_f) selection intensities varies between 1.0 and 2.0 (top pair of curves: $h_f^2 = 0.5$, $h_{wf}^2 = 0.1$ (□ and *, respectively); bottom pair of curves: $h_f^2 = 0.9$, $h_{wf}^2 = 0.5$ (+ and ■, respectively)). The ratio of selection intensities is 2.0 for the top curve of each pair and 1.0 for the bottom curve of each pair.

underestimate its benefits. As discussed above, however, at least for low-heritability traits, such benefits can only accrue after establishing QTL from fairly long term tests, and then only after showing that they are useful in other generations and environments.

QTL mapping as a research tool, and in comparison to other tree improvement approaches in biotechnology and tree breeding

As a research tool for dissection of quantitative trait architecture, QTL analysis should, we believe, be given very high priority. It will allow many hypotheses that have been the subject of theory and speculation for years to be examined. Examples include the nature of gene action, the number of genes controlling quantitative traits, the significance of epistasis in hybrid breakdown, and the evolution of adaptive and morphological trait complexes. The recent work by Doebley *et al.* (1990) on the evolution of maize reproductive morphology provides a nice example of some of the evolutionary insights possible via quantitative trait dissection. Included also will be testing the very concept of QTL themselves; can quantitative traits routinely be reduced to a discrete number of unitary effects, or are interactions among QTL, genetic backgrounds, and environments so complex and variable that current models, which treat QTL main effects almost exclusively, prove to generate reifications?

As a vehicle for gene cloning in trees, however, particularly conifers, its power is seriously limited. For conifers, these limitations stem from the large, repetitive genomes that make gene cloning and chromosome walking very difficult (Neale and Williams 1991), and from the large physical distances between genetically mapped loci, probably exceeding 4000 kilobases per centimorgan (cf. Meagher *et al.* 1988). For conifers and other tree species, including those with relatively small genomes, it suffers from the lack of transposon tagging systems; from inefficient or unproven transformation techniques; from lack of inbred, mutant, and isogenic lines; and from long generation times, all of which singly and interactively impede classical genetic strategies for fine mapping of gene locations. Until major new advances are made in these technologies for trees, it will be difficult to justify genome mapping and QTL analyses on the basis of gene isolation. Moreover, for conserved genes that have homologues or functional analogues in easily manipulated herbaceous species (e.g., *Arabidopsis*), it may be more effective to clone genes there, and then use them to identify the relevant genes in trees. As the number of genes cloned from model species increases and understanding of gene function expands, such opportunities will greatly increase. Note that even for rapidly evolving genes, such as those related to morphologically diverse structures such as reproductive organs, conserved domains can often be located and used to identify homologues in distant species. This has recently been accomplished for floral homeotic genes and other transcription factor encoding genes, where conserved domains have been identified within or outside of the plant kingdom and used to isolate similar genes from plants or from heterologous plant families (Jackson *et al.* 1991; Ma *et al.* 1991; Pnueli *et al.* 1991; Ruberti *et al.* 1991; Takasuji *et al.* 1991; Vollbrecht *et al.* 1991). Such approaches may also be possible for traits commonly thought to be unique to woody perennials (such as secondary xylem growth, seasonal dormancy, and delayed reproduction) once the genic basis for control of related structural and developmental traits are elucidated in model species. In fact, when identified in trees, such genes would be ideal candidates for QTL studies that attempt to go beyond linkage to random markers, and may actually begin to identify QTL themselves. Should this

be accomplished for a number of the major genes underlying quantitative traits, the problems of linkage equilibrium discussed above would be reduced greatly (to that within, rather than between, genetic loci).

Because of the many concerns about application of MAS to trees, we feel that for the near to middle term, MAS should augment, but not displace, operationally oriented research in conventional tree breeding and biotechnology. Alternative methods for speeding selection and breeding, such as by early phenotypic selection and flower stimulation, have already proven to be useful. In our opinion, biotechnological goals such as improved clonal propagation via somatic embryogenesis and the insertion of foreign genes with clear economic value, such as for insect and herbicide resistance, have likelihoods of medium-term payoffs that are equivalent to or higher than MAS. Advancing clonal propagation and transformation therefore appear to be more defensible as applied research goals than MAS, at least until information on quantitative trait architecture in trees is available.

Conclusions

The main conclusions we draw are:

(1) Experience with QTL identification in agronomic crops is of little value for helping to predict the usefulness of MAS in forestry.

(2) For conifer-type breeding programs, QTL–marker associations will be limited to specific genetic backgrounds and environments as a result of linkage equilibrium, interactions of QTL effects with genetic backgrounds, G × E interaction, and changes of QTL allele frequencies and environments among generations.

(3) Some exceptions where MAS may find use include interspecific hybrid programs; study of highly heritable, difficult to assay traits; and within-family selection of weakly heritable traits by using very large sample sizes and high selection intensities.

(4) Quantitative trait dissection should be given high priority in basic research where it may reveal unsuspected aspects of quantitative trait architecture. It should not, however, be justified on the basis of gene isolation from trees unless major technical hurdles are solved.

(5) Until the strength and consistency of QTL–marker effects in trees are known, MAS should augment, but not supplant, operationally oriented research programs in conventional breeding, early selection and precocious flowering techniques, tissue culture propagation, and genetic engineering.

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