FUSIFORM RUST - A MODEL FOR MARKER ASSISTED SELECTION IN LOBLOLLY PINE?

P.L. Wilcox[1,2], H.V. Amerson[2], D. O'Malley[2], S. Carson[1], M.J. Carson[1], G. Kuhlman[3], R.R. Sederoff[2]

[1] Forest Research Institute Ltd., Private Bag 3020, Rotorua, New Zealand.

[2] Forest Biotechnology, Dept. of Forestry, North Carolina State University, Raleigh, NC 27695, U.S.A.

[3] U.S.D.A. Forest Service Laboratories, Athens GA., U.S.A.

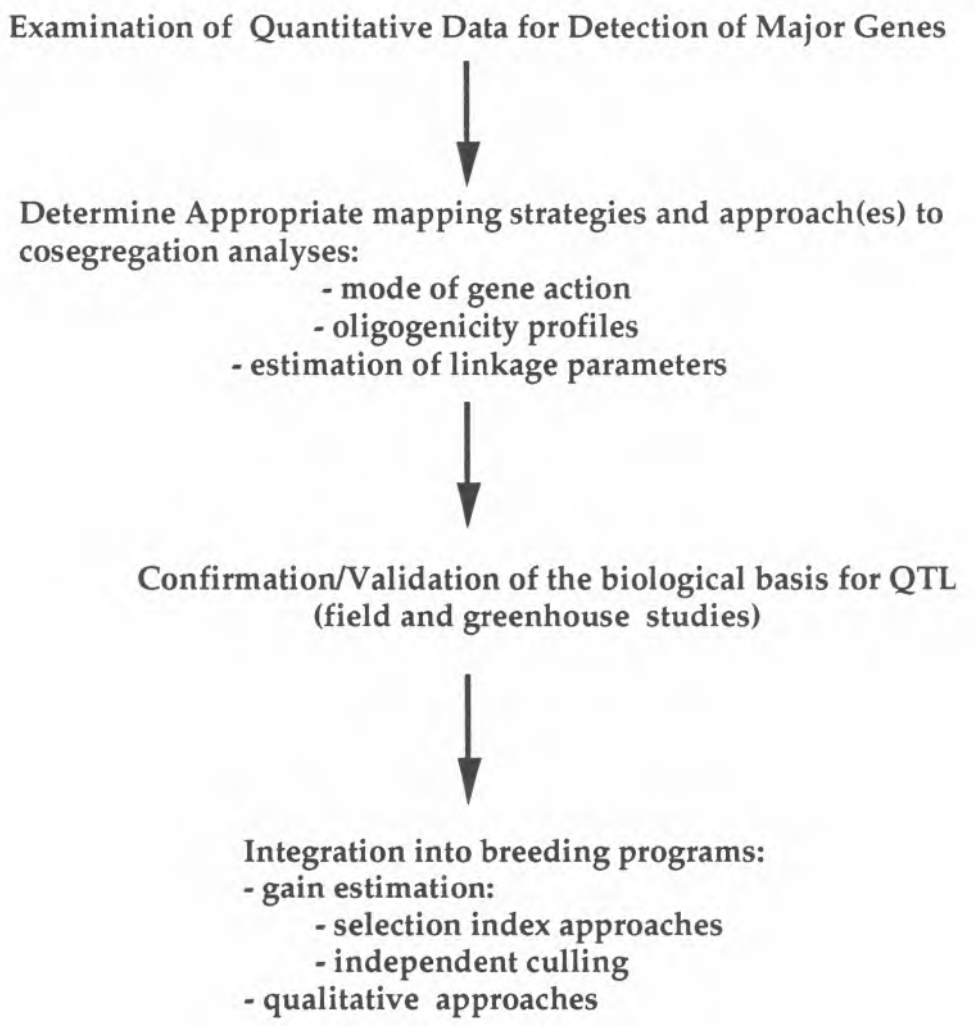
Abstract. Recent advances in DNA marker technology have enabled forest geneticists to better dissect the genetic bases for complex traits such as host pathogen interactions. Information from such experiments can be directly integrated into breeding programs, with the potential for significantly advancing the rate of gain in resistance breeding. Here we describe the steps involved in marker assisted selection, including hypothesis development regarding major gene presence and mode of action, mapping resistance loci with DNA markers via cosegregation analysis, and the subsequent use of this information for breeding purposes. This approach effectively integrates some of the recently developed tools of molecular biology with the more traditional quantitative genetics to solve problems in tree breeding that have not previously been considered tractable.

INTRODUCTION

The advent of more advanced DNA marker technology has led to widespread experimentation focused on dissecting the genetic basis of continuous traits in many plant and animal species. The seemingly common occurrence of a few loci of disproportionately large contribution to genetic variance (referred to hereon as 'major genes') has led to reconsideration of the use of such discrete markers for selection of continuously distributed traits in breeding programs. There are four stages involved in applying DNA markers to tree breeding (Figure 1): i) *identification* of traits and individuals that have major genes contributing to genetic variance; ii) finding associations between markers and major gene loci via *cosegregation analysis* of segregating populations; iii) biological *validation of the statistical associations* between markers and trait loci; and iv) *implementation* of this

information into existing breeding programs. In this paper we briefly outline each of these stages with particular reference to rust resistance breeding in loblolly pine *(Pinus taeda L.)*.

Figure 1. Stages Involved in Marker Assisted Selection



Marker assisted selection (MAS) in forest tree breeding is potentially useful for a variety of reasons, including i) the potential for early selection based upon marker genotype rather than waiting up to many years before obtaining breeding values from the phenotype; and ii) the high levels of heterozygosity in many tree species as well as the relatively few generations of breeding means the likelihood of detecting meaningful associations is considerable (if trees do indeed have major genes).

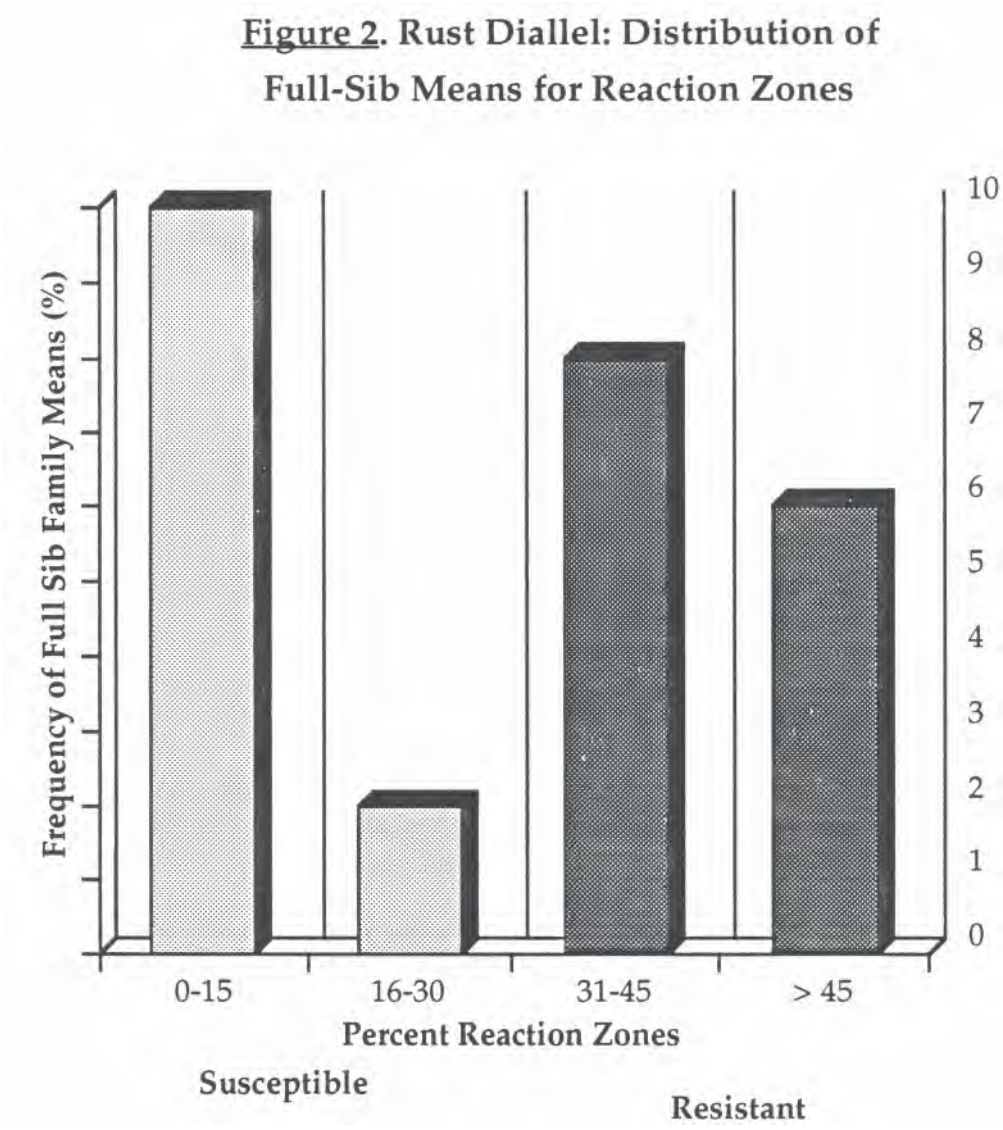
Because of the high cost and relatively low throughput of the technology, experiments to detect marker-trait associations have to be carefully planned, therefore requiring the correct choice of trait, marker technique, and mapping

population(s). To date, most experiments involving DNA markers for dissection of quantitative traits are in the data collection phase. In choosing the appropriate trait, forest geneticists have considered high heritability an indicator of oligogenicity (few genes of large effect) - wood density is an example (Williams and Neale 1992). An alternative trait is disease resistance, not because of heritability, but rather the likelihood of major genes due to the interaction of multiple, but discrete, pathogenic and resistance mechanisms (Strauss et. al. 1992) - despite the lack of evidence for single gene defense mechanisms in the vast majority of forest tree pathosystems.

Resistance in loblolly pine to the causal agent of fusiform rust, *Cronartium quercuum* f. sp. *fusiforme (Cqf)*, is a potential candidate for the presence of major genes, and ultimately the integration of marker information into existing breeding programs, not only because of the potential for major genes but also because of the development of early screening procedures for resistance. Such procedures can potentially be used for detecting candidate loci associated with field resistance. This in turn may ultimately reduce screening cost if markers are used as a substitute or complement to further greenhouse screening, as the costs of greenhouse screening may ultimately be greater than genotyping using DNA markers. This may also apply to specialist breeding programs such as seedling seed orchards.

THE FIRST STAGE: LOOKING FOR MAJOR GENES IN QUANTITATIVE DATA

The first stage in MAS is the examination of quantitative data for evidence of major gene segregation (Figure 1). Examination of data from partial diallels which have been screened for resistance has shown strong evidence for segregation of major genes in both slash pine *(Pinus elliotii)* (Kinloch and Walkinshaw 1989) and loblolly pine (M. Carson 1983 unpubl., Wilcox unpubl. data). In the studies involving loblolly pine, M.Carson found bimodal distributions of half and full sib family means for symptom types such as rough galls and reaction zones (Figure 2). Subsequent examination of the data showed an unexpected pattern when the more resistant genotypes were crossed with the less resistant clones (where resistance was based on half sib family means): full sib family percentages showing the resistant symptom type were equal to that of full sib families of two resistant clones, implying gene action is dominant, and few loci are involved in altering the threshold of resistance in the full sib families. According to expectations of a polygenic basis for resistance where gene action is additive (Carson and Carson 1989), one would not expect to see either bimodal distributions of full and half sib family means nor percentage infection of the high X low resistance families similar to that of high X high resistance families. Subsequent examination of four more partial diallels involving five or six clones suggests the pattern of dominant gene action and bimodal distributions of family means predominates: of the four diallels examined, eight symptom type/diallel



combinations have been examined to date: six combinations exhibit the aforementioned pattern with the other two combinations showing little or no segregation whatsoever. Two tester mating designs involving a larger number of clones have also been examined: one showing a similar pattern to the diallels, whereas the other showed a less clear pattern. Whilst statistical analysis of this data is required before we can be more confident that major genes for resistance are present, patterns evident in the diallels are strongly suggestive of few segregating major genes. We have therefore hypothesized that greenhouse resistance is oligogenic, where gene action is dominant rather than additive.

In all of the above experiments with loblolly pine, the pathogen source used was genetically heterogeneous, being derived from gall mixtures.

Although this data has assisted us in deciding upon resistance as a potentially oligogenic trait, it is by no means mandatory to have such data before choosing any given trait. Researchers working with other plant species are commonly finding major genes for a wide variety of traits including growth and yield characteristics (e.g., Stuber et. al. 1992) which were at one stage thought of as having too many potential component loci to be oligogenic. Whether or not this is the case for forest tree species is yet to be decided.

THE SECOND STAGE: FINDING ASSOCIATIONS BETWEEN TRAITS AND MARKERS

Mapping trait loci in highly heterozygous organisms of long generation length is by no means an easy task: standard approaches in annual crop species such as backcrosses and intercrosses utilizing isogenic lines are not usually possible in forest tree species. There are however a variety of approaches available to forest geneticists to map quantitative traits depending upon factors such as the nature of the mapping population (full or half sib), mode of gene action and marker technology used. Quantitative trait mapping strategies include the three generation cross approach (e.g. Williams and Neale 1992), within half-sib family mapping (O'Malley et. al. 1992, Wilcox et. al. 1992, Liu et. al. 1993), pseudo-testcross (Grattapaglia and Sederoff 1993) and utilization of interspecific hybrid crosses (e.g., Bradshaw unpubl.). Pseudo-intercross approaches have also been used to map traits in *Prunus persica* (Chapparro et. al., in press). In addition, for coniferous species, the above mapping strategies may be used in combination with haploid and/or diploid tissue for genotyping with DNA markers. In addition, for disease resistance mapping, it is necessary to control or eliminate heterogeneity in pathogen virulence as well as environmental variance.

Because forest pathosystems typically lack well defined (iso- or near-isogenic) lines of both host and pathogen, a combinatorial experimental design has been used to identify informative heterogeneous families. A putative heterozygous *(Rr)* mother tree was chosen (clone 10-5 - a resistant clone) , along with four of its half-sib progeny, where all five clones were crossed to a highly susceptible pollen parent (rr). The rationale for using the half sib progeny as parents is that the desired segregation may not occur if clone 10-5 is homozygous at the major gene locus for resistance. All of the resulting full-sib families have been challenged with inoculum from six single aeciospore lines (SALs) which vary in their virulence to the progeny of the mother clone. The desired SAL genotype is effectively homozygous for avirulence, as this will be the most informative given the putative *Rr* X *rr* host parental cross. Resistance has been scored for several greenhouse symptom types in the progeny of each family X SAL combination at three, six and nine months.

RAPD markers segregating in the haploid megagametophytes of all progeny are being used to construct RAPD linkage maps of the parent clone, 10-5, as well

as the half-sib progeny of 10-5. Maps will be used to find associations between markers and resistance that is maternally inherited (as we are using a putative susceptible homozygote pollen donor). Two SALs have been targetted: three and six month data indicate SALs NC 2-36 (50% expression of percentage galled symptom type at six months) and 2-40 (80% galled, therefore segregation of gall phenotypes) will be most informative for a range of symptom types. Maps for families A (full sib progeny of 10-5 X susceptible parent) and B are currently being constructed using haploid megagametophytes from the NC 2-36 SAL combination. These maps should be representative of each family regardless of pathogen inocula, hence detection of marker-trait associations for SAL NC 2-40 will entail genotyping the megagametophytes from this combination for markers approximately every 10 cM, rather than repeating the entire mapping exercise.

STEP 3: BIOLOGICAL VALIDATION OF ASSOCIATIONS BETWEEN MARKERS AND MAJOR GENE LOCI

For markers to be useful in breeding programs, validation of (statistical) marker - trait associations will be necessary. Validation may involve replicating experiments with different progeny from the same cross(es), testing in different environments, and in the case of long lived perennial species, at different ages. In our experiments, two validation steps are necessary. Firstly, validation of 'greenhouse' rust resistance should be possible by using related families, where the associations found in one family between a given chromosomal region and resistance to a given SAL (or set of SALs) should also hold for another family. However, even if resistance maps to different chromosomal regions for different families (locus heterogeneity), this does not necessarily invalidate associations. The second validation step will be to test associations derived from greenhouse screening in field experiments. Because the linkage maps we produce will be specific to individual clones, markers associated with resistance in a given family may not be linked to resistance in non-related families, therefore we are restricted to families that have been screened in the greenhouse *and* planted in field tests. Marker-resitance associations may be different for different families, thus may be of limited use only. However, use of clone 10-5 seedlots has been widespread in both greenhouse and field experiments, offering opportunity for validation in field experiments.

STAGE FOUR. INTEGRATION OF MARKER-TRAIT ASSOCIATION INTO EXISTING BREEDING PROGRAMS.

The ultimate use of marker-trait associations for breeders will be for selection of genotypes with superior breeding value. There are a variety of ways in which markers can be used for improvement of quantitative traits. In the case of rust resistance, marker-trait associations can be utilized in either a quantitative or qualitative manner. Some examples of both are outlined in this section.

With continuously distributed traits, quantitative approaches to gain estimation appear to be sufficiently adaptable to allow the evaluation of marker-trait information in breeding programs. For example, Lande and Thompson (1990) outlined the application of index selection theory to combined selection based on marker and phenotype. This approach has been used by Zhang and Smith (1992) to show combined marker and phenotypic selection will be more efficient than either alone in animal breeding programs. Simulations by Strauss et. al. (1992) for forest tree breeding suggested combined selection will be more efficient when within family heritabilities are low, and between family heritabilities are low to moderate. Use of index selection theory for threshold traits such as rust resistance has been developed (Danell and Ronningen 1981), as has theory for combining threshold and continuously distributed traits (Cue and Hayes 1985). It should theoretically be possible to evaluate gain and estimate index weights for both multitrait and family indices. A further approach may be two-stage selection, where only genotypes with (greenhouse screened) resistance loci are planted out for further resistance screening in genetic tests, effectively increasing selection intensity. A further use of marker information may be to select for resistance in situations where there is no variance in the trait: selection of individuals within families that have been screened for resistance in the greenhouse and also planted out in field tests is possible. In this case, selection for resistance can be made on individuals planted in field tests even if no resistance variation exists, by using marker genotype, whereas other traits may be selected based upon field information.

In contrast with continuously distributed traits, markers for resistance may also be used in a qualitative manner to obtain gain via the alignment of multiple defense mechanisms in a predetermined fashion. This approach utilizes the potential for markers in combination with histological and biochemical techniques, to dissect resistance into discrete mechanisms, each of which may be controlled by several loci. With this information, it may be possible to take a multiple population approach to breeding for resistance, where each population has all resistance mechanisms present in each family, but different populations having different loci controlling each mechanism. To offset pathogen evolution, the production populations would therefore be constructed of population mixes, rather than alone. This will approach potentially allows breeders to manipulate mechanisms and loci, whilst maintaining sufficient diversity to reduce risk of catastrophic losses due to pathogen evolution.

CONCLUSIONS

There are four stages involved in the using DNA markers for selection purposes, three of which are considered essential. The first stage is the selection of trait and segregating population for mapping. This stage may turn out to non-essential due to the presence of major genes for the majority of traits as well as

the high levels of heterozygosity at trait loci for forest trees. The next stages involve finding associations between trait and marker loci via cosegregation analyses; followed by biological validation of these statistical associations. The final stage is the implementation of this information in breeding programs, for which there are a variety of potential quantitative approaches. In addition, for more discrete traits (such as disease resistance), qualitative approaches to achieving gain are potentially possible.

Rust resistance has the potential to be a model trait for marker assisted selection, since seedlings resistance can be screened at six to nine months of age, and is potentially more cost effective to screen breeding populations with markers. Furthermore, resistance appears to be oligogenic - at least in greenhouse screening - and offers the potential for a variety of approaches to be employed when using marker information for selection.

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