RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND THEIR POTENTIAL USE IN MARKER ASSISTED SELECTION IN SOUTHERN PINE IMPROVEMENT

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Abstract.--A *new* class of molecular markers based on restriction fragment length polymorphisms (RFLPs) is described. The use of RFLPs in exposing the genetic basis of phenotypic variation is then explored from a tree breeders perspective. It is concluded that RFLP markers developed in conjunction with multi-generation pedigrees and properly designed experiments will eventually make it possible to apply marker assisted selection to southern pine improvement programs.

Keywords: restriction fragment length polymorphisms (RFLPs), linkage, fusiform rust resistance, marker assisted selection, gene mapping.

INTRODUCTION

Genetic markers by definition are monogenically inherited traits with heritability equal to one. Quantitative geneticists, using linkage concepts, have developed a body of theory that attempts to utilize the perfect heritability of genetic markers to increase the efficiency of selection for traits of low heritability (Jayacker 1970, Mather and Jinks 1971, Thoday 1961). This theory, termed marker assisted selection, seems especially appropriate for use in forest tree improvement, since the long generation interval and large size of forest trees cause extensive progeny testing to be very expensive. Many methods and applications of this approach have been described and tested with agronomic crops and farm animal species (Soller 1978, Soller and Beckmann 1983, Soller and Plotkin-Hazan 1977, Stuber et al. 1982, Tanksley et al. 1981, Tanksley and Rick 1980.

Most markers traditionally studied by geneticists have been either morphological or molecular in nature (Tanksley 1983). Forest geneticists have traditionally identified and studied molecular markers -- primarily monoterpene and enzyme variants (Conkle 1981, Squillace et al. 1980). Most applications of these molecular markers in forest trees have been aimed at understanding the genetic structure and mating systems of both natural and artificial populations (El-Kassaby and Ritland 1986, Knowles 1985, Neale and Adams 1985). Although monoterpenes and isoenzymes have proven to be very useful in specific applications, they are not expected to be generally useful in marker assisted selection efforts due mainly to their limited number and lower level of polymorphism (Beckmann and Soller 1983). Limited numbers of markers decrease the probabilities of finding markers closely linked to important loci, and low levels of polymorphism decrease the probability of finding informative pedigrees.

Within the last few years, a new class of molecular markers, restriction fragment length polymorphisms (RFLPs), has been developed which should overcome the limitations of the present marker systems (Botstein et al. 1980). RFLPs are expected and in many cases have been shown to have the following desirable features: (1) almost limitless in number, (2) highly variable or polymorphic, (3) co-dominant expression, and (4) lack of pleiotropic effects on other characters (Beckmann and Soller 1983, Tanksley 1983). RFLPs have recently been discovered in the chloroplast DNA of gymnosperms (Neale et al. 1986, Wagner et al. 1987) and there is hope that RFLPs will soon be available for nuclear and mitochondrial DNA in the southern pines.

The purpose of this paper is twofold: (I) to describe in detail RFLPs and RFLP loci and (2) to begin exploring the use of RFLP loci in forest genetic experiments and tree improvement programs.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

The fact that deoxyribonucleic acid (DNA) is the informational molecule that encodes fundamental life processes was only convincingly demonstrated in 1944 (Avery et al. 1944). Nine years later the precise structure of DNA was discovered (Watson and Crick 1953), and eleven more years passed before the complete genetic code was finally deciphered (Anon. 1966). Despite these remarkable advances, detailed studies of specific regions of the DNA molecule itself proved impossible. Then, in 1970 a new class of enzymes called restriction endonucleases was discovered that allowed sequence-specific assays of the DNA molecule (Smith and Wilcox 1970). With the aid of these enzymes, greatly accelerated progress in many areas of molecular genetics has been made in the last two decades (Watson et al. 1987).

Restriction Enzymes

An understanding of restriction enzymes and their relationship to RFLPs requires some knowledge of molecular genetics, including the structure of DNA and various chemicals and enzymes, and laboratory procedures related to the manipulation of DNA. Watson et al. (1987) provide an excellent overview of the subject at a more advanced level, while Drlica (1984) provides a more general introduction to the subject.

Restriction enzymes are generally extracted from bacterial cells, where they provide a defense mechanism against the functioning of foreign DNA. The restriction enzymes bind only to specific base sequences (usually 4 to 8 bases long) in the foreign DNA and subsequently cut both strands of DNA at the recognition (restriction) site, thus fragmenting the invading DNA and generally destroying its function. The microorganism's DNA is protected from this fragmentation by the chemical modification (methylation) of its own recognition sites, which effectively blocks the action of the enzymes.

Figure 1 illustrates the cleavage of DNA by a particular restriction enzyme, MstII. The MstII restriction enzyme cleaves DNA within the recognition site CCTGAGG between the second and third base from the 5' end, leaving three unpaired nucleotides at the 5' ends. These so-called "sticky ends" allow the insertion of any similarly restricted (MstiII double-stranded DNA

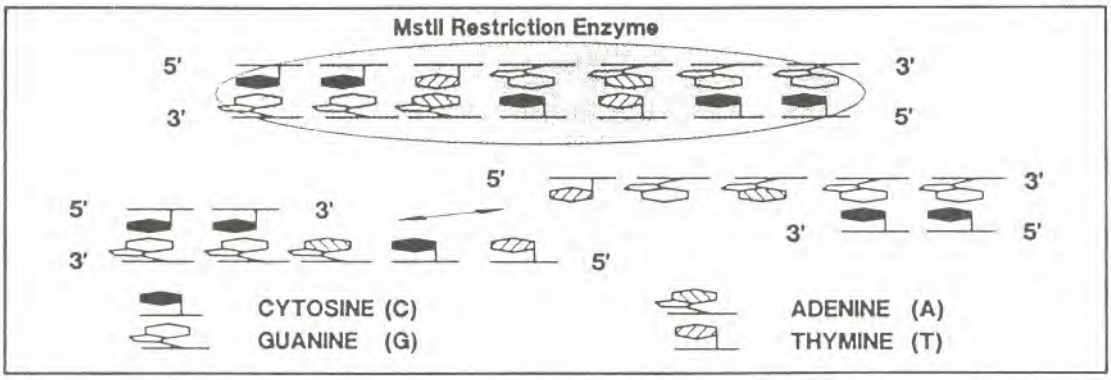

Figure **1. Restriction enzyme MstII** recognition site **and cleavage of** DNA.

fragment into the gap created by the restriction. The insertion of foreign DNA in this way produces a "recombined" or "genetically engineered" DNA molecule. This application of restriction enzymes is one of their primary uses, and without restriction enzymes recombinant DNA technology would not be possible.

However, restriction enzymes are not limited to recombinant DNA work, where the major goal is to manipulate and restructure genomes. Perhaps an even more important and immediate application for these enzymes is to help geneticists expose variation between individuals at the

most fundamental level, the DNA molecule itself (Beckmann and Soller 1986). This level of genetic variation, DNA polymorphism, need not be directly involved in phenotypic variation to be useful to geneticists and breeders. For example, many regions of DNA in eucaryotic genomes are not part of functional genes nor are they involved in the regulation or expression of functional genes (Watson et al. 1987). Yet many of the most productive applications of DNA polymorphisms (including molecular marker systems, genetic fingerprinting, and phylogenetics) are actually enhanced by the absence of selection pressure and/or pleiotropic effects that is characteristic of DNA polymorphisms from non-functional DNA regions.

Restriction Enzyme Analysis of DNA Polymorphisms

The technology and procedures used to expose DNA polymorphisms rely on the unique power of restriction enzymes to fragment DNA in a sequence-specific manner. Restriction enzymes are used to cut the DNA molecule under study into fragments, and the number and lengths of those fragments (the fragment length array) depend on the number and distribution of the particular enzyme's recognition sites. The fundamental idea is that two DNA molecules are considered to be polymorphic with respect to one another if they produce different fragment length arrays when cut with the same enzyme. Such an enzyme is said to have exposed a DNA polymorphism which is observed as a restriction fragment length polymorphism (RFLP).

Two common sources of variation generally account for RFLPs (White and Lalouel 1988): (1) two DNA molecules may differ in the number of restriction sites cut by a particular enzyme, and/or (2) two molecules may differ in the length of DNA sequences separating common restriction sites. The former may arise as a result of point mutations that either destroy a restriction site or create a new restriction site. The latter may arise as a result of variable numbers of repeating sequences (VNR) occurring between restriction sites.

For small haploid genomes (such as DNA from most viruses and some bacteria), the array of DNA fragments produced by a restriction enzyme digest may consist of only a few fragments (perhaps less than 25 or so), and in such cases the fragment array can often be sorted by length and directly visualized using agarose gel electrophoresis (Maniatis et al. 1982). When the gel is stained with a DNA-specific stain such as ethidium bromide and exposed to ultraviolet light, the DNA fluoresces in the visible spectrum. The pattern of electrophoretic bands produced by the digestion of a genome with one or more restriction enzymes is a karyotype (a restriction fragment length karyotype), and a comparison of such karyotypes from different individuals or populations can provide evidence of RFLPs. The procedure is illustrated in figure 2.

For large genomes, such as the nuclear (diploid) genome of humans and the even larger genome of gymnosperms, the individual fragments from a restriction digest cannot be visualized in the gel as separate bands due to the large number of fragments produced and their nearly continuous size variation. When the gel is stained and illuminated, a smear of DNA, rather than separate bands, is produced. Fortunately, a procedure known as Southern blotting (Southern 1975) can be used to expose restriction fragment length variation in DNA smears. The basic idea of this procedure is to allow hybridization (double-strand formation) between a single-stranded, P^{32}-labeled DNA clone (probe) and single-stranded, electrophoresed (target) DNA. The hybridization occurs only between homologous (complementary) base sequences and its result is observable with the aid of radioactivity and X-ray film.

Figure 3 illustrates the Southern blotting procedure which includes the following steps: (1) digest target DNA with a restriction enzyme, (2) size-separate digested DNA in gel electrophoresis, (3) denature (split into single-strands) size-separated DNA fragments, (4) transfer (by capillary action) single-stranded DNA fragments to a rigid support (e.g. nitrocellulose or nylon) membrane, (5) bind DNA fragments to the membrane with ultraviolet light or heat, (6) place membrane into a hybridization solution and add multiple copies of a P^{32}-labeled, single-stranded probe DNA, (7) allow hybridization reaction to occur between probe and target DNA, (8) wash membrane of excess, unbound probe DNA and expose to autoradiographic film, (9) develop film and observe bands. The observed bands reveal the length of the target DNA fragments that were homologous to the probe DNA.

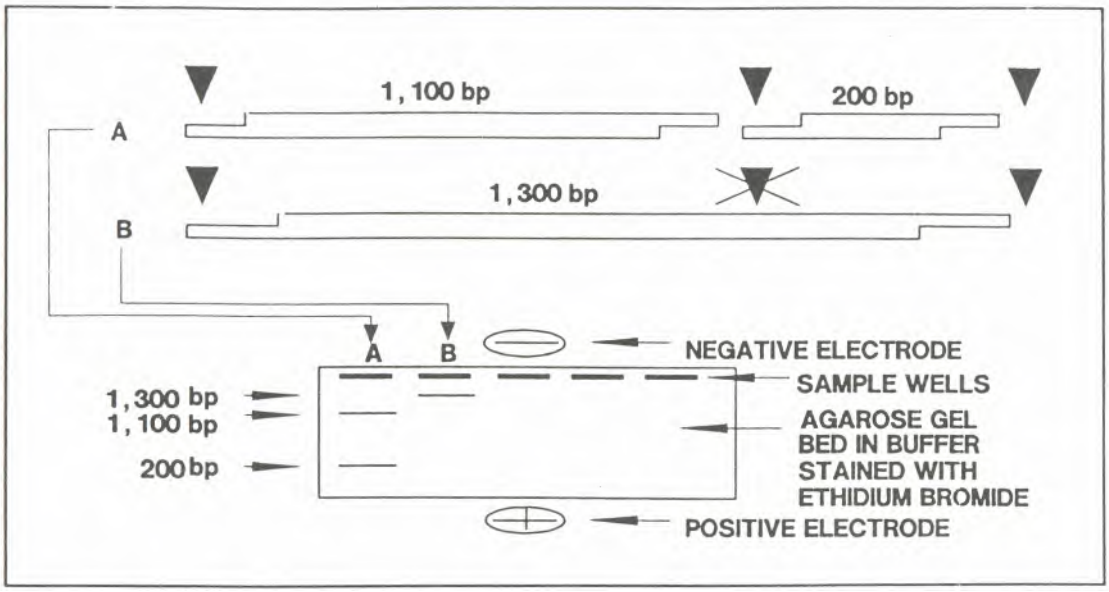

Figure 2. Restriction digest and electrophoretic separation of fragments from two individuals A and **B. B** is missing an MstII restriction site in this 1,300 base pair region.

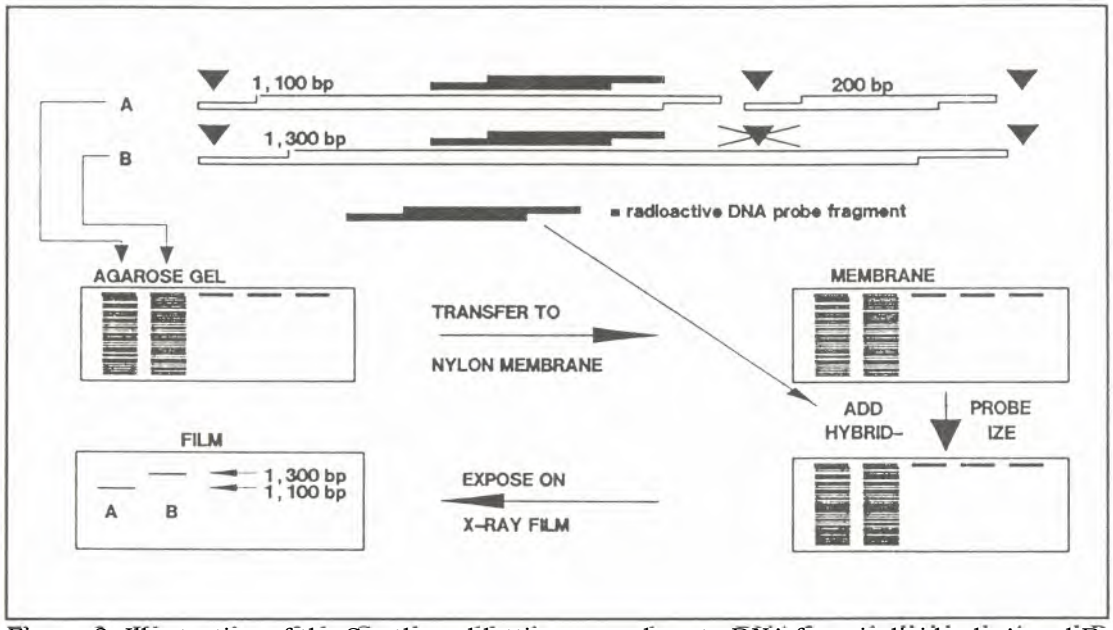

Figure 3. Illustration of the Southern blotting procedure to DNA from individuals A and B in figure 2. **See** text for description of each step.

Probe Libraries

The choice of a probe to use in Southern blotting is, of course, critical to the success of the procedure. To be useful, a probe must be homologous to the target DNA fragment(s) that expose polymorphisms. To assure homology, probes may be generated by randomly fragmenting DNA from the same genome (or a closely related one) under study. Using recombinant DNA technology, the fragments are inserted into a vector (such as a plasmid, virus, or a cosmid)

capable of preserving and reproducing (i.e. cloning) the inserted DNA fragments. Cloned fragments then must be examined to detect and eliminate those with highly repetitive sequences. This kind of probe library is called a unique-sequence random genomic library, and such libraries have proven to be useful in detecting RFLPs in many species (Helentjaris et al. 1986, Helentjaris and Gesteland 1983, White and Lalouel 1988).

Another type of library, called a cDNA library, is constructed by first extracting messenger RNA and then using an enzyme (reverse transcriptase) to produce a complementary DNA (cDNA) copy of each of the various RNA molecules extracted. The assortment of cDNA molecules produced in this way can each be inserted into a vector and handled in the same way that random genomic probes are handled. Because the cDNA probes are homologous to actively transcribed regions of DNA in the genome, they are homologous to functional regions of the genome and are potentially more useful, though more limited in number, than random probes.

A useful probe library (whether unique-sequence random genomic or cDNA) will contain many probes that are homologous to the target genome, but only a small percentage may be useful in exposing restriction fragment length variation. Each probe/enzyme combination has the potential of producing a different banding pattern, and many different probe/enzyme combinations may have to be screened before a suitable combination is discovered which exposes polymorphisms for the DNA samples under study. Fortunately, membranes can be reprobed many times (20 or more) and probes may also be multiplexed (pooled) in one hybridization experiment to allow the convenient screening of many probe/enzyme combinations. Given the growing number of restriction enzymes (>200) commercially available and hundreds of probes that can be generated and maintained in libraries, the possible enzyme/probe combinations that could be screened are enormous. Short of completely sequencing the DNA, no other system can provide such a rich reservoir of potential polymorphic markers.

RFLP Loci and Linkage

For haploid genomes, a given enzyme/probe combination which exposes a single band of equivalent length for each haploid individual analyzed is essentially equivalent to a genetic locus with a single allele. Such loci are not polymorphic in that one cannot distinguish individuals one from the other in the population based on these loci. On the other hand, if at least one sample exhibits a single band of different length than the others, then there is more than one allele at this locus and there is evidence of DNA polymorphisms in the population.

For diploid individuals, a unique-sequence probe that hybridizes to and exposes a single band on each homologous chromosome will produce only one band for homozygous individuals. The position of that band defines the allele carried by that homozygote, and the locus is defined by the particular enzyme/probe combination used. A cross between two homozygotes carrying different alleles for the locus is expected to produce heterozygous offspring with two bands, the position of the bands should correspond to the positions observed in the parents. If the inheritance pattern in the offspring is as expected, then the existence of the RFLP locus is confirmed, otherwise an anomaly discounts the locus. It is thus essential to confirm RFLP loci with inheritance data before accepting such loci as markers.

Upon finding RFLP loci, it is vital to begin arranging these loci into linkage groups. Loci whose alleles freely recombine during meiosis are unlinked and belong to different linkage groups, whereas those that do not freely recombine belong to the same linkage group. A direct and several indirect methods are available for linkage analysis (Ott 1985). The direct method relies on counting recombinants and non-recombinants, taking the proportion of recombinants as theta, the recombination fraction. Theta varies from 0, for complete linkage (i.e. same loci) to .5, for no linkage. In practice the direct method can only be applied under ideal conditions to organisms where inbred lines are available.

The indirect method of likelihood and LOD score appears to be of greater utility in studying linkage in forest tree pedigrees. The likelihood is the probability that the observed data occur under a specified model. In the linkage analysis case, the observations are genotypes, two

alleles present at each locus, and the model consists of only one parameter, theta. The object of the linkage (two point) analysis for a given pedigree and pair of loci is then to determine the likelihood of the observations over the range of possible theta (0 to .5), typically .01, .05, .1, .2, .3, and .4.

To accept a linkage hypothesis, a likelihood or odds ratio of 1000 to 1 is required, where the odds ratio is the probability of the observations at a specified theta (<.5) over their probability at theta =.5 (no linkage). An LOD score, obtained by taking the log (base 10) of the odds ratio, of 3 is equivalent to an odds ratio of 1000 to 1 and "proves" linkage. Alternatively, a linkage hypothesis is rejected when an LOD score of -2 (1 to 100 odds ratio) is reached. LOD scores for pairs of loci are computed for each unrelated pedigree and added to produce composite (current) LOD scores, one composite score for each value of theta. If an LOD score of 3 is reached for any theta, then that value which maximizes the LOD score is taken as the maximum likelihood estimate of theta.

Estimates of theta between all known loci allow the relative arrangement or map of these loci. The extension of the above two point analysis concepts to multi-point analysis greatly increases the precision of the order and theta estimates among all known loci (Lander and Green 1987, Lathrop and Lalouel 1984). The result is a likelihood for each possible or hypothesized order of loci and estimates of theta between each loci in the given order (Lander et al. 1987). Thus, likelihoods of alternative maps may be compared, analogously to comparing likelihoods of alternative theta. A strategy for mapping genetic loci in forest trees can be found in this volume (Neale and Tauer) and strategies proposed for another outbreeding organism (humans) in White et al. (1986) and Lalouel et al. (1986).

USE OF RFLP MARKERS IN FOREST GENETICS

For RFLP marker loci to be useful to tree breeders, they must be linked with phenotypes of economically important traits. Such linked markers can help to position the gene(s) controlling the expression of those traits on the genetic map. In this context it may be useful to consider the RFLP loci as X-variables and important economic traits as Y-variables. Assuming the availability of markers (X-variables), the basic problem for tree breeders reduces to one of generating the phenotypes (Y's) for traits of interest. By generating the required pedigrees and performing experiments that allow the clean expression of individual gene(s) as phenotypic variation among individuals within the pedigrees, breeders provide the critical information needed to utilize markers effectively to improve important traits.

Pedigrees

Pedigrees are important in understanding qualitative as well as quantitative trait variation. Quantitative Tait variation is typically analyzed with methods based on the covariance among family members. The genetic model in these analyses specify that the trait under study is controlled by a large number of independent loci each with an equal and additive effect. It is becoming apparent that more complex models are needed to better fit the genetics of many quantitatively inherited traits (Kempthorne 1988). Pedigree analysis utilizes independent pedigrees to distinguish between monogenic and polygenic traits and in the case of monogenic traits allows estimates of parameters of the genetic model (Elston and Stewart 1971, Lange and Elston 1975). In the case of polygenic traits large computer simulations may be the only means of uncovering the action and interaction of the individual genes. For either case, multi-generation pedigrees with large family sizes will be most efficient for providing the necessary data.

Experiments

Having generated a set of multi-generation pedigrees it becomes necessary to test and score the individuals for one or more traits. RFLP markers seem to offer the most immediate application to the genetic understanding and improvement of simply inherited traits such as disease resistance. An example of such a trait in forest trees is major gene white pine blister rust

(Cronartium ribicola J.C. Fischer ex Rabenh.) resistance (MGR) in sugar pine (Pinus lambertiana Dougl .). A rare, dominant allele at the MGR locus provides blister rust resistance against a wide variety of rust races (Kinioch and Littlefield 1977). In the South, the most important disease of forest trees is fusiform rust (Cronartium quercuum (Beck.) Miyabe ex Shirai f. sp. fusiforme), and individual loci expressing resistance have not been identified. This is probably due to experimental methods, since most inoculation experiments have utilized, by design, heterogeneous rust (multiple gall cultures) and pine (open-pollinated families) sources.

However, Snow et al. (1975) may have found a rare resistant gene in a slash pine (Pinus elliottii Engelm.) tree of south Mississippi origin. Open-pollinated progeny of this tree were not infected (no gall formed) when separately inoculated with two single gall rust cultures also of south Mississippi origin. In the same experiment these single gall cultures were found to infect about 50% of the progeny in other "resistant" open-pollinated families. Griggs and Walkinshaw (1982) verified this result in a five-tree diallel cross involving the "resistant-gene" containing parent. All progeny of this tree, excluding 25% in one reciprocal, were free of infection when inoculated with one of the single gall cultures of Snow et al. Progeny of the other parents were infected to various degree s, 25 to 91 percent, when inoculated with this same inoculum. These cases are unusual, but they serve to illustrate the possibility of exposing single gene expression in progeny of forest trees, N hen spurious genetic variation in both host and pathogen is reduced.

An idealized experi nental set-up for exposing single gene (host-pathogen) resistance responses can be envisioned for fusiform rust in southern pines. In this experiment, tested or presumed resistant and susceptible trees are mated to produce several pedigrees of n full-sibs. Each member of the sibship is then cloned to produce r ramets for each of m pathogen cultures. For each host by pathogen genotypic combination, the r ramets are inoculated with one of the m rust clones. The rust clones may be produced vegetatively or possibly by single urediospore cultures, or better yet, by inbreeding single urediospore cultures to homozygosity. The main features of this set-up are (1) control of spurious genetic variation in the rust by using homogeneous (and if possible homozygous) inoculum, (2) control of environmental variation in the inoculations by using k clonal pine replicates per inoculation, and (3) allowing multiple inoculations per pine genotype by using m*k clonal pine replicates per pine genotype.

Given the data from this experiment (Y's) and RFLP loci data (X's) pedigree analyses could provide insight on the genetic model and a map of the individual resistance loci. With RFLP and resistance loci mapped, simple marker assisted breeding procedures could be employed to increase the efficiency of tree breeding programs. The most likely areas of increased efficiency appear to be early selection and inter-species and inter-population gene introgression (Beckmann and Soller 1986, Tanksley et al. 1981). With a saturated map of RFLP loci, detailed investigations of quantitative trait inheritance become a reality (Lander and Botstein 1989, Paterson et al. 1988).

CONCLUSION

The new DNA technology has allowed geneticists for the first time to directly study variation in the DNA molecule itself, and to translate DNA polymorphisms between individuals into useful molecular marker loci. As these new molecular markers become readily available in forest tree species, as they almost certainly will, forest geneticists should be able to use these molecular markers to great advantage in their breeding and selection efforts. By developing advanced pedigrees and designing and installing the kinds of experiments required to expose gene action in these pedigrees, tree breeders provide critical information that will largely determine whether or not these new markers systems realize their potential for enhancing and accelerating the tree improvement process.

LITERATURE CITED

Anonymous. 1966. The Genetic Code. Cold Spring Harbor Symposia on Quantitative Biology, Vol. 31. Cold Spring Harbor, NY.

Avery, 0.T., C.M. MacLeod and M. McCarty. 1944. Studies on the chemical nature of the substance inducing transformation of Pneumonococcus types. J. Exp. Med. 79:137-158.

Beckmann, J.S. and M. Soller. 1983. Restriction length polymorphisms in genetic improvement: Methodologies, mapping and costs. Theor. Appl. Genet. 67:35-43.

Beckmann, J.S. and M. Soller. 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111-124.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32:314-331.

Conkle, M.T. 1981. Isozyme variation and linkage in six conifer species. p. 11-17. In: M.T. Conkle (tech. ed.), Proc. Symposium on Isozymes of North American Forest Trees and Forest Insects. July 27,1979. Berkeley, CA. USDA, Gen. Tech. Rep. PSW-48.

Drlica, K. 1984. Understanding DNA and gene cloning. John Wiley & Sons, New York.

El-Kassaby, Y. and K. Ritland. 1986. Low levels of pollen contamination in a Douglas-fir seed orchard as detected by allozyme markers. Silvae Genetica 35:224-231.

Elston, R.C. and J. Stewart. 1971. A general model for the analysis of pedigree data. Hum. Hered. 21:523-542.

Griggs, M.M. and C.H. Walkinshaw. 1982. Diallel analysis of genetic resistance to Cronartium quercuum f. sp. fusiforme in slash pine. Phytopath. 72:816-818.

Helentjaris, T. and R. Gesteland. 1983. Evaluation of random cDNA clones as probes for huma restriction fragment length polymorphisms. J. Mol. Appl. Gen. 2:237-247.

Helentjaris, T., M. Slocum, S. Wright, A. Schaefer and J. Nienhuis. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms Theor. Appl. Gen. 72:761-769.

Jayacker, S.D. 1970. On the detection and estimation of linkage between a locus influencing a quantitative character and a marker locus. Biometrics 26:451-464.

Kempthorne, 0. 1988. An overview of quantitative genetics. p. 47-56. In: B.S. Weir, E.J. Eisen, M.M. Goodman and G. Namkoong (eds.), Proc. 2nd International Conference on Quantitative Genetics. Raleigh, NC. Sinauer Associates, Sunderland, MA.

K inloch, B.B. and J.L. Littlefield. 1977. White pine blister rust: Hypersensitive resistance in sugar pine. Can. J. Bot. 55:1148-1155.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and plantations: Jack pine and black spruce. Can. J. For. Res. 15:902-908.

Lalouel, J.-M., G.M. Lathrop and R. White. 1986. Construction of human genetic linkage maps: II. Methodological issues. p. 39-47. Cold Spring Harbor Symposium on Quantitative Biology, Vol. 51. Cold Spring Harbor, NY.

Lander, E.S. a7 d D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RF LP linkage maps. Genetics 121:185-199.

Lander, E.S. and P. Green. 1987. Construction of multi-locus linkage maps in humans. Proc. Natl. Acad. Sci. USA. 84:2363-2367.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Lange, K. and R.C. Elston. 1975. Extension to pedigree analysis. I. Likelihood calculations for single and complex pedigrees. Hum. Hered. 25:95-105.

Lathrop, G.M. and J.-M. Lalouel. 1984. Easy calculation of lod scores and genetic risks on small computers. Amer. J. Hum. Genet. 36:460-465.

Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mather, K.M. and J.L. Jinks. 1971. Biometrical genetics. Chapman and Hall, London.

Neale, D.B. and W.T. Adams. 1985. The mating system in natural and shelterwood stands of Douglas-fir. Theor. Appl. Genet. 71:201-207.

Neale, D.B., Wheeler, N.C. and R.W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152-1154.

Ott, J. 1985. Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721-726.

Smith, H.O. and K.W. Wilcox. 1970. A restriction enzyme from Hemophilus influenza. I. Purification and general properties. J. Mol. Biol. 51:379-391.

Snow, G.A., R.J. Dinus and A.G. Kais. 1975. Variation of pathogenicity of diverse sources of Cronartium fusiforme on resistant slash pine. Phytopath. 65:170-175.

Soller, M. 1978. The use of loci associated with quantitative effects in dairy cattle improvement. Anim. Prod. 27:133-139.

Soller, M. and J.S. Beckmann. 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Appl. Genet. 67:25-33.

Soller, M. and J. Plotkin-Hazan. 1977. The use of marker alleles for the introgression of linked quantitative alleles. Theor. Appl. Genet. 51:133-137.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Squillace, A.E., O.O. Wells and D.L. Rockwood. 1980. Inheritance of monoterpene composition in cortical oleoresin of loblolly pine. Silvae Genetica 29:141-151.

Stuber, C.W., M.M. Goodman and R.H. Moll. 1982. Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. Crop Sci. 22:737-740.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Mol. Biol. Report. 1:3-8.

Tanksley, S.D., H. Medina-Filho and C.M. Rick. 1981. The effect of isozyme selection on metric characters in inter-specific backcross of tomato-- basis of an early screening procedure. Theor. Appl. Genet. 60:291-296.

Tanksley, S.D. and C.M. Rick. 1980. Isozymic gene linkage map of the tomato: Applications in genetics and breeding. Theor. Appl. Genet. 57:161-170.

Thoday, J.M. 1961. Location of polygenes. Nature 191:368-370.

Wagner, D.B., G.R Furnier, M.A. Saghi-Maroof, S.M. Williams, B.P. Dancik and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. USA. 84:2097-2100.

Watson, J.D. and F.H.C. Crick. 1953. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. Nature 171:964-967.

Watson, J.D., N.H. Hopkins, J.W. Roberts, J.A. Steitz and A.M. Weiner. 1987. Molecular Biology of the Gene. Benjamin/Cummings Publ. Co., Menlo Park, CA.

White, R. and J.-M. Lalouel. 1988. Chromosome mapping with DNA markers. Scientific American 258:40-48.

White, R., M. Leppert, P. O'Connell, Y. Nakamura, C. Julier, S. Woodward, A. Silva, R. Wolff, M. Lathrop and J.-M. Lalouel. 1986. Construction of human genetic linkage maps: 1. Progress and perspectives. p. 29-38. Cold Spring Harbor Symposium on Quantitative Biology, Vol. 51. Cold Spring Harbor, NY.