Financial feasibility of marker-aided selection in Douglas-fir

G.R. Johnson, N.C. Wheeler, and S.H. Strauss

Abstract: The land area required for a marker-aided selection (MAS) program to break-even (i.e., have equal costs and benefits) was estimated using computer simulation for coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the Pacific Northwestern United States. We compared the selection efficiency obtained when using an index that included the phenotype and marker score with that obtained using only the phenotype. It was assumed that MAS was restricted to within-family selection, that the rotation age was 50 years, and that growth rate (h^2 = 0.25), tree form (h^2 = 0.25), and (or) wood density (h^2 = 0.45) were the objects of improvement. Several population quantitative trait loci (QTL) models, selection population sizes, and interest rates were considered. When large selection population sizes were employed (500 trees per family) MAS gave considerable increases in efficiency of within-family selection; however, results showed that the combination of small selection population sizes (100 trees per family) and many QTL of moderate effect could lead to losses in gain from MAS compared with phenotypic selection. For many reasonable selection scenarios and the simplified assumptions in our model, the land base required for breeding programs to break-even is smaller or near to the limit of those in place under operational breeding programs in the region. Considerably more research is needed to reasonably predict whether MAS would be cost-effective in practice. However, before some of the basic research needed to implement MAS can be done, organizations need to establish large blocks of full-sib families to allow for QTL identification.

Résumé : Les auteurs ont estimé la superficie en plantations nécessaire pour équilibrer les coûts et les bénéfices associés à un programme de sélection assistée par marqueurs (SAM). L'étude a été réalisée à l'aide de simulations informatiques pour le Douglas vert de la côte (*Pseudotsuga menziesii* (Mirb.) Franco), dans la région du pacifique nord-ouest, aux États-Unis. Deux méthodes de sélection ont été comparées, l'une basée sur le phénotype et la seconde basée simultanément sur le phénotype et la valeur du marqueur. Les effets de la SAM ont été évalués dans un cadre de sélection intra-familiale, en assumant un âge de révolution de 50 ans. Les caractères d'amélioration suivants ont été étudiés : le rythme de croissance (h^2 = 0,25), la forme de l'arbre (h^2 = 0,25), et/ou la densité du bois (h^2 = 0,45). Les auteurs ont considéré plusieurs modèles de population au niveau des loci contrôlant les caractères quantitatifs (QTL), ainsi que plusieurs tailles de population de sélection et plusieurs taux d'intérêt. Lorsque de grandes populations de sélection étaient considérées (500 arbres par famille), la SAM entraînait une sélection intra-familiale considérablement plus efficace. Cependant, les résultats ont démontré que la SAM pouvait entraîner une diminution du gain comparativement à la sélection phénotypique, lorsqu'on était en présence de petites populations de sélection (100 arbres par famille) et de plusieurs QTL à effet modéré. En fonction de plusieurs scénarios de sélection réalistes et selon les hypothèses simplifiées du modèle, la superficie en plantations requise pour équilibrer les coûts et les bénéfices de tels programmes d'amélioration est plus petite ou près de la limite de la superficie qu'occupent les programmes opérationnels d'amélioration dans la région. Beaucoup plus d'efforts de recherche sont nécessaires afin de prédire avec un bon degré de certitude si la SAM serait rentable dans la pratique. Cependant, avant que la recherche de base nécessaire à la mise en œuvre de la SAM puisse être réalisée, les organismes doivent établir des dispositifs formés de grandes parcelles de descendances biparentales afin de faciliter l'identification des QTL.

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Introduction

The use of molecular markers in breeding research is commonplace and their use in applied breeding programs is being investigated in numerous agronomic crops (reviewed in Mohan et al. 1997). In forest tree species, molecular markers are also being pursued, and there have been a number of reports of statistical associations with quantitative traits such as growth rate, wood quality, and shoot phenology (e.g., Groover et al. 1994; Bradshaw and Stettler 1995; Devey et al. 1995; Grattapaglia et al. 1996*a*, 1996*b*; Byrne et al. 1997*a*, 1997*b*; Wu 1998). This gives forest tree breeders hope that marker aided selection (MAS) can be used to increase selection efficiency.

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Use of MAS in applied programs has thus far given mixed results. MAS is widely accepted to be a powerful and economically profitable means for introgression of major genes into selected populations. For example, Toojinda et al. (1998) successfully used MAS to introgress rust resistance quantitative trait loci (QTL) in barley and Bernacchi et al. (1998) used MAS to introgress wild alleles in tomato. However, neither Stromberg et al. (1994) nor Openshaw and Frascaroli (1997) found that MAS increased gain over phenotypic selection using markers for yield in maize (*Zea mays* L.). Young (1999) found very few examples in the literature of released germplasm that was the result of MAS and suggested that more information on genomics is needed before MAS will become commonplace in applied breeding. In this paper, we focus exclusively on MAS as a tool to supplement phenotypic selection during population improvement of quantitative traits, which is the predominant focus for breeding of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and most other forest tree species.

There is no reason to believe that gains cannot be increased in Douglas-fir using MAS if sufficient resources are available. Increases in the efficiency of breeding programs through the use of QTL can come from two sources: more accurate assessment of the genotype through the use of QTL and time savings achieved through early selection on seedling genotypes for traits expressed only in mature trees. However, because of the costs of genotyping large numbers of molecular markers on large numbers of progeny and the long time between selection and harvest, it is unclear whether MAS could be profitable. In addition, there are limits to increasing gains over traditional tree breeding activities. For example, in one breeding simulation study (Johnson 1998*a*), the correlations between predicted breeding values using phenotypic information (family and individual data) and simulated genotypes for traits with a heritability of 0.20 were estimated to be 0.56 and 0.66 for half-sib and full-sib breeding programs, respectively. It would therefore be impossible to double the efficiency of phenotypic selection, as the maximum correlation possible is 1.0.

It is anticipated that the increase in selection efficiency from MAS will come from the increased precision of within-family selection. Given the large number of progeny tested for families in most Douglas-fir breeding programs, family mean heritabilities for most traits tend to be 0.8–0.9. Thus, the correlation of family means and actual genotypic values (the square root of heritability) should be about 0.89–0.95. These values provide limited opportunity for increased among-family selection efficiency.

Linkage equilibrium, the independence of QTL and marker alleles at the population level, further complicates MAS for family selection in forest trees (reviewed in Strauss et al. 1992; Williams 1997) and requires that MAS for within-family selection be customized for each family. Markers that are associated with a positive QTL allele in one family may be unassociated with the QTL or associated with a negative QTL allele in others. Once QTL–marker associations for a family are known, they can be used in subsequent generations; however, recombination will reduce selection efficiency (e.g., Edwards and Page 1994; Kerr et al. 1996).

The effect of a QTL allele can also vary in differing genetic and environmental backgrounds. If dominance is associated with a locus, then the average effect of a QTL allele will vary with gene frequency at the family level (see Falconer and Mackay 1996, p. 114). Epistasis among loci can also modify the effect of a QTL allele. Numerous studies have also shown that QTL effects vary depending on environment (e.g., Tinker et al. 1996; Lu et al. 1997; Sari-Gorla et al. 1997).

Linkage equilibrium, QTL × genetic background interactions, and QTL × environment interactions, collectively, also make it difficult to use MAS for early selection, one of its most heralded benefits (e.g., Tauer et al. 1992; Williams and Neale 1992). Because of recombination and variation in QTL effects with differing genetic backgrounds, it is necessary to screen for QTL effects in each new full-sib family; thus it requires several years to allow expression of economic traits (e.g., tree volume).

Because of the problems associated with early selection and among-family selection that are noted above, we have confined our analysis to studying how MAS can increase the efficiency of within-family selection that is practiced simultaneously with standard phenotypic selection (cf. O'Malley and McKeand 1994).

Douglas-fir has a number of characteristics that make the economic use of MAS especially challenging:

(1) Because its native montane habitat is environmentally diverse, breeding zones tend to be small compared to other conifer breeding programs (e.g., in the southeastern U.S.A.), thus reducing the area over which any increased gains can be spread.
(2) Rotation ages of 40–60 years are common, forcing financial gains from MAS to be discounted for long periods. Selection costs, however, occur early and are, by comparison, discounted little.
(3) The very high level of genetic diversity found in Douglas-fir at the phenotypic and molecular level (e.g., Campbell 1979; Adams et al. 1998) suggests that valuable QTL alleles may differ substantially among families.
(4) There is no evidence from breeding programs that traits of broad economic importance are controlled by QTL of major effect; large detection–selection populations and many markers are therefore likely to be required for QTL detection and MAS.
(5) Clonal propagation, while technically feasible via both embryogenesis and rooted cuttings, is rarely utilized in commercial programs (Talbert et al. 1993), limiting the amount of nonadditive genetic variance that can be captured by MAS. Current use of full-sib families can capture one-quarter of the dominance variance. However, dominance variation can only contribute to gain in the current generation, since it is only the additive gain that is carried from generation to generation. The majority of gain in Douglas-fir growth is expected to come from the additive genetic variation since it is approximately threefold greater than dominance variation (Yanchuk 1996).

Computer simulation studies have been used to estimate the efficiency of MAS in agronomic crops, however, under breeding scenarios that are very different from those that are relevant to forest trees (e.g., F_2 and backcrosses: Zhang and Smith 1992; Edwards and Page 1994; Gimelfarb and Lande

1994; Hospital et al. 1997; Moreau et al. 1998; van Berloo and Stam 1998; Xie and Xu 1998). Kerr et al. (1996) used computer simulation to estimate gains and the economic returns from MAS in a forest tree breeding program. However, they assumed that QTL information could be applied at the population level for family and within-family selection, and their rotation lengths were much shorter than those for Douglas-fir.

Before industry is likely to create significant MAS programs, there must be a reasonable chance that MAS will be profitable. The objective of this paper is to explore its profitability using Monte-Carlo simulation and available marker technologies. Our approach follows that of Lande and Thompson (1990), who developed a methodology to evaluate the efficiency of using molecular markers and phenotypes in a selection index compared with phenotypic selection alone. Given conditions appropriate for Douglas-fir and many other species of forest trees, most notably linkage equilibrium, long rotations, and restriction of MAS to within-family selection, we examine the size of the land base required under different combinations of QTL structure and genotyping costs to capture sufficient financial return to justify MAS.

Methods

The breeding program we chose to model is based on a complementary mating design with three different tests. As in most complementary designs, a general combining ability (GCA) test is used to identify superior parents using either a polycross or female tester. At the same time that the GCA tests are being established, full-sib family blocks in which selections will be made for the following generation are also established. Full-sib families (blocks) are chosen on the basis of mid-parent values calculated from the GCA tests. The third test is a QTL detection study in which five full-sib families are constructed by single-pair mating 10 unrelated parents in our breeding program. Five hundred progeny would be tested for each family. Each tree would be cloned and six ramets tested in each of two environments to increase heritabilities, so that QTL loci with small effect could be detected. A framework map of 120 evenly spaced loci (30 cM apart) would be used to map the traits.

We assume in these simulations that a seed orchard will be established with selections from the top 30 full-sib families, as determined by the GCA tests. Gain from the within-family selections from these 30 families is what will be examined in the simulations. The first option was to choose the best two individuals per family using only the available phenotypic data. The MAS option was to genotype these 30 families with molecular markers and use both the marker information and the phenotypic information to make within-family selections. By only genotyping the best 30 families the cost of genotyping is reduced because only a portion of the total breeding population is analyzed. These seed orchard selections will comprise half of the breeding population in the following generation; the remaining selections will come from additional families not represented in the orchard. Thus MAS will contribute fully to the seed orchard and only partially to the subsequent breeding populations. In this breeding program, the marginal costs and gains that are associated with MAS are tied to the top 30 families from which the seed orchard candidates will be drawn and to the costs of developing markers and finding QTL. All other aspects of the program are the same whether or not one chooses to use MAS.

Because of our assumption of linkage equilibrium and varying QTL effects, each family must be examined separately to determine whether the QTL and marker alleles are segregating, the linkage phase of the marker alleles with respect to the QTL alleles, and to verify that any measurable QTL effect exists in the particular genetic background. For simplicity in modeling selection efficiency, we also assume that

(1) QTL epistasis is insignificant.
(2) Regression is adequate for determining the presence and effect of QTL within full-sib families (Haley and Knott 1992; Martínez and Curnow 1992; Haley et al. 1994; Knott et al. 1997).
(3) QTL × environment interaction is negligible within breeding programs for the QTL used for MAS.
(4) Highly polymorphic SSRs (simple-sequence-repeat, or microsatellite, markers) will be developed and used to construct the framework maps.
(5) We will find segregating flanking markers for each QTL on either side of the QTL. These markers are, on average, 15 cM from the QTL. The marker alleles differ for each parent, such that four different marker alleles are available in a family for each marker locus.
(6) Each QTL has two alleles that affect the trait value positively or negatively.

QTL models and heritabilities

The simulation scenarios used six QTL models (Table 1) and two narrow-sense heritabilities. Heritabilities represented those that are commonly found for individual tree volume growth and bole form ($h^2 = 0.25$), and wood specific gravity ($h^2 = 0.45$). Locating and quantifying QTL with modest population sizes and low heritability is difficult (Beavis 1998; Beavis 1994); therefore, instead of trying to model low heritability QTL for all the QTL models, we arbitrarily set 20% of the additive variance to be a function of low heritability and low magnitude QTL, and did not assign this variation to any modeled QTL linkage groups. This level was increased to 40% for our "near-polygenic" model. This also reflects the expected reality that there will be inadequately sized breeding trials for the foreseeable future to enable QTL of very small effect to be recognized.

The QTL models were (see Table 1 for details) (*i*) 2, two major QTL, each contributing 40% of the additive genetic variation; (*ii*) 4, four major QTL, each contributing 20% of the additive genetic variation; (*iii*) 4D, four major QTL with dominance variation; *(iv)* Geo, a geometric progression where there are *n* QTL having an effect of approximately r^n (r set to 0.72, for this six QTL model); (*v*) 2+5, a model that simulates seven moderate QTL that each control 10–15% of the additive variance; and (*vi*) 1+10, a model with 11 QTL, all but one of which contribute 5% of the additive variance (and where 40% of the additive variance is unaccounted for by known QTL).

Simulation

Parental allele frequencies and haplotypes were based on population frequencies of 0.5 for the two QTL alleles. This gene frequency generates the maximum number of segregating parents. Therefore, QTL locus did not always segregate in each family. In this example, the QTL will only be segregating in 75% of the families because 25% of the time both parents will be homozygous at the QTL locus. Each parent had two different alleles at each marker locus, thus the marker loci were always segregating. Marker–QTL haplotypes for the full-sib progeny were established based on the estimated frequency of crossovers.

After establishing marker and QTL genotypes for the progeny, genotypic values for the trees were determined by summing the QTL effects and adding the residual additive genetic variation by sampling from the appropriate normal distribution which represented either 20% (40% for QTL model 1+10) of the within-family additive genetic variation $N(0, 0.1\sigma_a^2)$ Phenotypes were assigned by adding the residual environmental component that came from sam-

Table 1. Six gene action models simulated.

QTL number	Model 2		Model 4		Model 4D		Model Geo		Model 2+5		Model 1+10	
	Additive variance (%)	Dominance variance ratio	Additive variance (%)	Dominance variance ratio	Additive variance (%)	Dominance variance ratio	Additive variance (%)	Dominance variance ratio	Additive variance (%)	Dominance variance ratio	Additive variance (%)	Dominance variance ratio
1	40	0	20	0	20	0	29	0	15	0	10	0
2	40	0	20	0	20	25	15	0	15	0	5	0
3			20	0	20	50	15	0	10	0	5	0
4			20	0	20	75	7	0	10	0	5	0
5							7	0	10	0	5	0
6							7	0	10	0	5	0
7									10	0	5	0
8											5	0
9											5	0
10											5	0
11											5	0

Note: All models except 1+10 assume 20% of the additive variance is unaccounted for by QTL; model 1+10 assumes 40% is unaccounted for by known QTL. Dominance variance ratio = genetic variance/additive genetic variance.

pling the appropriate normal distribution $N(0,\sigma_e^2)$ to obtain the desired heritabilities.

Regression was then used to develop a marker score using simultaneous flanking markers (Edwards and Page 1994). With this method, flanker scores were first calculated separately for each marker QTL haplotype. For a single QTL we would have had the following linkage groups:

Male Parent	Female Parent
A1------QTL------B1	A3------QTL------B3
A2------QTL------B2	A4------QTL------B4

Considering first the male parent's markers, if a progeny had A1–B1 then it was given a flanker score of +1, if it had A2–B2 it was given a score of –1, and if it had either A1–B2 or A2–B1 (a crossover) it was given a score of 0. The same procedure was followed for the markers from the female.

After flanker scores were assigned, marker scores were derived by regressing the phenotype on flanker scores and then using the predicted phenotypic value as the marker score. The regression models for each full-sib family were constructed with SAS's REG procedure (SAS Institute Inc. 1990), using the forward option. The significance level for entry into the model was set to 0.05. This significance level was shown to maximize selection efficiency in a preliminary study. For a single QTL model the regression model would be

$$\text{Phenotype} = \text{Flanker score}_{\varphi} + \text{Flanker score}_{\sigma}$$

As suggested by Lande and Thompson (1994), both the phenotype and the marker score were used in a selection index to estimate the genetic value of the tree, such that

$$\text{Estimated genetic value} = (\beta_{\text{phenotype}} \times \text{Phenotype}) + (\beta_{\text{marker}} \times \text{Marker score})$$

Where, $\beta_{\text{phenotype}}$ is the index coefficient for the phenotype and β_{marker} is the index coefficient for the marker score.

Index values (β's) for using the marker score and phenotype were calculated using standard formula as

$$\beta = P^{-1}G$$

where β is the vector of index weights, P is the 2 × 2 correlation matrix of the phenotype and marker score, G is the 2 × 1 array of the correlation of phenotype and marker score with the true genotypic value.

This simplifies to

$$[1] \qquad \beta_{\text{phenotype}} = r_{\text{ap}} - r_{\text{am}} r_{\text{pm}}$$

$$[2] \qquad \beta_{\text{marker}} = r_{\text{am}} - r_{\text{ap}} r_{\text{pm}}$$

where r_{ap} is the correlation between the additive genetic value and the phenotype, equivalent to the square root of within-family heritability (h_{p}), r_{am} is the correlation of marker score with additive genetic value = $p^{0.5}$ where p is the proportion of the additive variance associated with the marker loci, estimated by $\sigma^2_{\text{marker}}/\sigma^2_{\text{a}} = (r_{\text{pm}})^2/h^2_{\text{p}}$, and r_{pm} is the correlation between the phenotype and marker score

Because correlations were used to establish the index coefficients, the phenotypes and index scores were standardized by subtracting the mean and dividing by the standard deviation. The index coefficients (weights) were multiplied by the marker scores and phenotypic values and summed to obtain index scores.

Genetic gain was directly calculated by selecting the top two individuals in each family, first using the phenotype alone and then with the index. The gain from each method of selection was calcu-

Table 2. Gain assumptions derived from one generation of Douglas-fir breeding in the Pacific Northwest (for variables assessed at ages 10 to 15).

Trait	Heritability	Total 1st-generation gain (%)	Proportion of gain from within-family selection	Expected gain from within-family selection (%)	Value per hectare range (1% gain)	Value used in financial model
Growth	0.25	15	1/3	5.0	US$50–US$250	US$150
Stem form	0.25	10	1/3	3.3	US$125–US$350	US$250
Wood density	0.45	6	1/2	3.0	US$125–US$500	US$312

lated as the average genetic value of the two selections minus the family mean.

This process was performed 200 times for each QTL model – heritability combination. Average gain and the standard deviation of gain were calculated for each set of 200 simulations. The efficiency ratio of using MAS over phenotypic selection was calculated as

$$[3] \quad \text{Efficiency ratio} = \frac{(\text{Gain from MAS index selection})}{(\text{Gain from phenotypic selection alone})}$$

Cost–benefit analyses

The cost–benefit analyses used the following assumptions and parameters based on ongoing or planned operational breeding programs in the Pacific Northwest:

(1) The production population for a breeding program (the seed orchard or clonal stool population) will arise from the top 30 full-sib families in the breeding population. These 30 families will be identified by a concurrent GCA test. Only these 30 families will be genotyped using molecular markers. This assumes that all full-sib families in the breeding program will be established in relatively large family blocks.

(2) The breeding–testing cycle is 13 years. Seed orchards have no pollen contamination, begin production at age 6, and reach full capacity at age 10.

(3) Rotation age is 50 years. One thinning will occur at age 25 when one-tenth of the financial harvest gain will be captured. Therefore, the first gains from thinning will begin at 31 years from selection (6 + 25), and gains from final harvest will begin at 56 years from selection (6 + 50).

(4) Breeding populations in succeeding generations will be comprised of one-half of the selections from the current elite population of 30 families and one-half from other families in the breeding program. Therefore, any additional gains from MAS will contribute to future generations, but at a reduced rate (50%). Gains from one generation (cycle) of breeding must be captured in the life-span of a single seed orchard.

(5) Present gains from intense selection in breeding programs have yielded the age-15 gains shown in Table 2 (Dan Cress, Pacific Northwest Tree Improvement Cooperative, personal communication). The breakdown of family and within-family gains are based on the ratios of observed family mean to within-family heritabilities, and selection intensities used in first-generation breeding programs. Increased selection efficiency resulting from MAS are assumed to only affect gains from within-family selection. Benefits were estimated as (efficiency ratio – 1) multiplied by the estimate of within-family gain.

(6) We used an estimated cost of US$0.65 per marker genotype and US$1.05 per DNA extraction, based on reasonable costs for SSR analyses from commercial and academic laboratories. For each QTL in the QTL model, it was assumed that two markers would be needed. Thus, for QTL model 1, each tree would cost US$1.05 for DNA preparation plus 2 QTL × 2 markers × US$0.65 = US$2.60 for scoring markers, for a total cost of US$3.65 per tree. Marginal costs were assumed to be only those associated with obtaining marker genotypes; we assumed that current personnel are sufficient to perform any additional analyses and that population sizes would be the same regardless of whether one chooses to use MAS.

(7) The cost for developing a set of SSR markers that would allow the selection of a framework subset of markers spaced at about 30 cM throughout the genome was estimated at US$200 000. Molecular marker studies of the Douglas-fir genome suggest a genetic length of between 2800 and 3500 cM (Krutovskii et al. 1998); the number of markers used in the QTL detection studies was therefore approximated at 3500/30 ≈ 120 markers.

(8) Dollar values for gain were estimated after discussion with industry personnel. The actual dollar value of a 1% gain in any of these traits is highly variable depending on log prices, product market, and site class of plantation. Of the three traits presented (growth, tree form, and wood density), only growth rate probably follows a linear trend in value. Both wood density and form are likely to be threshold traits; however, for simplicity we model them as linear. We present a range of values in Table 2 that we feel are realistic for most organizations and indicate the midpoint values we chose to model. Benefits were discounted to net present value using three discount rates: 4, 6, and 8%. Based upon the marginal cost of MAS, the land base needed to break-even was calculated.

An overall financial analysis that considered the costs of QTL detection and marker development was conducted. Field testing costs were estimated at US$8 per tree, for a total cost of: 5 families × 500 clones × 6 ramets × 2 sites × US$8 = US$240 000. Genotyping costs for these 2500 clones would be US$197 625, with another US$30 000 added for data management and analyses. The total cost of the QTL detection study would therefore be US$240 000 + US$197 625 + US$30 000 = US$467 625. With the cost of SSR marker development, the total cost would be US$667 625.

Because the QTL detection study could apply to multiple traits or breeding programs we assumed that benefits from selecting on all three traits would be used to offset these costs. To simplify matters, we assumed that each of the three traits had the same QTL structure. The discounted benefits of MAS for all three traits were used to calculate the number of hectares this gain would need to be spread across for benefits to equal the total cost of improving all three traits.

A separate set of analyses were done to examine the additional costs and benefits of genotyping 30 full-sib family blocks for obtaining additional gains from MAS on a trait-by-trait basis assuming that QTL were already known and SSR markers were already available.

Results and discussion

Selection efficiency

MAS contributed positively to selection efficiency in most

Table 3. Efficiency ratios comparing the measured gain in genetic value from selecting the top two selections per family using phenotypic selection alone, and index selection using the phenotype and marker score; for two heritabilities, 0.25 and 0.45.

QTL model	h^2 = 0.25					h^2 = 0.45				
	Phenotype		Index		Efficiency ratio	Phenotype		Index		Efficiency ratio
	Mean	Std. dev.	Mean	Std. dev.		Mean	Std. dev.	Mean	Std. dev.	
100 trees per family										
2	4.60	3.37	5.09	3.37	1.11	5.39	3.23	6.44	3.27	1.19
4	4.40	3.42	4.57	3.47	1.04	5.65	3.18	6.24	3.02	1.10
4D	3.56	3.16	3.75	3.34	1.05	5.29	3.18	5.45	3.16	1.03
Geo	3.77	3.37	4.26	3.21	1.13	5.98	3.13	6.15	3.03	1.03
2+5	4.56	3.11	4.49	3.02	0.99	6.40	2.87	6.42	2.85	1.00
1+10	4.53	3.22	4.31	3.26	0.95	5.92	3.28	6.03	3.12	1.02
300 trees per family										
2	4.47	3.26	6.12	3.52	1.37	6.32	3.65	7.45	3.77	1.18
4	5.15	3.60	6.96	3.08	1.35	7.16	3.13	8.96	3.06	1.25
4D	4.40	3.03	5.36	3.39	1.22	5.70	3.30	7.02	3.47	1.23
Geo	4.81	3.89	6.23	3.52	1.30	6.69	3.32	8.48	3.19	1.27
2+5	4.94	2.85	6.03	3.32	1.22	7.28	3.05	8.68	2.86	1.19
1+10	5.07	3.28	5.31	3.36	1.05	7.06	3.16	7.46	2.98	1.06
500 trees per family										
2	4.74	3.36	7.09	3.16	1.49	7.06	3.38	7.67	3.49	1.09
4	4.86	3.51	7.63	3.31	1.57	7.64	3.33	9.53	2.94	1.25
4D	4.13	3.26	6.72	3.97	1.63	6.23	3.22	8.19	3.54	1.32
Geo	5.27	3.43	7.55	3.14	1.43	7.28	3.23	9.01	2.76	1.24
2+5	5.21	3.26	7.57	3.14	1.45	7.58	3.13	10.01	2.71	1.32
1+10	5.60	3.30	6.25	3.39	1.12	7.55	3.03	8.42	2.68	1.12

cases (Table 3). For the larger family sizes, the low heritability traits gave higher MAS efficiencies than the high heritability trait (Table 3). Efficiency also increased with increasing family size. This was the result of being able to more accurately estimate the effect of segregating QTL. With a large selection population (n = 500), MAS could increase the efficiency of selection up to 63% (1.63) for a weakly heritable trait and up to 32% for a moderately heritable trait (1.32 in Table 3).

For more complex QTL structures and smaller selection populations, gains in efficiency were modest to negative (i.e., efficiency < 1.0). For example, under the geometric model (Geo) with 6 major QTL and 300 trees per family, the gain in efficiency was 30% for the weakly heritable traits and 27% for the moderately heritable trait. With 100 trees per family, gains in efficiency were negligible or negative except for the two major QTL model (2), which gave an increase of 11–19%. The combination of a complex QTL structure, small family size, and low heritability reduced the efficiency of MAS compared with that of phenotypic selection up to 5% (efficiency index of 0.95 for QTL model 1+10).

QTL of small effect were generally of limited use for MAS in these simulations, and attempts to use these QTLs with a small family size could cause a loss in efficiency compared with phenotypic selection alone. QTL model 1+10, for which most QTL explained 5% of the additive variance, gave little to no increase in efficiency. Thus, if QTL structure is truly this polygenic, it will be difficult for MAS to be profitable. QTL model 2+5, where most QTL explained 10% of the additive genetic variance, was much less stringent; it gave considerably larger efficiencies than QTL model 1+10 at 300 and 500 trees per family. However, even a reasonably strong QTL model, such as model 2, and low heritability had very little benefit with 100 trees per family; 300 trees or more were required to cause a significant increase of selection efficiency over phenotypic selection.

The levels of increased efficiency we estimated are similar to those from other simulation studies (Zhang and Smith 1992; Edwards and Page 1994; Whittaker et al. 1995; Kerr et al. 1996; Hospital et al. 1997), including the common result that MAS efficiencies tend to be highest for low heritability traits (e.g., Lande and Thompson 1990; Knapp 1994; Gimelfarb and Lande 1995).

The estimated gains from MAS varied from –0.25% to 3.13% for growth, –0.16% to 2.07% for form, and 0.01% to 1.22% for wood density.

Gain efficiencies were also examined by comparing the correlation of the index values with the true genotype and the correlation of the phenotype with the true genotype (data not shown). This should give an indication of the gains that would be predicted from theoretical gain equations since

$$\frac{\text{Index selection gain}}{\text{Phenotypic selection gain}} = \frac{ir_{ia}\sigma_a}{ir_{pa}\sigma_a} = \frac{ih_i\sigma_a}{ih_p\sigma_a} = \frac{r_{ia}}{r_{pa}}$$

where i is the selection intensity, σ_a is the square root of the additive genetic variance, h is the square root of heritability of the value being selected upon (either the index score (i) or phenotype (p)), and r is the correlation between the value being selected upon and the additive genetic value.

Increases in gain from comparing the correlations were 45% larger than those calculated with measured gain. On average the correlations increased selection efficiency 30%; the

average increase in efficiency from Table 3 is 21% (1.21). Therefore, efficiency gains predicted from theoretical equations could possibly be overestimating gains achieved when using high selection intensities.

Cost–benefit analyses

If one assumes that markers are available and that the important QTL have already been identified by prior research, the number of hectares a program needed for discounted benefits to equal genotyping costs is shown for growth and wood density in Tables 4A and 4B. The results for form (not shown) were nearly equivalent to those of growth; the land areas needed to break-even for form were approximately 90% of those for growth, regardless of the interest rate, QTL model, and family size.

For the lower heritability traits, optimal family size varied by QTL model (Table 4A). One hundred trees per family was best for QTL model Geo. Three hundred and 500 trees per family resulted in very similar numbers of hectares needed to break-even for all QTL models except model 1+10, where 500 trees was best. For wood density, the higher heritability trait, 100 trees was optimal for the two simpler QTL models (2 and 4), 300 trees per family was optimal for the moderately complex QTL models (4D and Geo), and 500 trees per family was optimal for the more complex QTL models (2+5 and 1+10).

Table 5 shows the number of hectares needed to recover all costs involving SSR development, QTL detection, and scoring markers for all three traits for 30 full-sib families. Five hundred trees per family was best for all QTL models. As expected, economic parameters had a large impact. The land area required to break-even varied greatly depending on discount rate and whether an organization needed to recoup costs in the first generation of breeding or could consider gains from subsequent generations. By considering benefits past the first generation, the number of hectares needed to break-even are reduced substantially compared with that for a single generation of gain. For example, at an interest rate of 6% the acreage required to break-even for multiple generations was 70% of the acreage needed when only first generation gains were considered. Where one accepts the gains from subsequent generations, the value of a 1% increase in growth over 1000 ha varied from US$611 (8%) to US$1893 (6%) to US$6869 (4%). The values of a 1% increase in growth for a single generation were US$481, US$1334, and US$3983 for discount rates of 8, 6, and 4%, respectively. Doubling the discount rate from 4 to 8% increased the land area required by approximately eightfold for all modeled scenarios using only one generation of gain (Table 5).

Implications for applied breeding programs

Do the simulations indicate that MAS can be cost-effective in operational programs in the near future? Unfortunately, there is insufficient information to make a confident prediction in favor of MAS for a number of reasons.

First, although some studies in forest trees have suggested that major QTL exist for growth, form, and wood property traits (see reviews by Sewell and Neale (1998), their Table 12.1 and Wilcox et al. (1997)), most of these were limited in scope and relevance to Douglas-fir breeding programs. The results suggested that some of the traits could be under the control of very few QTL (an oligogenic QTL model). The most significant QTL for growth and wood density, for those studies that found QTL, explained 5 to 29% of the phenotypic variation, but most were in the 10% range. None of these studies employed a population size large enough to make a precise estimate of QTL magnitude (cf. Beavis 1998); most had less than 200 progeny and none had more than 300. When comparing results of six *Pinus radiata* D. Don QTL studies to those found for corn, Wilcox et al. (1997) came to the conclusion that many loci probably contribute to the genetic variation in these traits; noting that the relatively large QTL found in some studies were probably an artifact of family size. In addition, multiple families and environments germane to breeding programs were not examined in most of the studies reviewed by Sewell and Neale (1998). Thus, it is impossible to predict whether the reported QTL operate on a population level or in multiple environments, as assumed in our models. If QTL do not operate on a population level or in multiple environments then their usefulness will be limited.

Second, SSR markers are under development (Amarasinghe et al. 1999), but a reliable set of highly polymorphic markers that cover the genome still appears to be some years away. Our estimate of US$200 000 to develop a set of SSR markers is a rough approximation; the cost could easily be double this amount depending on technical difficulties that might be encountered as a result of the large, repetitive genomes of conifers. A set of RFLP (restriction fragment length polymorphisms) markers are available and their use for QTL studies are underway in Douglas-fir (Jermstad et al. 1997; Neale et al. 1997). The costs of using RFLPs are approximately four times those of SSRs and they tend to be less polymorphic. In addition to increasing costs fourfold, the distance between QTL and polymorphic markers would increase for RFLPs, reducing the effectiveness of MAS. Land areas needed to break-even with RFLPs were 8 to 10 times higher than those for SSRs (data not shown).

RAPD (randomly amplified polymorphic DNA) markers and maps are available (e.g., Krutovskii et al. 1998) but would be difficult to employ in MAS because of their dominance, biallelism, and difficulty in consistent recognition of loci across families ALFP (amplified fragment length polymorphism) markers could be developed for Douglas-fir but are costly and would preclude the efficiencies we assumed by genotyping only the markers near to previously identified QTL.

Although SSRs appear most promising at present, progress on the major genome projects are expected to yield new technologies for high throughput genotyping, perhaps in conjunction with one of several DNA chip technologies under development (Lemieux et al. 1998). This has the potential to reduce costs 10- to 100-fold over the next 5 to 10 years, and increase marker density, which would have dramatic impacts on MAS profitability.

Third, very few Douglas-fir tree improvement programs have sufficient numbers of progeny for individual families that are required for efficient QTL detection. Second-generation breeding programs in the Northwest Tree Improvement Cooperative tend to plant 20 progeny on each of six sites, for a total of 120 per family. Weyerhaeuser programs plant 24 progeny on four to eight sites, for a total of

Table 4. Number of hectares (×1000) needed in a operational deployment zone to break-even with MAS for (A) volume growth and (B) wood specific gravity, ignoring costs of QTL detection.

(A) Volume growth.						
	One generation to recoup cost			Multiple generations to recoup cost		
	Discount rate			Discount rate		
QTL model	4%	6%	8%	4%	6%	8%
100 trees per family						
2	5	15	42	3	11	33
4	25	74	206	14	52	162
4D	18	55	152	11	39	119
Geo	10	31	85	6	22	67
2+5	—	—	—	—	—	—
1+10	—	—	—	—	—	—
300 trees per family						
2	4	13	37	3	9	29
4	8	24	66	5	17	52
4D	13	39	107	8	27	84
Geo	14	40	112	8	28	88
2+5	21	62	171	12	43	135
1+10	144	431	1197	84	304	942
500 trees per family						
2	6	17	46	3	12	36
4	8	25	68	5	17	54
4D	8	22	62	4	16	49
Geo	15	46	128	9	32	100
2+5	17	50	140	10	36	110
1+10	99	297	823	58	209	648
(B) Wood specific gravity.						
100 trees per family						
2	2	7	19	1	5	15
4	7	22	60	4	15	48
4D	25	73	204	14	52	160
Geo	38	114	316	22	80	248
2+5	408	1219	3385	237	859	2664
1+10	102	304	844	59	214	664
300 trees per family						
2	7	22	61	4	16	48
4	9	27	75	5	19	59
4D	10	29	80	6	20	63
Geo	12	36	99	7	25	78
2+5	19	57	158	11	40	124
1+10	97	290	805	56	204	633
500 trees per family						
2	25	76	210	15	53	165
4	15	45	126	9	32	99
4D	12	36	99	7	25	78
Geo	23	67	186	13	47	147
2+5	19	57	159	11	40	125
1+10	80	239	664	46	168	522

96 to 192 per family. These numbers are not sufficient to use marker-aided selection in most of the QTL models we studied, and are inadequate for the rigorous QTL detection studies that we assumed would precede MAS.

Fourth, we have not accounted for all costs and potential efficiencies of an operational MAS system. We did not account for the additional personnel that are likely to be required to collect samples, analyze data, and manage the much more complex breeding program that would exist under MAS. However, a more detailed analysis of these costs and efficiencies would only be meaningful after the major gaps in information about QTL structure are narrowed, and the possibility for quantum leaps to improve marker efficiency and reduce genotyping costs are better understood.

Finally, our simulations suggest that relatively large land bases will be required to confidently recoup the costs of an

Table 5. Number of hectares (×1000) needed in a operational deployment zone to recoup the costs of SSR development, QTL detection, and scoring 30 additional full-sib families for the three traits, each with the same QTL structure.

	One generation to recoup cost			Multiple generations to recoup cost		
	Discount rate			Discount rate		
QTL model	4%	6%	8%	4%	6%	8%
100 trees per family						
2	74	222	616	43	156	485
4	173	515	1431	100	363	1126
4D	246	735	2042	143	518	1606
Geo	121	360	1001	70	254	787
2+5	—	—	—	—	—	—
1+10	—	—	—	—	—	—
300 trees per family						
2	37	112	310	22	79	244
4	39	116	323	23	82	254
4D	55	163	454	32	115	357
Geo	47	139	387	27	98	304
2+5	65	195	543	38	138	427
1+10	310	925	2569	180	652	2021
500 trees per family						
2	35	105	291	20	74	229
4	31	91	254	18	64	200
4D	27	80	223	16	57	176
Geo	43	129	357	25	91	281
2+5	41	121	337	24	86	265
1+10	171	512	1420	99	361	1118

Table 6. Approximate land area in production for second generation Douglas-fir breeding programs in the Pacific Northwest.

Program	Approximate ha (×1000)
Weyerhaeuser low elevation, Washington	480
British Columbia Ministry of Forests	385
NWTIC, Vernonia and Ryderwood	277
NWTIC North Oregon Coast Range: coastal	215
NWTIC North Oregon Cascades	207
Weyerhaeuser low elevation, Oregon	202
NWTIC North Oregon Coast Range: inland	168
NWTIC Central Oregon Coast	127

Note: NWTIC, Northwest Tree Improvement Cooperative.

MAS program in Douglas-fir if these traits are polygenic (QTL models 2+5 and 1+10). The current deployment areas for second-generation breeding programs in the northwest are shown in Table 6. These programs are considerably larger than first-generation programs, increasing the potential for economic benefits from MAS (Johnson 1998*b*). Under selection for all three traits, recouping QTL detection costs, only considering one generation of benefits, and a 6% discount rate, all but one of the second-generation programs has the approximately 129 000 ha area needed to cover the costs of MAS (cf. Tables 5 and 6). If the QTL for these traits are more like the 1+10 model, however, most of the programs could be profitable at the 4% discount rate, but none at the higher rates. If one can depend on other organizations to develop inexpensive markers and find the QTL, all the programs could profitably use MAS (Tables 4A and 4B).

For traits such as volume growth that are expected to be highly polygenic at a population level, and to show dominance and QTL × environment interactions (cf. Strauss et al. 1992), a very high throughput marker technology such as a DNA chip, whose costs are not closely related to marker number, will probably be needed. Such a method could allow full genotyping of all framework loci or even of a much denser set of loci, in each family, obviating the need to first detect QTLs and then use only these population QTLs during MAS.

However, to be ready to take advantage of this technology, which should be available in less than a decade, industries will need to modify their breeding programs significantly to increase the sizes of their elite families (e.g., two- to fourfold). Unless breeding programs produce these large families in the near future, MAS will not be possible in Douglas-fir regardless of developments in marker technology.

Implications for further research

A set of framework markers, and tests of sufficient size and age for reliable QTL detection are prerequisites for detailed assessments of the financial value of MAS. Development of SSRs or other highly polymorphic markers would allow the needed research on QTL detection to begin while large progeny tests are established for QTL detection and operational MAS. Current RFLP and RAPD maps limit our ability to find QTL and use them economically. To spread costs, marker development would ideally be supported by a combination of public sector scientists, breeding organizations, and industries that intensively manage Douglas-fir around the world.

There is an urgent need to establish large field trials of in-

dividual families that can be used for QTL detection once a marker set is developed. While markers can be developed at any time, these trials need to be planted a decade or so before QTL studies could be reliably carried out. These trials should

(1) Have large numbers of genotypes (e.g., 500 or more) so that all major QTL are detected and their magnitudes are estimated reliably.
(2) Use multiple ramets per genotype, if possible, to increase heritability so that QTL of small magnitude can be identified.
(3) Be on multiple sites so that QTL × environment interactions can be quantified.
(4) Examine a number of full-sib families so that major population QTL can be discerned from family-specific QTL.

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