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MARKER ASSISTED SELECTION FOR BREEDING VALUE IN FOREST TREES

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ABSTRACT

Marker assisted selection provides a way to increase the efficiency of within family selection in breeding. The efficiency of MAS depends upon the heritability and the amount of additive genetic variation that is explained by genetic markers. In most forest tree breeding programs, a population approach is used that makes it difficult to relate quantitative trait locus (QTL) effects within family to the additive genetic variation in the population. However, in half-sib families where the pollen is a random sample of the breeding population, QTLs that segregate in the gametes from the common parent can be defined in terms of the average effect of the QTL alleles relative to the breeding population. QTL effects defined in this way can be viewed as components of breeding value and related to the conventional breeding schemes for advanced generation breeding. Selection for markers that trace components of the additive genetic variation of the breeding population in specific full-sib families could provide greater gain than conventional phenotypic selection within families. The genetic architecture of low heritability traits in forest trees is reviewed from the perspective of the additive genetic variation explained by QTLs.

Key words: selection, breeding, genetic markers, QTL

INTRODUCTION

Great progress has been made in constructing genomic maps of forest trees in the last few years (*e.g.*, BRADSHAW & STETTLER 1995, DEVEY *et al.* 1994, GROOVER *et al.* 1994, NELSON *et al.* 1993). Genomic mapping now provides an important way to study forest genetics. In human genetics, LANDER and SCHORK (1994) described this new genetic paradigm as complex trait analysis. Classical genetic analysis requires a 1:1 correspondence between genotype and phenotype, and genetic control is recognized through Mendelian segregation ratios of phenotypically distinct classes among progeny in families. Quantitative genetic analysis assumes polygenic inheritance and is based on phenotypic variances and family means. The phenotype is viewed as the result of an interaction of the genotype with the environment. Genetic control is explained through the concept of heritability. Complex trait analysis is based on the associations of phenotypic measurements or categories with genetic markers that segregate in Mendelian ratios. Complex traits are substantially controlled by one or a few major genes (*i.e.*, quantitative trait loci, QTLs), with an environmental component such that Mendelian ratios are obscured at the level of the phenotype.

QTLs discovered by genomic mapping could be useful in plant breeding. In an F_2 wide cross family of tomato, DEVICENTE and TANKSLEY (1993) identified QTLs that had effects opposite from those expected based on the parental phenotypes. These QTLs were associated with transgressive segregation (*i.e.*, individuals with phenotypic values that exceed the parental phenotypic distributions). Their work suggests a strategy for the systematic improvement of crops through the identification of useful genes in crosses between cultivars and phenotypically inferior germplasm. STUBER *et al.* (1992) identified genetic factors contributing to heterosis in a hybrid between two elite maize inbred lines. However, approaches to genetic improvement based on crosses among inbred progeny could be difficult to incorporate in tree improvement strategies due to the long generation interval and inbreeding depression typical of forest trees.

Knowledge of major genes for economic traits that are marked by DNA polymorphisms could be useful in tree breeding programs. Wood properties (*e.g.*, specific gravity) and disease resistance have been identified as candidate traits for marker assisted breeding in forest trees (WILLIAMS *et al.* 1992, NANCE *et al.* 1992). The heritability of wood specific gravity is high, but measurement is difficult. QTLs that influence wood properties have been detected (GRATTAPAGLIA 1994, GROOVER *et al.* 1994, BRADSHAW & STETTLER 1995). Disease resistance in many plants is under simple genetic control and generally requires difficult assays. Recent work in loblolly pine has shown that major genes play an important role in resistance to fusiform rust disease (WILCOX 1995). In both of these cases, markers for major genes could be followed in crosses before the

trait is expressed, thus have special value for tree breeding (STRAUSS *et al.* 1992). Marker assisted breeding for these major gene effects in forest trees could accelerate the rate of gain from tree improvement. However, the gains from manipulation of certain major genes is likely to be specific to particular circumstances and a general theoretical measure of efficiency will be difficult to derive.

A different approach to the use of markers in breeding was outlined by LANDE and THOMPSON (1990). Genetic markers could be used to increase the efficiency of phenotypic selection for low heritability traits by identifying components of genetic variation due to major quantitative trait loci (QTL). The accuracy of within family selection could be increased by choosing offspring that have favorable QTL genotypes and a superior phenotype for breeding in the next generation. LANDE & THOMPSON (1990) expressed the efficiency of marker-assisted selection (MAS) as a ratio, using the quantitative expression for response to phenotypic selection as the denominator, thus providing a basis for comparison of selection methods. Within family phenotypic selection is an important component of advanced generation breeding plans in forest trees, despite the poor response compared with family selection (*e.g.*, MCKEAND & BRIDGWATER 1992, WHITE *et al.* 1993, VAN BUIJTENEN & BURDEN 1990, COTTERILL 1986). The critical questions for tree breeders are how much benefit could MAS provide in the context of a conventional tree improvement program, and what will be the cost?

The prospects for MAS in forest trees were reviewed by STRAUSS *et al.* (1992). Their conclusions were pessimistic in the context of traditional tree improvement programs, but they emphasized the value of QTL information for fundamental research on tree genetics. In this paper, we reexamine the prospects for MAS and for understanding the genetic architecture of growth and volume in forest trees, and we address the use of markers to trace the transmission of additive genetic variation for quantitative traits from parents to progeny in the context of an advanced generation tree breeding program. Growth and volume are complex integrative traits that are likely to respond to many different environmental factors and be influenced by genes involved with different physiological and developmental processes. QTLs for these traits could also have extensive genotype × environment interactions (G×E). However, the response to selection for growth and volume in tree breeding programs has been successful, and it is important to know the role major genes played in achieving these gains. Studies in other plants do detect G×E for QTL effects, but a large portion of the effects are stable across environments (PATERSON *et al.* 1991, TANKSLEY 1993, HAYES *et al.* 1994). The frequencies of major genes in some populations of domesticated animals and plants could be already be fixed, but the distribution, frequency and abundance of major gene effects in undomesticated species such as forest trees is largely unknown. We draw heavily on FALCONER'S (1989) textbook on quantitative genetics and acknowledge this source for general statements on quantitative genetics theory and breeding strategies. We treat breeding issues from a conceptual point of view in an effort to show how molecular markers can be integrated with breeding theory for outbred organisms.

QUANTITATIVE GENETICS AND SELECTION RESPONSE

Heritability is a measure of the extent of genetic control for a quantitative trait. The phenotypic variation in a population can be attributed to genetic and environmental causes: $\sigma^2_P = \sigma^2_G + \sigma^2_E$. Genetic variation in quantitative traits can be partitioned into additive variance and dominance variance: $\sigma^2_G = \sigma^2_A + \sigma^2_D$, assuming no epistasis. The σ^2_A portion of σ^2_G is readily transmitted to progeny and contributes to breeding value. Narrow sense heritability (h^2) is the ratio of the additive variance and the total phenotypic variance: $h^2 = \sigma^2_A / \sigma^2_P$. Selection theory is based on heritability and the intensity of selection. The response (R) of a population to phenotypic selection can be predicted by the equation:

$$R = ih^2\sigma_P$$

where i is the selection intensity and σ_P is the phenotype standard deviation. The selected individuals are crossed in a mating design and their offspring planted in tests for the next generation.

For most growth and volume traits in forest trees, h^2 is low, usually << 0.5 (CORNELIUS 1994, ZOBEL & TALBERT 1984, WRIGHT 1976). Gain from phenotypic selection is limited when h^2 is small because the phenotypic value of an individual is a poor predictor of its genetic value. The genetic value of an individual is determined by progeny tests where the offspring from different families are compared in a common environment. When h^2 is low, family selection is much more efficient than phenotypic selection. The heritability of family mean differences is higher than the heritability of the deviations of individuals from their family means. Family mean heritability is expressed as:

$$h^2_F = h^2 [1 + (n-1)r] / [1 + (n - 1)t]$$

where r is the correlation of breeding values (r = ½ for full-sibs and r = ¼ for half-sibs) and t is the intraclass correlation coefficient (usually, t < r).

VAN BUIJTENEN and BURDEN (1990) distinguished between "forward selection" and "backward selection" in tree breeding strategies. In backward selection, the best parents in the current generation are chosen on the basis of progeny performance. In forward selection, the best progeny are selected as parents for the next generation on the basis of phenotype. Backward selection requires large numbers of families but selection is based on genotypic values estimated through family means. Intensive family selection is effective for low heritability traits, but inbreeding increases rapidly over generations and genetic variability is lost quickly (FALCONER 1989).

Ideally, more gain should be obtained from within family (WF) selection in advanced generations than in the early generations of tree breeding programs (*i.e.*, forward selection), but WF selection is based on phenotypic values. Progeny testing candidates for WF selection could double the generation interval, thus is seldom feasible. Larger family sizes allow a higher selection intensity, but the point of diminishing returns is reached at relatively small family sizes (n << 100). WF selection is best carried out in large family block plantings after the trees have reached selection age (*e.g.*, 6 – 8 years in loblolly pine). The generation interval is several years more than the selection age because the selected individuals must be induced to flower, mated in some breeding design, and seed collected (18 months after pollination for pine). Combined selection is an approach that balances selection among families and within families (*e.g.*, Figure 1). The lower ranking families are culled and the best offspring

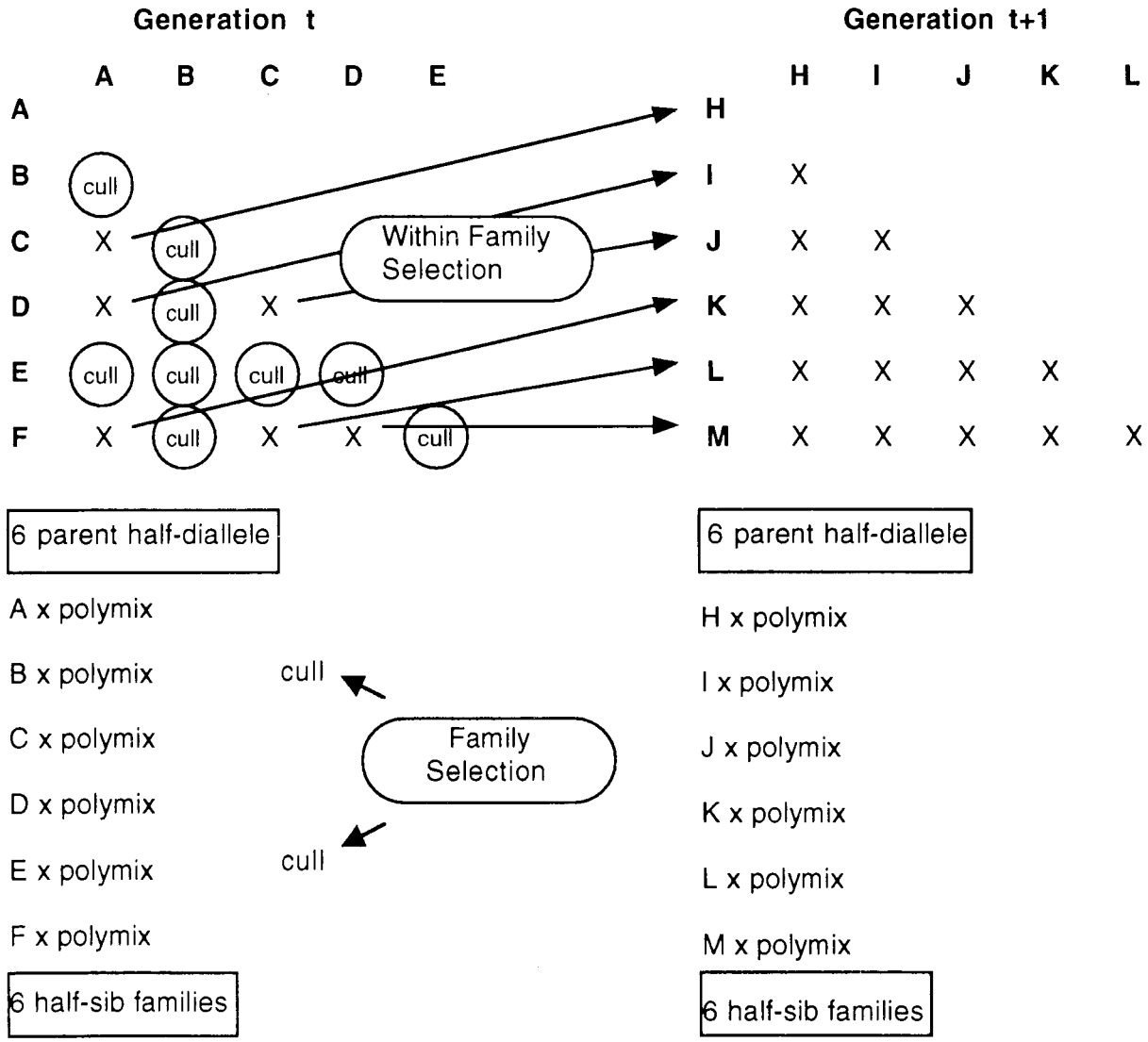

Figure 1 A hypothetical advanced generation combined selection scheme with complementary mating design for a single subline of tree program, where the polycross pollen mix that generates the half-sib families is collected from parents in many different sublines

are chosen for breeding from the remaining families. Response to combined selection for growth and volume in advanced generation breeding could be less rapid than response from family selection in the early generations of breeding.

IMPROVEMENT OF SELECTION RESPONSE USING GENETIC MARKERS

For forest trees, ways to make breeding more efficient are especially valuable because of the long generation intervals. The equation for response to selection suggests the ways that more progress could be made. Selection intensity could be increased, but that requires a disproportionate increase in family size. Heritability could be increased by decreasing environmental variation, or by clonal propagation of individuals within family so that a clone mean heritability is used for selection rather than the conventional h^2_W (SHAW & HOOD 1985). Alternatively, knowledge of segregating QTLs could provide genotypic values for a portion of the genetic variance. The progeny that have the most favorable QTL genotype can be identified using tightly linked genetic markers, and the best among those individuals can be selected as parents for the next generation (Figure 2).

LANDE and THOMPSON (1990) described Marker Assisted Selection (MAS) as a selection index. Phenotype was regressed on genetic markers to help choose markers to include in the index ($I = b_z z + b_m m$, where z was a column vector of quantitative traits and m was a "net molecular score"). The relative efficiency of MAS compared with phenotypic selection was expressed as a ratio of the response to selection using markers and phenotype, divided by the response to selection using phenotype alone. That ratio simplified to:

$$\text{Relative Efficiency} = [\, p/h^2 + (1-p)^2/(1-h^2p) \,]^{1/2}$$

where p was the proportion of σ^2_A associated with genetic markers. For traits with a low heritability (*e.g.*, 0.2) where genetic markers explain a relatively large portion of the additive genetic variance (0.4), the relative efficiency of MAS can be large (> 1.5).

The effectiveness of MAS is determined by the amount of σ^2_A that can be traced from parent to progeny by markers (DEKKERS & DENTINE 1991). The efficiency of MAS also is strongly dependent on h^2. For example, if h^2 in the example above is doubled to 0.4, the relative efficiency becomes 1.2. If h^2 is halved to 0.1, the relative efficiency becomes 2.1. For many inbred crop plants, the population of immediate interest is a single inbred pedigree with multiple generations (F_2, F_3, etc.) that is derived from a cross of inbred lines. The magnitude of σ^2_A and σ^2_D can be determined and related to QTL effects (*e.g.*, MORENO-GONZALEZ 1993). However, estimating the proportion of WF σ^2_A that is explained by QTLs is more complicated for forest trees because σ^2_A and h^2 are defined on a population of trees rather than on a specific pedigree.

Heritability, h^2, is estimated from the resemblance among relatives for populations of many families in forest trees and most other species. A within family (WF) heritability is sometimes calculated:

$$h^2_W = h^2 (1 - r) / (1 - t)$$

where r is the correlation of breeding values (r = 1/2 for full–sib families and r = 1/4 for half-sib families), and t is the intraclass correlation coefficient. (Usually, t < r.) The proportion of the phenotypic variance that can be attributed to females and males can be illustrated by evaluating the observational and causal components of variation in a factorial mating design (COMSTOCK & ROBINSON 1948):

$$\sigma^2_{Female} = \tfrac{1}{4}\,\sigma^2_A$$
$$\sigma^2_{Male} = \tfrac{1}{4}\,\sigma^2_A$$
$$\sigma^2_{F\times M} = \tfrac{1}{4}\,\sigma^2_D$$
$$\sigma^2_{Within} = \tfrac{1}{2}\,\sigma^2_A + \tfrac{3}{4}\sigma^2_D + \sigma^2_E$$

Within families, σ^2_A is equally contributed by females and males, thus $\frac{1}{4}\,\sigma^2_A$ is due to within female variation and $\frac{1}{4}\,\sigma^2_A$ is due to within male variation. Similarly, in a population of half-sib families, $\frac{1}{2}\sigma^2_A$ comes from the female parents and the other half from the males. The variance among half-sib families (female families for forest trees) accounts for $\frac{1}{4}\sigma^2_A$, and the remainder of the additive variance, or $\frac{3}{4}\sigma^2_A$ is within the family ($\frac{1}{4}\hat{\sigma}_A$ from female and $\frac{1}{2}\sigma^2_A$ from males). Thus, $h^2_W = (\frac{3}{4}\sigma^2_A) / (\frac{3}{4}\sigma^2_A + \sigma^2_D + \sigma^2_E)$. Therefore, the expected segregation variance (*i.e.*, σ^2_A) transmitted contributed by the common parent to a half-sib family is $PWA = (\frac{1}{4}\,\sigma^2_A)/(\frac{3}{4}\,\sigma^2_A + \sigma^2_D + \sigma^2_E)$, which is $<\frac{1}{3}\,h^2$ (Table 1).

In an outbred breeding population, the effect of a single gene can be related to σ^2_A through the concept of breeding value (*e.g.*, DENTINE & COWAN 1990, GODDARD 1992). Breeding value is the average effect of an individual's genes determined from the mean genotypic value of its progeny (FALCONER 1989). If the individual is mated to a random sample of parents in the population, the individual's breeding value is twice the deviation of its progeny mean from the population mean. σ^2_A is the variance of individual breeding values for the breeding population. For one locus, the breeding value of an individual is the sum of the average effects of the two alleles that constitute its genotype (Table 2). The average effect of an allele is the mean value of the progeny obtained by mating a gamete carrying that

Table 1 The expected proportion of the within half-sib family phenotypic variance attributed to σ^2_A transmitted by the common parent, $P_{WA} = \frac{1}{4}\sigma^2_A / (\frac{3}{4}\sigma^2_A + \sigma^2_D + \sigma^2_E)$, for several values of heritability, h^2

h^2	P_{WA}
1.00	0.3333
0.80	0.2500
0.60	0.1765
0.40	0.1111
0.20	0.0526
0.00	0.0000

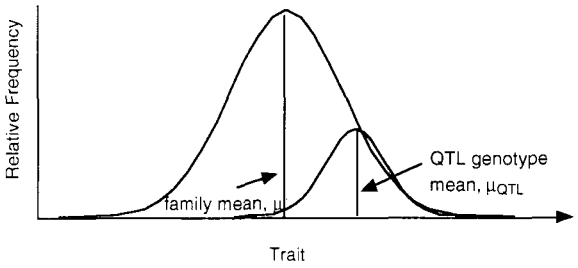

Figure 2 Hypothetical fequency distribution for progeny in a family showing the distribution of individuals within the family that have a favorable QTL genotype

allele to a random sample of gametes from the population. The breeding value for the whole genome of a parent is obtained by summing the individual locus breeding values, assuming no epistasis.

Breeding value can be estimated for individuals based on their progeny performance. A single locus approximation of breeding value was defined by DENTINE and COWAN (1990) as a 'chromosome substitution effect', which is the difference between the two groups of progeny that received alternative alleles of a marker locus from a common heterozygous parent that was mated to a random sample of parents from the population. Assuming no epistasis and that QTLs are not linked, chromosome substitution effects are *the average effects* of QTL alleles that are transmitted to a broad sample of genetic backgrounds present in a half-sib family (Figure 3). We will call QTLs defined in half-sib families average effect QTLs, in the same sense as chromosome substitution effects.

DENTINE and COWAN (1990) developed a method for estimation of chromosome substitution effects that takes account of the uncertainty of inheritance of markers from the common parent when the offspring is a heterozygote. Their method relies on multiple markers and knowledge of marker gene frequencies in the breeding population. Not all offspring in a half-sib family are informative for QTL detection. GAILLARD and SMITH (1992) calculated the average proportion of progeny in which the transmission of common parent gametes is known with certainty. In conifers, PCR-based markers can be assayed in the haploid megagametophyte tissue of seeds and genomic maps constructed (*e.g.*, NELSON *et al.* 1993). If the megagametophyte is taken from germinating seeds, these methods allow the gametic contribution of the common seed parent to a seedling to be determined unambiguously and used to estimate average effects of QTLs.

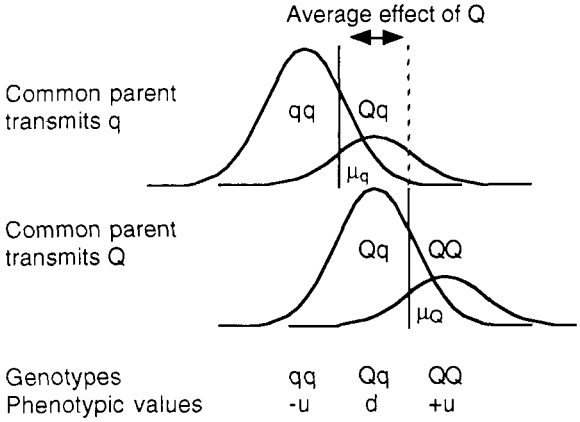

Figure 3 Expected progeny distributions for offspring that receive alternative QTL alleles from the common parent in a half-sib family, assuming 2 alleles, gametes from other parents randomly sample the population, and $p < q$, showing the effect of chromosome substitution, $\mu_Q - \mu_q$ (following DEKKERS & DENTINE 1991)

QTLS AND MARKER ASSISTED SELECTION IN OUTBRED ORGANISMS

Breeding programs for outbred organisms depend on the genetic variability of the whole breeding population. We have shown how σ^2_A is distributed within and among families and how much σ^2_A is available to be explained by QTLs within families. We have also shown how QTL effects that are attributed to the common parent of a half-sib family can be defined in terms of average effects of alleles that can be viewed as components of breeding value. Now we show how σ^2_A defined at the population level can be traced from parents to offspring in full-sib families using markers, and relate average effect QTLs to breeding theory and practice.

In breeding programs, selection increases the frequency of favorable genes in the breeding population and thereby increases the population mean from one generation to the next. Selectin the genes with the largest average effects (*i.e.*, breeding value) has the greatest effect on the population mean. FALCONER (1989) suggests that the genes with the largest breeding value are likely to be dominant, occur at low frequency

Table 2 Single locus model for breeding value that shows the average effect of the alleles at a locus, where A and a are the 2 alleles with frequency p and q in the breeding population, and the genotypic means for AA, Aa, and aa are +u, d, and –u , and the average effect of the two alleles is α_1 and α_2, and the effect of gene substitution is $\alpha_1 - \alpha_2 = \alpha$ (from FALCONER 1989, page 116)

Gamete type from common parent	Gamtes from populations and frequencies		Genotypes and frequencies		Genotypic means	Average effects of alleles A and a (μ_a–μ and μ_A–μ)
a	a A	q p	aa Aa	q p	–u d	
	Mean of individuals that received a				–qu + pd = μa	–p[u +d(q – p)] = α_2
A	a A	q p	Aa AA	q p	d +u	
	Mean of individuals that received A				pu + qd = mA	q[u + d(q – p)] = α_1
	Population mean				u(p–q) + 2dpq = m	$\alpha_1 + \alpha_2 = \alpha^*$

*) α is the effect of gene substitution, or the change in population mean expected by replacing a with A. Assuming no epistasis, the breeding value of an individual is the sum of the a for over all loci

in the breeding population, and have large effects. The role of gene frequency in the breeding population is crucial in understanding breeding value. The average effect of a high frequency allele will be small, and selecting such a gene will have little effect on the population mean even if the effect of the gene is large in full-sib families where the allele segregates. QTL effects detected in a single full-sib family cannot be interpreted in terms of *average effect* in the breeding population.

The average effect of QTL alleles transmitted by a heterozygous common parent to a large half-sib family is a measure of the value of the QTL for breeding. Average effect QTLs for low heritability traits could be difficult to detect because so little of the within family phenotypic variation is *expected* to be due to σ^2_A (*e.g.*, segregation variance is ~14% of phenotypic variation in a half-sib family when h^2 is 0.5, Table 1). However, if inheritance of the trait is oligogenic rather than polygenic, then heterozygosity will vary among parents and the segregation variance for some parents could be much greater for some parents than for others. How frequent are QTLs with large average effects? Even if QTLs with large average effects are rare at an individual locus, there are genes at many loci that could influence quantitative traits.

Marker assisted selection could be carried out within full-sib families of an advanced generation tree breeding program using markers for QTLs with large average effects (Figure 1). Advanced generation breeding populations can be subdivided into small breeding groups or sublines that allow unrelated matings among sublines in future generations to avoid inbreeding depression (LOWE & VAN BUIJTENEN 1986, MCKEAND & BRIDGWATER 1992). Combined selection (among families and within families) is done using complementary mating designs that generate polymix half-sib families to evaluate the breeding value of the current generation parents and partial dialleles for within family selection. In the scheme presented by MCKEAND and BRIDGWATER (1992), the polymix pollen is collected from many parents in many sublines and selection for breeding value based on these families allows for comparison of parents from different sublines in the larger breeding population. These polymix half-sib families provide an opportunity to identify and/or quantify average effects that could then be selected within full-sib families within sublines using markers. The initial gene frequency of a QTL that is rare in the breeding program will be ½m in at least one subline of m parents. Different sublines could be fixed for different average effect QTLs over a few generations of breeding. Linkage disequilibrium for linked loci will persist for several generations within sublines, so the marker:trait associations detected in one generation could be useful in the future. Some families should have more segregating QTLs than others, depending on the expected heterozygosity (h_e) of the parents for these loci. Additional efficiency of MAS could be obtained by placing several parents that have segregating major gene effects in the same subline.

Marker assisted selection for breeding value in forest trees could be facilitated by the development of a selection index following a similar approach taken in animal breeding (*e.g.*, HOESCHELE & ROMANO 1993). FERNANDOand GROSSMAN (1989) applied a best linear

Table 3 Mixed model analysis of variance for QTL effects that partitions genetic variation within family using clones of individual progeny, assuming a balanced design and treating QTLs as fixed effects and the genetic variation not explained by QTLs as a random effect (folowing KNAPP & BRIDGES 1990)

Source	Degrees of freedom	Observational Expected Mean Squares	Causal Expected Mean Squares
QTLs (Q)	q–1	$\sigma^2_e + c\sigma^2_{I:Q} + cn\phi^2_Q$	$\sigma^2_E+c\sigma^2_{G:Q}+cn\phi^2_Q$
Individuals w/in Q (I:Q)	q(n–1)	$\sigma^2_e + c\sigma^2_{I:Q}$	$\sigma^2_E+c(\sigma^2_{GB}-\phi^2_Q)=$ $\sigma^2_E+c\sigma^2_{G:Q}$
Residual	qn(c–1)	σ^2_e	σ^2_E
Total	nc–1		

* where q is the number of multilocus QTL genotypes, n is the number of individuals per QTL genotype, c is the clonal replication factor; σ^2e is the variance of clones of individuals, $\sigma^2_{n:Q}$ is the variance of individuals within QTL genotypes, and ϕ^2_Q are the fixed QTL effects; σ^2_E is the variance due to the environment , and $(\sigma^2_{GB}-\phi^2_Q) = \sigma^2_{G:Q}$ is the genetic variation among individuals within QTL genotypes

unbiased predictor (BLUP) approach to marker assisted selection in animals. They used a mixed linear model that provided for simultaneous evaluation of fixed effects, effects of QTL alleles associated with markers, and effects of alleles at the remaining QTLs, using known relationships and phenotypic and marker information. This approach can accommodate individuals with partial or no marker information. CANTET and SMITH (1991) presented a reduced animal model for obtaining best linear unbiased predictor (BLUP) estimates of additive effects for average effect QTLs and for the remaining portion of the breeding value. HOESCHELE (1993) showed that an animal model using BLUP, and that included breeding values and QTL effects associated with only some genotyped individuals, did not require QTL equations for animals that are not marker genotyped and do not provide relationship ties among genotyped descendants. With no marker data, the model reduces to the standard animal model for phenotypic data. Thus, only the families with parents that have a high breeding value need be genotyped using molecular markers.

QTL DETECTION: USE OF CLONES AND FAMILY SIZE

KNAPP and BRIDGES (1991) explored a linear genetic model for QTL effects (Table 3). Their model is used here only to illustrate concepts about QTLs because the ideal case for analysis using linear models is unrealistic for QTL analysis. The model assumes that QTLs are already known well enough to be considered fixed effects, the QTLs are not linked, and the experiment is balanced and completely randomized. The number of multilocus QTL genotypes, q, is:

$$q = x^k$$

where x is 3 for a F_2 family and 2 for a backcross family, and k is the number of QTLs. If the individuals within a family are clonally propagated, then variation can be partitioned among individuals as fixed QTL effects, ϕ^2_Q and within individuals as random effects, $c\sigma^2_e$, where c is the number of ramets per clone. These observational components of variation can be interpreted as genetic components. The genetic variation not explained by QTL genotypes, $\sigma^2_{I:Q}$, is a random effect nested within QTL genotypes. Without clonal propagation (*i.e.*, c = 1), the two sources of variation at the bottom of Table 3 cannot be separated, but significance tests for QTL effects are not affected. The same expected mean square is used for F-tests in both cases, $c\sigma^2_{n:Q} + cn\phi^2_Q$.

The key feature of the model of KNAPP and BRIDGES (1991) is that genetic variance among individuals that is not explained by QTLs, is a random effect nested within QTL genotypes. Thus, the phenotype of an individual is explained by a fixed QTL effect, a random genetic effect, and environmental error. More than one individual per multilocus QTL genotype is needed to obtain an estimate of a QTL effect that is not confounded by the unexplained genetic variance nested within QTL genotypes. The effect of a QTL is the expected value of the mean of several individuals with the same multilocus QTL genotype. When the genetic variation not explained by QTLs is large, the value of clonal propagation for detecting QTLs is small for low heritability traits. KNAPP and BRIDGES (1991) argued that power to detect QTLs depends upon the precision of estimation of QTL genotypic means. The standard error of a QTL genotypic mean is:

$$\sigma_{QTLm} = [\sigma^2_{G:Q}/n + \sigma^2_E/cn]^{1/2}$$

where c is the clonal replication number and n is the number of progeny. The variance of a QTL genotypic mean has a genetic component, $\sigma^2_{G:Q}$, and an environmental component, σ^2_E. Clonal replication affects only the environmental component of the variance, whereas increasing the number of progeny decreases both the genetic and environmental components. For a fixed number of experimental units, the standard error should be smallest for c = 1, and the effect of increasing c and increasing n will be equal only when all of the genetic variation is explained by QTLs (KNAPP & BRIDGES 1990).

Clonal propagation can play a valuable role in QTL analysis (BRADSHAW & FOSTER 1992), but clonal replication is not necessary for the analysis of low heritability traits. The justification for clonal propagation is efficiency because vegetative propagation usually costs less than genotyping progeny for genetic markers. Given a large family size, clonal propagation could allow replication of QTL experiments to test additional environments in an efficient way, as well as provide some increased precision for analysis of estimation of QTL effects at low cost. Vegetative propagation allows the within family genetic variation to be partitioned, but these variance components include non additive genetic variation that is not readily transmitted to progeny. Some species are not easily propagated vegetatively, and care must be taken to minimize environmental variability due to differences in clonal plant quality (*e.g.* HAINES 1994). For QTL analysis, a larger progeny size is preferred to clonal propagation except where the cost of genotyping prohibits increasing n.

In practice, QTL effects are usually studied using a family size in the low hundreds. QTL effects are estimated on each locus separately, then interactions of the QTLs are evaluated. KNAPP *et al.* (1992) showed that sometimes this approach can be misleading, compared with the ideal situation where effects are estimated simultaneously. The ideal situation for estimating QTL effects requires that each QTL genotype be represented by more than one individual. For small family sizes, some multilocus QTL genotypes probably are not represented by any individuals. KNAPP and BRIDGES (1991) pointed out that unbalance could reduce power to detect QTLs. Family size is an important consideration as well for MAS (GIMBELFARB & LANDE 1994). A very large number of progeny would be needed just to be certain that at least one of the best multilocus genotypes was among the progeny. For example, an F_2 family size of n = 191 is needed to be certain (Prob > 0.95) that at least 1 individual will be available that is homozygous for the favorable allele at each of 3 unlinked QTLs (Prob (k > 1) = 1 – Prob (k = 0) = $(1 - 0.25^3)^n$), and for 4 QTL, n = 766. In a cross of 2 outbred individuals, each heterozygous for 3 QTLs, 191 progeny would be needed to be certain (Prob > 0.95) of obtaining at least 1 offspring that carried the favorable allele at each of the unlinked QTLs. If QTLs are linked, even larger n is needed. QTL detection is a complex issue and it is important to validate QTL effects in independent samples of the same or related families where this is possible.

QTLS WITH AVERAGE EFFECTS

While QTLs have been detected in many plants using F_2 or backcross families, detection of QTLs with average effects have been reported almost exclusively for half-sib families of animals. BOVENHUIS and WELLER (1994) presented a likelihood analysis of QTL effects in a population of dairy cattle that accounts for both the effect of the marker and a linked QTL in segregating populations. They also reviewed the development of statistical methods for analysis of animal pedigrees. When they applied these methods to dairy cattle, a QTL for butterfat was found linked to the casein genes. This QTL effect accounted for additive genetic variance equivalent to 3.6% of the phenotypic variation. The heritability of butterfat was 13% and BOVENHUIS and WELLER (1994) suggested that this QTL effect represents approximately 28% of σ^2_A. GEORGES *et al.* (1995) studied the inheritance of milk yields using a granddaughter design where 14 bulls were mapped using microsatellite genotypes from 1500 sons, and yield records were obtained from 150 thousand granddaughters. They reported average effect QTLs that individually accounted for 28 to 179% of the expected segregation variance from the bulls. The effectiveness of marker-assisted selection for average effect QTLs has been investigated for granddaughter designs in dairy cattle (*e.g.*, HOESCHELE & ROMANO 1993, HOESCHELE 1993, HYLAND & QUAAS 1991, KASHI *et al.* 1990).

There has been little work reported to date on QTLs for low heritability growth and volume traits in forest trees using half-sib families. GRATTAPAGLIA (1994) found 3 QTLs for circumference at breast height that explained 11 – 15% of the phenotypic variation in a half-sib family of hybrid *Eucalyptus* (n = 300). The common parent was a *E. grandis* clone and the pollen parents were different *E. urophylla* genotypes. The trees were 6.5 years old. The h^2 of CBH is probably low to intermediate. Preliminary results from our studies of shoot elongation in a half-sib family of 2 year old *Pinus taeda* (n ~ 255) suggested 2 significant effects (P < 0.005) that explained 7 – 8% of the phenotypic variation, or approximately half of the expected segregation variance. The h^2 of shoot elongation in year 2 is approx-

imately 0.5 (BRIDGWATER 1990, MCKEAND & BRIDGWATER 1993). Extra care is needed in field testing to obtain such large heritability estimates for growth and volume traits.

WHAT IS THE NATURE OF GENETIC VARIATION THAT UNDERLIES SELECTIVE BREEDING AND EVOLUTION?

A fundamental question in evolutionary biology is how often adaptation involves a major gene (ORR & COYNE 1992)? Stated another way, can a small number of genes be responsible for the majority of the change in fitness during evolutionary change? ORR and COYNE (1992) concluded that there is weak evidence for the widely held view that adaptation almost always involves many genes of small effect. Several studies have demonstrated cases where adaptation has resulted from a small number of major genes. Genomic mapping studies are needed to address the this fundamental issue for cases where a simply inherited, visually distinguishable polymorphism is not involved. QTLs for growth and volume traits could be manifestations of different adaptive mechanisms in trees. Tree growth and volume are complex integrative traits that are affected by many different physiological processes and environmental factors. A better understanding of the genetic control of these traits could be helpful in resolving the genetic basis for adaptation as well as making gains through artifical selection in breeding programs. The genetic variation that is important for both selective breeding and for evolution is σ^2_A. In conifers, average effects provide a valuable approach to testing ideas about the genetic variation underlying evolutionary change.

The identity of DNA sequences that are responsible for QTL effects are largely unknown. DOEBLEY (1993) speculated that the genes likely to have large effects on the phenotype are the ones that sense that environment and redirect plant growth and development to better meet current environmental circumstances. QTL studies in crop plants have found genotype × environment interaction (*i.e.*, QTL expressed in one environment but not another) but a large portion of QTL effects are stable across environments (TANKSLEY 1993). G × E has been viewed as a problem from the perspective of breeding, where stability of effects is desirable. However, if DOEBLEY (1993) is right, then QTLs could be part of a mechanism that allows trees to physiologically adjust to year-to-year climatic extremes that occur during their long lifespans. Longevity poses another problem interpreting QTLs for forest trees. The low juvenile:mature correlation that delays phenotypic selection until trees are several years old could be caused by the expression of different genes at different stages of development. QTLs detected using the final phenotypic values could be the ones most consistently important for growth, but knowing how trees get to be tall year by year could be important as well.

CLONAL PROPAGATION AND MARKER ASSISTED BREEDING

Clonal propagation has long been a goal of forest biotechnology (*e.g.*, HAINES & NIKLES 1987, GUPTA *et al.* 1993). The genetic variability of trees, σ^2_G, is large and clonal propagation of the best offspring genotype in an elite full-sib family could provide a large gain in productivity. For many tree species, vegetative propagation becomes progressively more difficult as the plant tissues mature. In principle, somatic embryogenic cultures of individual seeds would be initiated, plantlets generated, and cultures placed in frozen storage for several years while the clonal plantlets were tested to determine which line was best (*e.g.*, GUPTA *et al.* 1993). To be certain (Prob > 0.95) that at least 1 of the lines is from the top 5%, 59 or more individual genotypes should be evaluated (Prob of a line not in top 5% = $(1 - 0.05)^{59} < 0.05$). Whether or not the best clone can be identified depends on the statistical precision of the clonal testing.

Among juvenile individuals in a family, markers could be used to select genotypes for vegetative propagation, that contain favorable genes for disease resistance, for wood quality, or for growth and volume for those elite families where QTLs have already been discovered (Figure 2). With a few exceptions, vegetative propagation systems for forest trees are often genotype-dependent and currently are unable to provide the large numbers of propagules needed for reforestation with a small number of the most highly productive genotypes (HAINES 1994). A clonal multiplication factor $c > 10^5$ would be needed, compared with perhaps $c > 10^2$ or 10^3 now for most conifers. Until the clonal multiplication factor is high enough, the selection of specific genotypes for clonal propagation will be subordinate to production of large numbers of propagules from elite full-sib families. Genetic gains will be limited to the means of the elite families. For an intermediate multiplication factor, markers provide a way to capture greater gains than the full-sib family means. Vegetative propagation in full-sib families also captures non additive genetic variation. The systems of breeding required to develop non additive genetic variation in trees for exploitation through clonal propagation are beyond the scope of this review. Immediate gains can be achieved by clonal propagation and selection of juveniles based on markers, but longer-term breeding will likely continue to be based on additive genetic variation.

DISCUSSION

A major emphasis of current work in crop plants using molecular markers is the identification of the gene sequences that underlie phenotypic differences. Clearly, forest trees are difficult subjects for genetic investigations and gene identification is more easily carried out in plants such as *Arabidopsis*. However, the potential advantages of MAS over conventional breeding methods in forestry are large compared with crop plants. Complex trait dissection provides a new capability for genetic analysis within families. In crop plants, recombinant inbred lines provide the basis for within family genetic analysis. Except for clonal replication studies, within family genetic analysis in forest trees was seldom explored because multi generation controlled inbreeding studies are not feasible.

LANDE and THOMPSON (1990) argued that MAS could increase the efficiency of within family phenotypic selection for low heritability traits. KNAPP (1994) reviewed the application of selection indices to MAS in plant breeding. He concluded that phenotypic and-marker information are difficult to incorporate in a selection index for most crops because direct estimates of the within family heritability and the proportion of the additive genetic variation explained by markers within a family are needed to obtain the relative weights for the two kinds of information. These parameters are estimable only from recombinant inbred lines or doubled haploids. Thus, KNAPP (1994) compared the efficiency of marker-only selection relative to phenotypic selection, and showed that MAS is more efficient when the proportion of additive genetic variation explained by markers is > 1/2 and heritability is < 1/2. While these parameters are not estimable within families of forest trees, it is reasonable to expect that values of these parameters could be favorable for MAS in some families.

MAS for low heritability traits such as volume and height growth could be valuable in tree breeding if families for QTL mapping could be identified from breeding records. Detecting average effects segregating in a half-sib family could be difficult because the expected segregation variance transmitted by the common parent in a half-sib family is small. However, the magnitude of individual QTL effects segregating in some selected families could be large even when the heritability of the trait is low, as was found in half-sib families of dairy cattle (GEORGES *et al.* 1995). Careful-analysis of existing breeding records in animals has led to the identification of some pedigrees where major gene effects are segregating (*e.g.*, PIRCHNER 1988). Segregation of major genes in populations can sometimes be inferred from biometrical data alone (*e.g.*, CONNER 1993, LE ROY & ELSEN 1992).

How could MAS be used in tree breeding? Breeding value (*i.e.*, average effect) is important in forest tree breeding because genetic gains from population breeding programs are now captured as seeds produced in open-pollinated seed orchards. Complex trait dissection could be carried out for parents with high breeding values (*i.e.*, highly selected individuals) or in pedigrees where biometrical analysis suggests segregating major gene effects. MAS increases the precision of selection for these genetic effects within full-sib families. The selected progeny could be mated to provide a breeding subgroup where MAS could be effective. QTLs could be fixed in a few generations of breeding. Trait dissection studies using half-sib families could also enable retrospective studies using mature trees in existing genetic tests. Large half-sib families could be assembled from diallele tests where a set of full-sib families share a common parent. The number of full-sib families used to estimate breeding value (GCA) in conventional genetic tests assuming polygenic inheritance is generally small (4 – 6). A lower number of full sib families would be desirable for estimation of average effect of QTLs because the sampling variance of gene frequencies is large. However, the existing field tests could be especially useful for validation of QTL effects discovered in younger plantings.

CONCLUSIONS

The major factors limiting the application of MAS in tree breeding are the cost of genotyping, the large family sizes needed for QTL detection and MAS, and a lack of knowledge of the magnitude of QTL effects and their biological significance for tree growth and development. Technological advances are driving the cost of genotyping lower, and it is not yet clear what kind of molecular marker will be the most economical in the future. To integrate MAS with conventional breeding programs would require an expansion of current capabilities for breeding and genetic field tests because of the large family sizes required for marker:trait association. Special sublines could be set up for families that have evidence of major gene effects. The last limitation is the most important. An understanding of the genetic architecture of height and volume traits could help to better predict gains from both MAS and conventional breeding schemes. Whether or not molecular markers can increase the efficiency of selection for the low heritability traits remains to be determined. However, research on these traits will result in better understanding of the genetic and evolutionary processes that shape the biology of forest trees.

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REFERENCES

BOVENHUIS, H. & WELLER, J. I. 1994: Mapping and analysis of dairy cattle quantitative trait loci by maximum likelihood methodology using milk protein genes as genetic markers. *Genetics* **137** :267–280.

BRADSHAW, H., JR. & FOSTER G. S. 1992: Marker-aided selection and propagation systems in trees: advantages of cloning for studying quantitative inheritance. *Canadian Journal of Forest Research* **22**:1044–1049.

BRADSHAW, H. D., JR. & STETTLER, R. F. 1995: Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form and phenology traits in a forest tree. *Genetics* **139**: 963–973.

BRIDGWATER, F. E. 1990: Shoot elongation patterns of loblolly pine families selected for contrasting growth potential. *For. Sci.* **36**:641–656.

VAN BUIJTENEN, J. P. & BURDON, R. D., 1990: Expected efficiencies of mating designs for advanced-generation selection. *Canadian Journal of Forest Research* **20**:-1648–1663.

CANTET, R. & SMITH, C. 1991: Reduced animal model for marker assisted selection using best linear unbiased prediction. *Genetics, Selection, Evolution* **23**:221–233.

COMSTOCK, R. E. & ROBINSON, H. F. 1948: The components of genetic variance in populations. *Biometrics* **4**:254–266.

CONNER, J. 1993: Tests for major genes affecting quantitative traits in wild radish, *Raphanus raphanistrum*. *Genetica* **90**:41–45.

CORNELIUS, J. 1994: Heritabilities and additive genetic coefficients of variation in forest trees. *Canadian Journal of Forest Research* **24**:372–379.

COTTERILL, P. P. 1986: Genetic gains expected from alternative breeding strategies including simple low cost options. *Silvae Genetica* **35**:212–223.

DEKKERS, J. & DENTINE, M. R. 1991: Quantitative genetic variance associated with chromosomal markers in segregating populations. *Theoretical and Applied Genetics* **81**:212–220.

DENTINE, M. R. & COWAN, C. M. 1990: An analytical model for the estimation of chromosome substitution effects in the offspring of individuals heterozygous at a segregating marker locus. *Theoretical and Applied Genetics* **79**:-775–780.

DEVEY, M. E., FIDDLER, T. A., LIU, B. H., KNAPP, S. J. & NEALE, D. B. 1994: An RFLP linkage map for loblolly pine based on a three-generation outbred pedigree. *Theoretical and Applied Genetics* **88**:273–278.

DEVICENTE, M. C. & TANKSLEY, S. D. 1993: QTL analysis of transgressive segregation in an interspecific tomato cross. *Genetics* **134**:585–596.

DOEBLEY, J. 1993: Genetics, development and plant evolution. *Current Opinion on Genetic Developemnt* **3**:865–872.

DOEBLEY, J. & STEC, A. 1993: Inheritance of the morphological differences between maize and teosinte: comparison of results for two F_2 populations. *Genetics* **134**:559–570.

DOEBLEY, J. & STEC, A. 1994: Teosinte branched 1 and the origin of maize. *Maize Genetics Cooperation Newsletter* **68**:88.

FALCONER, D. S. 1989: Introduction to Quantitative Genetics, 3rd ed. Longman Scientific & Technical, Essex, England. 438 pp.

FERNANDO, R. L. & GROSSMAN, M. 1989: Marker assisted selection using best linear unbiased prediction. *Genetics, Selection, Evolution* **21**:467–477.

GAILLARD, C. & SMITH, C. 1992: Proportion of paternal marker gametes identified in progeny. *Journal of Animal Breeding and Genetics* **109**:337–346.

GEORGES, M., NIELSEN, D., MACKINNON, M., MISHRA, A., OKIMOTO, R., PASQUINO, A. T., SARGEANT, L. S., SORENSEN, A., STEELE, M. R., ZHAO, X., WOMACK, J. E. & HOESCHELE, I. 1995: Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* **139**:907–920.

GIMELFARB, A. & LANDE, R. 1994: Simulation of marker assisted selection in hybrid populations. *Genetic Research* **63**:39–47.

GODDARD, M. E. 1992: A mixed model for analyses of data on multiple genetic markers. *Theoretical and Applied Genetics* **83**:6–7.

GRATTAPAGLIA, D. 1994: Genetic mapping of quantitatively inherited economically important traits in *Eucalyptus*. Ph.D. Thesis, North Carolina State University, Raleigh

GROOVER, A., DEVY, M., FIDDLER, T., LEE, J., MEGRAW, T., MITCHELL-OLDS, T., SHERMAN, B., VUJCIC, C., WILLIAMS, C. & NEALE, D. 1994: Identification of quantitative trait loci influencing wood specific gravity in an outbred pedigree of loblolly pine. *Genetics* **138**:1293–1300.

GUPTA, P. K., PULLMAN, G., TIMMIS, R., KREITINGER, M., CARLSON, W. C., GROB, J. & WELTY, E. 1993: Forestry in the 21st century: the biotechnology of somatic embryogenesis. *Bio/Technology* **11**: 454–459.

HAINES, R. J. & NIKLES, D. G. 1987: Mass propagation of superior families and individual genotypes of some conifers in Queensland. *Australian Forestry* **50**:106–111.

HAINES, R. 1994: Biotechnology in Forest Tree Improvement. FAO, Rome.

HALEY, C. S. 1991: Use of DNA fingerprints for the detection of major genes for quantitative traits in domestic species. *Animal Genetics* **22**:259–277.

HALEY, C. S., KNOTT, S. A. & ELSEN, J. 1994: Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136**:1195–1207.

HAYES, P. M., LIU, B. H., KNAPP, S. J., CHEN, F., JONES, B., BLAKE T. & FRANCKOWIAK, J. 1993: Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. *Theoretical and Applied Genetics* **87**:392–401.

HOESCHELE, I. 1993: Elimination of quantitative trait loci equations in an animal model incorporating genetic marker data. *Journal of Dairy Science* **76**:1693–1713.

HOESCHELE, I. & ROMANO, E. O. 1993: On the use of marker information from granddaughter designs. *Journal of Animal Breeding and Genetics* **110**:429–449.

HYLAND, F. C. & QUAAS, R. L. 1991: Benefits from marker-assisted selection under varying assumptions. *Journal of Dairy Science* **74**:282.

KASHI, Y., HALLERMAN, E. & SOLLER, M. 1990: Marker-assisted selection of candidate bulls for progeny testing programmes. *Animal Production* **51**:63–74.

KNAPP, S. J. & BRIDGES, W. C. 1990: Using molecular markers to estimate quantitative trait locus parameters: power and genetic variances for unreplicated and replicated progeny. *Genetics* **126**:769–777.

KNAPP, S. J., BRIDGES, W. C. & LIU, B. H. 1992: Mapping quantitative trait loci using nonsimultaneous and simultaneous estimators and hypothesis tests. *In:* Plant genomes: methods for genetic and physical mapping. Beckman, JS ed. Kluwer Academic Publishers, Dordrecht, Netherlands p. 209–237.

KNOTT, S. A. & HALEY, C. S. 1992: Aspects of maximum likelihood methods for the mapping of quantitative trait loci in line crosses. *Genetical Research* **60**:139–151.

KNOTT, S. A. & HALEY, C. S. 1992: Maximum likelihood mapping of quantitative trait loci using full–sib families. *Genetics* **132**:1211–1222.

LANDE, R. & THOMPSON, R. 1990: Efficiency of marker--assisted selection in the improvement of quantitative traits. *Genetics* **124**:743–756.

LANDER, E. S. & SCHORK, N. J. 1994: Genetic dissection of complex traits. *Science* **265**:2037–2048.

LE ROY, P. & ELSEN, J. M. 1992: Simple test statistics for major gene detection: a numerical comparison. *Theoretical and Applied Genetics* **83**:635–644.

LOWE, W. J. & VAN BUIJTENEN, J. P. 1986: The development of a sublining system in an operational tree improvement program. *In:* Proc. IUFRO Conf. Joint Meeting of Working parties on Breeding Theory, Progeny and seed orchards. Williamsburg, VA. p. 281–317.

MCKEAND, S. E. & BRIDGWATER, F. E. 1992: Third-generation breeding strategy for the North Carolina State University – Industry Cooperative Tree Improvement Program. *In:* Proc. IUFRO Conf. S2.02–08, Breeding Tropical Trees. Solving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species. Oct. 8–18, 1992. Cartagena and Cali, Colombia.

MCKEAND, S. E. & BRIDGWATER, F. E. 1993: Provenance and family variation for juvenile growth characterisitics of *Pinus taeda* L. and the impact on early selection for growth. *Studia Forestalia Suecica* **191**:5–10.

MEUWISSEN, T. H. & VAN-ARENDONK, J. A. 1992: Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. *Journal of Dairy Science* **75**:1651–1659.

ORR, H. A. & COYNE, J. A. 1992: The genetics of adaptation: a reassessment. *American Naturalist* **140**:725–742.

MORENO-GONZALEZ, J. 1993: Efficiency of generations for estimating marker-associated QTL effects by multiple regression. *Genetics* **135**:223–31.

NANCE, W. L., TUSKAN, G. A., NELSON, C. D. & DOUDRICK, R. L. 1991: Potential applications of molecular markers for genetic analysis of host-pathogen systems in forest trees. *Canadian Journal of Forest Research* **22**:1036--1043.

NELSON, C. D., NANCE, W. L. & DOUDRICK, R. L. 1993: A partial genetic linkage map of slash pine *Pinus elliottii* Engelm. var. *elliottii:* based on random amplified polymorphic DNAs. *Theoretical and Applied Genetics* **87**:145–151.

PATERSON, A. H., LANDER, E. S., HEWITT, J. D., PETERSON, S.LINCOLN, S. E. & TANKSLEY, S .D. 1988: Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature,* UK **335**:721–726.

PATERSON, A. H., DAMON, S., HEWITT, J. D., ZAMIR, D., RABINOWITCH, H. D., LINCOLN, S. E & LANDER, E. S. 1991: Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics* **127**:181–197.

PIRCHNER, F. 1988: Finding genes affecting quantitative traits in domestic animals. *In:* Weir BS, Eisen EJ, Goodman MM, Namkoong G eds. Proc 2nd International Conference on Quantitative Genetics. Sinauer Associates Inc., Sunderland Massachusetts, USA. p 243–249.

SHAW, D. V. & HOOD, J. V. 1985: Maximizing gain per effort by using clonal replicates in genetic tests. *Theoretical and Applied Genetics* **71**:392–399.

STRAUSS, S. H., LANDE, R. & NAMKOONG, G. 1992: Limitations of molecular-marker-aided selection in forest tree breeding. *Canadian Journal of Forest Research* **22**:1050--1061.

STUBER, C. W., LINCOLN, S. E., WOLFF, D. W., HELENTJARIS, T., LANDER, E. S. 1992: Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* **132**:-823–839.

TANKSLEY, S. D. 1993: Mapping polygenes. *Ann Rev Genet* **27**:205–233.

WELLER, J. I., KASHI, Y. & SOLLER, M. 1990: Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. *Journal of Dairy Science* **73**:2525–2537.

WHITE, T. L., HODGE, G. R. & POWELL, G. L. 1993: An advanced-generation tree improvement plan for slash pine in the southeastern United States. *Silvae Genetica* **42**:359–371.

WILCOX, P. L. 1995: Genetic dissection of fusiform rust disease resistance in loblolly pine. Ph.D. Thesis, North Carolina State University, Raleigh

WILLIAMS, C. G. 1987: The influence of shoot ontogeny on juvenile-mature correlations in loblolly pine. *Forest Science* **33**:411–422.

WILLIAMS, C. G. & NEALE, D. B. 1992: Conifer wood quality and marker-aided selection: a case study. Marker aided selection: a tool for the improvement of forest tree species. *Canadian Journal of Forest Research* **22**:-1009–1017.

WRIGHT, J. W. 1976: Introduction to Forest Genetics. Academic Press, NY

ZOBEL, B. Z. & TALBERT, J. T. 1984: Applied Tree Improvement. Wiley and Sons, NY.