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# Genomic and physiological approaches to advancing forest tree improvement†

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**Summary** The recent completion of a draft sequence of the poplar (*Populus trichocarpa* Torr. & Gray ex Brayshaw) genome has advanced forest tree genetics to an unprecedented level. A "parts list" for a forest tree has been produced, opening up new opportunities for dissecting the interworkings of tree growth and development. In the relatively near future we can anticipate additional reference genome sequences, including the much larger *Pinus* genome. One goal is to use this information to define the genomic attributes that affect the phenotypic performances of trees growing in various environments. A first step is the definition of ideotypes that constitute optimal tree and stand-level performance. Following this, the genome can be systematically searched for genetic elements and their allelic variants that affect the specified traits. Knowledge of these alleles and their effects will facilitate the development of efficient tree improvement programs through genome-guided breeding and genetic engineering and further our mechanistic understanding of trait variation. Improved mechanistic understanding of tree growth and development is needed to develop process models that will allow us to anticipate and manage change in forest ecosystems. Here we consider the development of an ideotype for loblolly pine (*Pinus taeda* L.) and discuss genomic approaches for studying the component traits that will enable advances in process model development and the genetic improvement of this important conifer.

*Keywords: biotechnology, crown architecture, ideotype, marker assisted selection, net photosynthesis, pest resistance, physiological genetics, tree breeding.*

## Introduction

In this article, we consider the near-to-medium term applications of genomic and physiological research directed to advancing forest tree improvement practice. We focus our discussion on loblolly pine (*Pinus taeda* L.) in which genome-guided breeding, clonal selection and genetic modification can rapidly increase the rate of tree improvement. This is not meant to imply that plantation forests are more important than natural forests. Extensive natural forests, which are commonly managed with low intensity, provide environmental services that are critical to society, and genomic research will have an impact on their management especially in areas such as genetic conservation and species restoration (Mosseler et al. 2003).

As in all biological science, forestry research, and in particular forest genetics, has become increasingly reductionist. Forest genetics first used statistical techniques to partition phenotypic variation of morphometric traits into environmental, genetic and genetic × environment interaction components. Selection and breeding have been aimed largely at improving productivity, pest resistance and wood quality based on knowledge of variance components. Quantitative trait loci (QTL) have been discovered that correspond with phenotypic variation in many of these same traits (e.g., Bradshaw and Stettler 1995, Grattapaglia et al. 1996, Wu 1998, Kaya et al. 1999, Sewell et al. 2000, 2002, Weng et al. 2002, Myburg et al. 2003). Physiological genetics has aimed to elucidate the processes and mechanisms contributing to genetic variation in growth and development, including the variation due to specific QTL (e.g., Kubisiak et al. 1999, Frewen et al. 2000, Tschaplinski et al. 2006). Molecular-level variation (isozyme and DNA polymorphisms) used to identify QTL has been used also to estimate gene flow, mating structure and the extent of inbreeding (e.g., Schmidlting et al. 1999, Al-Rabab'ah and Williams 2002, 2004), furthering our understanding of the genetics of forest trees.

The pinnacle, thus far, of reductionism in forestry is the recent mapping of the *Populus* genome by whole-genome shotgun sequencing (Tuskan et al. 2006). The *Arabidopsis* genome has provided a good model for understanding *Populus*, and there is hope that *Populus* will provide a better model for *Pinus*. However, a *Pinus* reference genome sequence is needed if we are to understand fully this conifer's genetics and physiology, and its similarities to, and differences from, the angiosperms. Minorsky (2003) likened sequencing genomes to listing the parts of an airplane, an important step that by itself does not provide understanding of the engineered object (in

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this case the tree). Using a systems biology approach, we can begin to connect the pieces at different levels of complexity, ultimately building an *in silico* (Minorsky 2003) or virtual (Chory et al. 2000) tree growing and developing in various environments.

In some cases, important traits have been found to be largely controlled by one or a few genes. Where allelic variation occurs for these traits, breeding cycles can continue to recombine specific alleles and genotypes can be selected, tested and then deployed in the field. In other cases, traits are controlled by many genes, and these will be deduced and understood only by integrating genomic analysis with well-designed genetic and physiological experiments. In this paper, we explore the opportunities to incorporate and integrate genomic and physiological research to advance tree improvement practice, and we briefly discuss the opportunities and risks of employing genetic engineering to augment such efforts.

## Tree improvement in the genome era

Comprehensive information on the genomes of forest trees will change the way tree improvement is practiced. Technology for assessing the effects of single genes and their alleles on complex traits is being developed, which will make it possible to select and breed for specific genes or gene combinations. To make this a reality, we need to assign the genes and the allelic effects to traits in populations that are important for tree improvement and the development of our understanding of the tree phenotype from a mechanistic point of view. The concept of the ideotype provides a convenient conceptual bridge between physiological processes and the genes that underlie them.

An ideotype is a model representing the physical and chemical attributes of a plant for a specified end-use grown in a particular environment. Depending on the end-use and environment, ideotypes can be simple (as recommended by Martin et al. 2001), but usually they are complex because many desired attributes are envisioned and may encompass a range of environmental conditions. For example, the ideotype for biomass production in non-irrigated, high-density poplar plantations includes seven major attributes (growth, physiology, ecology, morphology, stem form, wood quality and roots), and each major attribute has at least two sub-attributes (physiological sub-attributes include high photosynthetic rate per unit leaf area, high ratio of photosynthesis to dark respiration, and leaves, cambium and fine roots that adjust osmotically to hydration) (Dickmann and Keathley 1996).

Ideotypes are often classified into a few classes such as isolation, competitor and crop, but more exacting descriptions are needed. For example, what are the specific individual tree traits that translate into high biomass yield per hectare to produce a crop ideotype. Although only a few traits controlled by only a few genes may significantly impact growth rate, other traits such as pest resistance and wood quality need to be incorporated into a genotype that is to be deployed in the field. Tree breeding programs already incorporate multi-trait selection, but can a complex array of genes be accommodated in a tree breeding program? Through genomics it will be possible to recombine specific genes with specific genotypes to produce desired ideotypes.

Besides rapid growth, idiotypes will possess attributes such as pest resistance, cold hardiness and high wood density, which insure attainment of high yields of wood with desired characteristics. The more we specifically target a particular forest product or growth environment the more refined ideotypes will become. Regardless of our overall goal, specific traits need to be identified and characterized at the gene and genome level. As an example of what traits might be included in a *Pinus* ideotype, we adapted and refined the Kärki and Tigerstedt (1985) conifer ideotype for loblolly pine (Table 1).

This general purpose loblolly pine ideotype is relatively simple, yet it includes the measurement of 22 component traits for the assessment of 15 composite traits (Table 1). These traits must be measured at specific times from the seedling or somatic embryo culture stage through the base (or selection) measurement age. At one end of the spectrum, assessment of a trait such as plantable index (Table 1) is completed before the material goes to the field and its phenotype is evaluated in the laboratory. Other traits, such as pest resistance and cold tolerance, need to be assessed either as stress events occur or by means of artificially imposed stress tests. In reality, the 22 component traits are themselves the manifestation of two or more sub-component traits, many of which are expressed in the field differentially over time and space and are often difficult to quantify. Sub-component traits and genes may act in concert such that they appear pleiotrophic, or they may be negatively correlated with each other or the component trait (Bongarten 1986).

Most tree improvement programs measure only a few traits (five or less) for objective (statistical) analysis, while using several other traits for subjective grading when making final selections. This incorporates the basic ideotype concept as the breeder is selecting trees with specified attributes. However, if we are to identify the genetic and physiological mechanisms that fully define a refined ideotype, we must assess quantitatively the genome and the component traits in experiments designed to develop predictive models for general use. The ultimate objective is to know what genes control the component traits and how they work together to control the composite trait, which will enable breeders to manipulate the attributes by selecting the best alleles for these genes.

Theoretically, component traits can be reduced to the products of the actions of single genes on single biochemical steps and physiological processes. Short- to medium-term strategies for what traits to reduce genomically should be driven by practical considerations about how to achieve the most rapid progress, while retaining flexibility for future improvement. If one to a few genes are found to be strongly correlated to a component trait, and if the relationship holds across environments, or if the genotype × environment interaction is predictable, and if the genes are known to control processes directly involved in the component trait, then no further reduction is warranted.

Genotyping (determining the alleles of an individual) requires only one sample of DNA per genotype, although alleles

Table 1. A proposed general purpose loblolly pine ideotype for use in varietal forestry near the cold and dry limits of the species' range. All component traits are measured at selection age unless otherwise indicated.

| Major attribute | Minor attribute | Composite trait | Component trait(s) |
|---|---|---|---|
| Growth | Rapid diameter and height growth | Stem volume index = height × $DBH^2$ | Height; $DBH^2$ |
| Development | Delayed reproductive maturity | Maturation index = (1/age of first cone) × number of cones | Number of cones; Age of first cone |
| | High somatic embryogenic (or rooting) potential | Plantable index = number of embryos (or shoots) × percent of embryos (or shoots) that develop to plants | Number of embryos (or shoots); Percent of embryos (or shoots) that develop to plants |
| Ecology | Good winter hardiness | Cold tolerance | Spring height growth in an abnormally cold winter |
| | Good drought hardiness | Water stress tolerance (maintenance of relative growth rate during stress; Tschaplinski et al. 2006) | Later summer growth after abnormally dry year (osmotic adjustment) |
| | Low competitiveness with other crop trees | Competition index | Competition effect (Montagnon et al. 2001) |
| | High nutrient use efficiency | Stem to leaf ratio | Stem biomass; Leaf biomass |
| Crown form | Few, Small branches | Branch index = number of branches between 1.5 m and 3 m × mean volume index of branches in whorl closest to 1.5 m | Number of branches between 1.5 m and 3 m; Mean length of branches in whirl closest to 1.5 m; Mean $diameter^2$ of branches in whorl closest to 1.5 m |
| | Large branch angle | Branch angle | Mean branch angle of branches in whorl closest to 1.5 m |
| Stem form | Stem straightness, main stem without defects | Stem form index = degree lean × number of defects | Number of degrees off $90^o$ from ground to 3 m; Number of visible defects from ground to top of tree |
| | Low main-stem taper | Stem taper index = diameter at 3 m/DBH | Diameter at 3 m; DBH |
| | Rapid diameter growth | Stem shape index = DBH/height | DBH; height |
| Wood quality | High wood density | Wood density index = wood density of whole core taken at 1.5 m | Whole core wood density |
| Rust resistance | Low disease incidence | Disease index = number of visible galls × gall length of gall closest to 1.5 m | Number of visible galls over whole tree, stem and branch; Length of stem gall closest to 1.5 m |
| SPB resistance | High resin yield | Resin yield index = mean 24 h resin yield at 1.5 m | 24-h Resin yield per tap at 1.5 m |

will need to be determined for many loci. Phenotyping (assessing trait values of an individual) requires temporal and spatial sampling over periods of varying duration and over multiple environments. Until recently, genomic technology has been limited to applications such as marker-assisted selection and breeding. However, in the near- and medium-term, quantitatively characterizing trait phenotypes will likely present the greatest challenge and expense.

For example, net photosynthesis ($P_n$) does not appear to be a promising sub-component trait (Martin et al. 2005) for genome analysis. Although $P_n$ has been shown to be positively related to growth (Ceulmans and Impens 1983, Johnsen and Major 1995, Johnsen et al. 1999), more often than not significant genetic variation has not been demonstrated (Ledig and Perry 1967, Ottosen 1990, Samuelson et al. 1992, Cregg 1994, Marshall et al. 2001, Yang et al. 2002). If significant genetic variation in $P_n$ exists, then it may only be expressed at specific times of the year (Johnsen et al. 1996, Marshall et al. 2001) or under specific environmental conditions (Major and Johnsen 1996), again requiring comprehensive sampling for informative and accurate phenotyping. Furthermore, we know that photosynthetic rate is a function of stomatal behavior and photochemistry of the light and dark reactions, and sub-components of the photosynthetic apparatus in conifers are largely paternally inherited (Major et al. 2007).

Carbon isotope discrimination ($\delta^{13}C$) provides a better tool

for estimating integrated photosynthetic rate and discerning genetic variation in trees (Johnsen et al. 1999). However, its use does not solve the difficulties associated with temporal and spatial variation. In addition, genetic correlations between $\delta^{13}C$ and growth are non-significant among families, and inconsistent and small within families of loblolly pine and slash pine (*Pinus elliottii* Engelm. var. *elliottii*) (Emhart et al. 2007).

The biochemical pathways for lignin biosynthesis are well understood (Peter and Neale 2004) and provide candidate sub-component traits for genome-level analyses. Angiosperms and gymnosperms share one pathway to produce guaiacyl (G) lignin, but angiosperms have an additional pathway that leads to syringal (S) lignin. One of the fastest growing of the original loblolly pine selections made by the North Carolina State University–Industry Cooperative Tree Improvement Program was discovered to carry a rare mutant allele (*cad-n1*) of the gene (*cad*) that encodes cinnamyl alcohol dehydrogenase (CAD) (MacKay et al. 1995, Gill et al. 2003, Yu et al. 2005). Trees with *cad-n1* have lower *cad* activity and less CAD, and modified lignin composition partly because *cad* expression is the final step in the G lignin transcriptional pathway (MacKay et al. 1997, Ralph et al. 1997, Stasolla et al. 2003). Several studies report that *cad-n1* heterozygotes have lower lignin contents (MacKay et al. 1995), higher specific gravities (Dimmel et al. 2002, Yu et al. 2005), better pulping characteristics (Dimmel et al. 2002) and higher growth rates (Wu et al. 1999, Yu et al. 2005). However, research has also provided contrasting results for all these traits for trees of different ages, grown on different sites and from different full-sib families (Dimmel et al. 2002, Yu et al. 2006). These studies indicate that the *cad-n1* mutation might be exploited in producing genotypes for pulp and biofuel production. The cumulative effects of the other genes in these pathways and their regulation and interaction must also be determined before genome-informed approaches can be applied to ideotype construction and tree improvement.

Disease and insect resistances are candidate traits for inclusion in genome research and incorporation into ideotypes for two reasons. First, genotypes can be field tested in areas where pest incidence is high, so phenotypic variation can be assessed relatively easily. At least for some of the important diseases, *ex situ* testing protocols have been developed that are accurate and affordable. Second, our understanding of the genetics, and in some cases the genomics of disease and insect resistance, is advancing rapidly (Martin et al. 2003, Thompson and Goggin 2006). Resistance to fusiform rust (caused by *Cronartium quercuum* (Berk.) Mayabe ex. Shirai f. sp. *fusiforme*) and pitch canker (caused by *Fusarium circinatum* Nirenberg & O'Donnell, formerly *F. subglutinans* f. sp. *pini*) diseases have been studied extensively in both loblolly pine and slash pine. Recent comparative studies have shown that these disease organisms have different strategies for invading and obtaining resources from their pine hosts (Davis et al. 2004). The differing strategies have apparently led to different resistance mechanisms and have been studied at the gene expression level (Morse et al. 2004).

For fusiform rust disease, a gene-for-gene system has evolved in which specific resistance (R) genes in the host are effective against specific avirulence (Avr) genes in the pathogen (Kinloch and Walkinshaw 1991, Nelson et al. 1993, Doudrick and Nelson 1994, Stelzer et al. 1999, Kubisiak et al. 2005). At least one R gene, *Fr1*, has been genetically mapped (Wilcox et al. 1996), and several more are known and are being mapped (Henry Amerson, personal communication). Proof of the corresponding Avr gene awaits genetic mapping, but a segregating fungal population has been produced and a mapping experiment is currently in progress (Kubisiak et al. 2007, Thomas Kubisiak, personal communication). A microarray-based gene expression study failed to identify candidates for *Fr1*, but showed several genes that were differentially regulated during disease (i.e., stem gall) development (Myburg et al. 2006). Mapping R and Avr genes is feasible and worthwhile, especially if the goal is to characterize the genes at the nucleotide level and to use this information to develop effective tools for tree improvement and better models for understanding disease resistance.

Crown architecture (Trousdell et al. 1963, Stenberg et al. 1994, Maier et al. 2002, Emhart et al. 2007), including leaf area, leaf distribution, branch length and other branch characteristics will likely be important sub-components of loblolly pine ideotypes, particularly when combined with process models that estimate total radiation interception (Emhart et al. 2007). However, simpler and standardized digital-based protocols need to be developed for measuring crown architecture traits. Measuring carbon allocation and carbon partition is tedious work, but as in crop plants, these processes will likely provide important sub-component traits (Cooke et al. 2003, Johnsen et al. 2004, 2007, Palenchar et al. 2004, Wullschleger et al. 2005).

Phenotypic characterization of drought and cold tolerance (Burr et al. 1990) requires high temporal and spatial sampling as well as opportune timing in observing sufficient genetic variation when families or clones are being field tested. It is important to identify the existence and magnitude of negative genetic correlations involving these traits. For example, is spring cold hardiness reduced if increased growth is attained through earlier bud break and shoot extension? In field tests, environmental conditions need to be monitored to assess when genetic variation is expressed in growth or survival responses during large, integrated physiological genetic experiments, and to examine critically elite genotypes before they are deployed commercially. If negative correlations are understood, deployment strategies can be modified to reduce risk.

Combining genomic with trait-specific ideotypic analyses will allow us to begin to develop an *in silico* or virtual tree. Can a process model be developed that simulates a tree's characteristics at some point in time under some environmental condition based only on the alleles it contains? This high level of reductionism may be necessary for adequate prediction although its tractability remains questionable. In this scenario, genes that affect the component or subcomponent traits must first be identified. Then the effects of the alleles for these genes and their interactions must be quantified.

Consider the possibilities if each trait is controlled by only

two genes and there are only three relevant alleles in the population, all with additive effects, and the epistatic interactions are all additive and confined to only pairs of genes. For the 22 component traits that affect the ideotype there are about 44 main effects (i.e., genes) each with three factors (i.e., alleles) and up to 946 interactions between pairs of genes. Parameterizing such a model is difficult, so we suggest taking one trait at a time, although alternative approaches should be considered (e.g., Meuwissen et al. 2001). First, determine the genes and the effects of the relevant alleles for Trait 1. Then, consider Trait 2 and determine the relevant alleles and also consider what effect the genes we associated with Trait 1 have on Trait 2. This process is continued for all 22 traits. Now we have a list of genes, their effect on each trait and the allele values for each gene on each trait. To validate, we could then take 10 random trees from the same population and genotype them for these genes. We could use information about the effects of these genes and alleles to predict the performance of each trait in each of the 10 trees. Finally, we could grow the 10 trees in the same environment and measure the traits and compare these data to our predictions to evaluate our success.

A way to approach this problem at this level of reduction is by association mapping using either candidate genes or a genome scan (for a recent review of these and other related methods see Vasemagi and Primmer 2005; see also Glazier et al. 2002, Rafalski 2002, Neale and Savolainen 2004, Hirschorn and Daly 2005). In the former, candidate genes for each trait are identified by some means (e.g., Pflieger et al. 2001, Tabor et al. 2002, Brown et al. 2003) and then allelic variants of the genes are found that act as allele-specific markers for the genes. The allele-specific markers are then tested for their effects on the trait in a large randomly mated population. For a genome scan, markers for densely mapped positions in the genome are developed and then tested for their statistical association with phenotypes. Alternatively, for well-studied organisms, one might consider using allele-specific markers for all (or a large proportion) of the genes in the genome. Either approach to genome scanning relies on linkage disequilibrium (LD) between the marker alleles and the alleles causing the variation. A large number of markers is required for most forest trees (highly heterozygous, outbreeding organisms) because LD is assumed and has been found in some cases to exist over fairly short distances (Brown et al. 2004, Krutovsky and Neale 2005). However, it is now becoming clear that, in the near future, genome-level information will afford the opportunity to conduct powerful candidate gene or genome scan experiments (Lander 1996, Tabor et al. 2002, Hirschorn and Daly 2005).

In this example, we envision a randomly mated population consisting of 1000 clones, replicated 10 times on each of 10 sites, genotyped for 10,000 candidate genes or mapped positions in the genome and phenotyped for these 22 traits. For each trait we would need to find the genes or genomic regions that influence phenotype and determine the value of each allele of these genes. We could then assess a much larger sample of trees, say 10,000 individuals from the same population, for the diagnostic genes, developing predictions for how these individuals would perform as clones. The goal would be to find rare individuals that are candidate clones for possessing all the positive attributes of our ideotype. Different individuals will likely contain combinations of genes suited for different uses (e.g., dimension lumber versus biofuel feedstock). Further breeding and selection could better refine the ideotypes destined for different uses.

A lesser level of reductionism might be useful for prediction and might be more tractable, especially for species for which less genome information is available or there is less potential for clonal propagation and testing. Here we suggest tracking larger genomic regions for their effect on the simple traits included in the ideotype. This is likely only possible within a family structured population, which is often available for species undergoing some degree of genetic improvement. The family structure provides large genomic regions of high LD, and a marker or markers specific for a given region descending from a particular ancestor can be assessed for their effect on traits much the same way as for candidate genes or more closely spaced markers. The key in this scenario is to know what ancestor the genome region descended from and which homolog (or allele) is being dealt with in any descendent. With this information in hand, the same types of analyses can be performed as for candidate gene or genome scan experiments. Particular alleles of ancestral parents are then used to identify trees with all positive attributes of the ideotype and validated by testing. Repeating this type of selection within a family-structured population in successive generations without intervening phenotypic evaluations is the essence of a form of marker-assisted breeding that is currently under development (Nelson and Echt 2004), and which should allow for a significant practical extension of marker-assisted selection.

A variation of the above approaches adds genome-wide gene expression information to the analyses. These analyses have been identified as expression QTL (e-QTL) studies in which the expression of each gene (i.e., RNA transcript level or expression phenotype) is quantitatively assessed in various tissues and environments (e.g., Cheung and Spielman 2002, Wayne and McIntyre 2002, Morley et al. 2004) and related to phenotypes of simple or even more complex traits in the same environments (Kirst et al. 2004). This approach promises to be a particularly powerful tool for identifying candidate genes whose expression is correlated to phenotype. Kirst et al. (2004) applied the approach to an interspecies *Eucalyptus* backcross family of 91 individuals that were clonally replicated and evaluated for stem diameter and lignin content and quality 20 months after planting. Transcript variation in about 2000 genes (2608 cDNAs) was assayed and 26 genes were found to be highly significantly correlated with stem diameter. Variation in one transcript (representing *Cald5H* also known as *F5H*) explained 38% of the trait variation. The expression of these genes was negatively correlated with stem growth and most of the genes are involved in the lignin biosynthesis pathway (consistent with results from transgenic work in poplar, e.g., Hu et al. 1999, Li et al. 2003). Furthermore, the amount and type of lignin found in the test trees were, as expected, based on the observed expression data and the known lignin

biosynthetic pathway.

Transcript levels of several genes have proved useful in predicting phenotypes. Many of these genes were co-regulated and their regulators (i.e., presumably transcription factors) mapped to different and distinct positions in the genome (Kirst et al. 2004). Two positions previously found to be growth QTL (Myburg et al. 2003) were co-located with the e-QTL, indicating that trans-acting factors affect the expression of these genes. In addition, the expression levels were more predictive than the allelic state of these QTL, which seems to follow as expression is a quantitative measure and alleles are categorical representations. The quantitative data contain more information because they integrate information from other interacting genes and thus can be powerful predictors. The question from a tree improvement perspective is whether there are meaningful amounts of genetic variation at these loci within the breeding populations. That is the case within the studied interspecies backcross family, but what about in intraspecies breeding populations? Here we have candidate genes, but we need to assess their allelic diversity and effects on trait value in particular populations. This can be done with the type of experiment described above, where the candidate genes are assessed and allelic differences are quantified. Once we know the DNA sequence variants that correlate to gene expression variation that correlate to trait variation, marker-assisted selection and breeding become possible.

Epistasis, the interaction of two genes in the expression of a single trait (discussed above), requires attention and study (Carlborg and Haley 2004), but what about pleiotrophy, where genes affect more than one trait leading to genetic correlation? Genetic correlation can also result when alleles of correlated genes cause correlation between the traits they control. In the case of pleiotrophy, we can think of genes that affect both height and diameter growth. Through allometry, the traits are positively genetically correlated. The alleles that promote faster height growth do more or less the same for diameter growth. However, a subset of the population may contain alleles of genes that cause average height growth and above average diameter growth. Such a population may not be optimally fit in the natural environment, but it may produce the best trees for use in plantations designed to maximize stem biomass yield because yield is a function of diameter to the second power, but of height only to the first. In the context of varietal forestry, such individuals could be selected and clonally propagated for direct gains. Selection for trait combinations that are useful only in artificially manipulated environments such as intensively managed biomass plantations is the essence of domestication.

In the case of epistasis, we can consider wood density and diameter growth. These traits tend to be negatively genetically correlated, presumably because faster growing trees produce lower density wood. However, faster growing trees may also need stronger wood, and density will probably contribute to strength along with other traits such as microfibril angle. Some portion of the population may possess combinations of alleles for higher wood density and more rapid diameter growth. Again, such trees would typically not be the fittest individuals in natural environments but in intensively managed plantations they may combine the highest stem volume yield with the greatest stem mass.

With a better understanding of the genes and alleles involved in controlling important traits, we may be able to identify and utilize naturally occurring variation in a way that markedly increases the output of desired products. Clearly, the number of traits and genes that create a phenotype is large, so similar observed phenotypes can be the result of different combinations of traits. This is likely desirable because combinations of elite varieties either planted together or across a landscape will help buffer the entire system from major losses caused by severe biotic and abiotic stressors.

Finally we may be at the stage where genomic information on specific traits begins to affect and improve our efforts to model the processes of tree growth and development. The *in silico* or virtual tree demands a process model that accurately predicts how the tree responds to any specific genetic manipulation or environmental perturbation. Thus, if we select and clone a tree with a particular set of alleles we should be able to simulate the growth and development of this tree and compare it to a randomly selected tree from the same base population. This would be excellent news for tree breeders and genetic engineers because they could then target specific genes and alleles for a desired outcome or the same outcome in a different environmental condition. We suggest that the proper integration of genome science, tree genetics and physiology, and systems biology will lead to the development of useful virtual tree models that can be used to guide genetic improvement and silvicultural prescriptions.

### *Genetic modification*

Although not an emphasis of this paper, the potential for genetic engineering to contribute to developing ideotypes cannot be ignored. Additional enhancements to selected varieties through genetic engineering have been achieved in many plants including some forest trees (e.g., Halpin et al. 1994, Hu et al. 1999) and can soon be generally contemplated (Boerjan 2005). Process models that are gene specific would clearly point to various alternatives for modifying traits that constitute desired ideotypes. These modifications must then be evaluated under real forest conditions to determine their efficacy and provide important information for improving the models. Iterative research programs will develop useful, new varieties and increase our basic understanding of the underlying physiological processes, which will lead to improved process models for further use in forest biology.

While many potential benefits can be envisioned and have been described for genetic engineering in forest trees (Boerjan 2005), there are also risks and these must be thoroughly evaluated. These risks can be placed into two general categories: (1) risks to plantations of genetically engineered trees; and (2) risks associated with the migration of transgenes (i.e., gene flow) into neighboring environments. The first type of risk occurs even if transgenes do not migrate. The second type of risk entails transgenes migrating and either reducing or increasing the fitness of trees in the recipient populations.

Transgenes are packaged in genotypes that combine to produce phenotypes that the environments acts on. For low or modified lignin genes, the trees could be affected by unusual environmental occurrences (i.e., unusual wind or ice events), or soil microbes could be affected by the introduction of a modified food source in the form of decaying wood and root systems (Pilate et al. 2002, Talukder 2006). For disease or insect resistance genes, the trees could be affected by reductions in growth rate or the pest populations could be changed as they necessarily adapt to the new resistance gene. Furthermore, the potential for these genes to migrate into the same or related species by gene flow (i.e., pollen or seed movement) must also be evaluated (Valenzuela and Strauss 2005, Williams 2005). When the potential for gene flow exists, the possible effects of the transgene in new environments and genetic backgrounds must be considered. What is the likelihood that the transgene will persist in the non-target populations and environments and at what frequencies, and given this, what will be the effect on the recipient population and ecosystem? These questions must be carefully considered. Finally, we agree with Talukder (2006) that both the benefits and the risks of transgenic options should be transparently discussed and researched so that honest and informed assessments of the value of the product can be made by all stake holders.

## Conclusions

Genomic research into the physiological processes of forest tree growth and development promises to provide new insights for process modeling and genetic improvement. Discovering the "parts list" provided by the genome and learning how the parts work together in the various processes under relevant environmental conditions is critically important to our ability to develop domesticated forest trees for high productivity in sustainable systems. In addition, this understanding will facilitate improvements in process models that will further our ability to manage and sustain highly productive stands of forest trees. Effective process models will enable tree breeders and genetic engineers to better define the ideotypes for specific combinations of environment and product end use. Genetic engineering provides many opportunities to enhance tree improvement and forest management, so we appeal to all parties to respect each other's concerns, commit to discussing the science openly and honestly, and allow the research to proceed for the benefit of society as a whole.

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