

Will genomics guide a greener forest biotech?

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Forest biotechnology has been increasingly associated with wood production using plantation forestry, and has stressed applications that use pedigreed material and transgenic trees. Reasons for this emphasis include limitations of available technologies to conform to underlying genetic features of undomesticated forest tree populations. More recently, genomic technologies have rapidly begun to expand the scope of forest biotechnology. Genomic technologies are well suited to describe and make use of the abundant genetic variation present in undomesticated forest tree populations. Genomics thus enables new research and applications for conservation and management of natural forests, and is a primary technological driver for new research addressing the use of forests trees for carbon sequestration, biofuels feedstocks, and other 'green' applications.

The roots of forest biotechnology

Two major drivers have influenced the focus of forest biotechnology. The first driver was the influence of would-be end users of forest biotechnology. Requests for forest biotechnology research and applications have come disproportionately from industry versus conservation groups, and the term forest biotechnology has been increasingly associated with applications for industrial plantation forestry with the aim of improved wood and fiber production. The second driver has been the limited ability of available technologies to address problems associated with natural forests. Technologies and approaches have been adapted from crop biotechnology and applied successfully to pedigreed material for industrial plantation forestry. But in general, the fundamental genetic attributes of undomesticated tree populations greatly limit the application of crop biotech approaches to natural forests.

In this Opinion article, I discuss the strengths and limitations of past forest biotechnology efforts, and point to two new drivers that are strengthening and broadening forest biotechnology. (i) Investment in forest genomics is developing more generalized technologies that are applicable to industrial plantations and to natural forests. (ii) Forest biotechnology is being expanded to address new or previously intractable problems, including the genetic dissection of adaptive traits vital to forest conservation and management, the use of forest trees as carbon sinks, and the use of forest trees as sources of biofuels.

Inherent genetic architecture of forest trees challenges limitations of available technologies

The past focus of forest biotechnology in part reflects the challenges posed by the genetic attributes of forest trees, and the limitations of extending available biotechnologies developed for agricultural crop species to forests. The genetic attributes of forest trees stand in stark contrast to those of domesticated annual crop plants. Forest trees typically have long generation times, are wind pollinated with outcrossing mating systems, and have not been subjected to significant domestication. Individual trees are highly heterozygous and carry a high genetic load (see Glossary), such that mating between related individuals results in inbreeding depression and, thus, precludes strategies involving creation of inbred lines. In contrast to crop species that have lost genetic variation during domestication, most natural tree populations contain large amounts of genetic variation that can be exploited in breeding programs. Furthermore, unlike crop species, forest trees are expected to have minimal population substructure and low linkage disequilibrium [1,2]. A practical consequence of low linkage disequilibrium is that linkage relationships between markers and alleles of genes controlling phenotypic traits are not consistent among individuals, which limits the application of marker-assisted selection and breeding. Collectively, the characters of inbreeding depression and low linkage disequilibrium combined with limitations of past technologies have skewed the focus of forest biotechnology towards pedigreed material and applications for industrial plantation forestry.

Glossary

Adaptive trait: phenotypic character that enables an organism to survive and reproduce in specific environments.

Additive genetic variation: describes the average breeding value or general combining ability of a parent.

Association genetics: test for statistically significant correlation between allele haplotypes and phenotypes.

Evolutionarily significant units: populations with unique evolutionarily histories, genetic constitution or adaptive traits.

Gene flow: movement of alleles among populations.

Genetic load: the collective deleterious recessive alleles within a genome.

Inbreeding depression: reduced fitness in progeny from mating between related individuals, caused by homozygosity for recessive deleterious alleles.

Linkage disequilibrium: degree of co-occurrence of two or more markers within a population, for example through close linkage.

Non-additive genetic variation: describes departure of progeny from specific crosses from that predicted by average breeding value of parents.

Population substructure: subpopulations among which there is a reduced amount of gene flow.

Transgenesis: introduction of genes into an organism.

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The reasons for this are discussed below for past forest biotechnology applications.

Clonal propagation and transgenesis

Clonal propagation combined with transgenesis is particularly attractive for short rotation plantation-based industrial forestry. Traditional tree breeding is a lengthy process that cannot efficiently capture non-additive genetic variation, primarily because inbred lines would suffer from inbreeding depression. Clonal propagation of elite genotypes allows for the capture of both additive and non-additive genetic variation, and the addition of transgenes can confer new or enhanced traits. The technical ability to create such clones has already been demonstrated, for example, fast-growing poplar clones carrying transgenes conferring herbicide resistance and insect resistance [3]. Transgenic approaches could also potentially produce trees with unique properties for niche markets, including production of technical materials [4] or biopharmaceuticals [5]. At the extreme, trees could be essentially domesticated for specific applications through rounds of selection and transformation [6–8].

However, there are significant limitations of clonal forestry and transgenics with regards to solving problems relevant to natural forests. Non-plantation forests typically have low or no cultivation, and genetic variability is one of the most crucial attributes for survival in addition to providing the raw input for adaptive evolution. Extensive use of clonal propagation is, therefore, perceived as risky. Transgenics have numerous potential applications for natural forests. For example, damage from introduced diseases and insects for which there is no natural genetic basis for resistance could be mitigated through introduction of transgenes conferring resistance [9]. However, research of the strategies and risks of introducing transgenics into natural populations is still in its infancy [10–12]. And in a practical sense, political, societal and regulatory restrictions make the application of transgenics to natural forests in the near future uncertain [13].

Quantitative trait loci and marker-aided selection

The lengthy traditional tree breeding process typically relies on identifying trees with desirable attributes, followed by indirectly evaluating their breeding potential by measuring phenotypic traits in their progeny. Most traits of interest to forest industry are quantitative in nature, can be costly to measure, and occur later in development (e.g. wood quality). To better understand the genetic regulation of quantitative traits and speed up the progeny testing process, research has focused on the ability to detect chromosomal regions carrying favorable alleles controlling quantitative traits, so called quantitative trait loci (QTL). Studies in both angiosperm and coniferous tree species have demonstrated the feasibility of this approach within pedigrees, and have identified quantitative trait loci influencing traits ranging from wood properties to adaptive traits (e.g. Ref. [14]). Marker-aided selection is an extension of QTL technology, in which progeny with desired genotypes within a given pedigree are identified using molecular markers linked to favorable QTL alleles.

However, QTL and marker-aided selection have limited application outside of pedigreed material. Limitations to QTL and marker-aided selection are exposed when consideration is given to the low linkage disequilibrium and high allelic variation present in forest tree populations [15,16]. Although linkage relationships between markers and QTLs can be established within pedigrees resulting from controlled crosses, historical recombination between markers and the QTL within populations means that QTL-marker relationships must be reestablished in each new pedigree examined, and are completely uncertain in unrelated individuals taken from natural populations.

Genetic markers and population genetics

Molecular genetic markers have been extremely useful for forest population genetics, a discipline supporting basic research into the evolution of species and populations, and supporting applications ranging from tree improvement to conservation and restoration. Molecular markers have been used to estimate population parameters including population structure, gene flow, hybridization, migration, mating systems and inbreeding. Knowledge of these attributes can be used to guide applications for management and conservation. For example, existing marker technologies can be used to determine levels of genetic diversity and inbreeding, two factors indicative of adaptive potential, which can help identify populations at risk. Existing markers can determine taxonomic relationships, a crucial component of establishing the legal basis for protection of endangered plant species. Contamination by non-local seed sources can erode the local adaptation of a population, and can potentially be detected using existing marker technology.

A major limitation of currently available markers is that they are neutral, meaning they are not within the actual genes that play a causative role in determining traits of interest. In addition, recombination and low linkage disequilibrium in forest tree populations means that linkage relationships between markers and alleles of genes controlling phenotypic traits are not consistent among individuals. This is a limiting factor for the application of marker technology to conservation and restoration applications because the markers have little or no predictive value for evaluating adaptive genetic attributes [17,18]. Along the same lines, a lack of difference in neutral marker genotypes does not preclude significant adaptive differences between individuals or populations. For example, common garden experiments in Scots pine showed distinct genetic differences in the time of bud flush among populations sampled along a climatic gradient, but molecular markers failed to distinguish these differences [19]. Markers identifying unique alleles of genes regulating such adaptive traits would be ideal.

New drivers in forest biotechnology – genomic technologies

From the previous discussion, it is apparent that forest biotechnology has been limited by the ability of available technologies to deal with inherent genetic features of forest trees and natural forest populations. However, this situation is rapidly changing with the development of new

genomic and related technologies that are compatible with or even exploitive of the genetic attributes of trees. Discussion of two crucial events is given below, the accumulation of crucial genomics data and infrastructure, and the development of forest population genomics.

The required critical mass of data and infrastructure for effective application of forest genomics is quickly being realized. At the heart of forest genomics is the need for informative DNA sequence, and the recent sequencing of the genome of a *Populus trichocarpa* is a watershed event, providing the first look into the structure and content of a tree genome containing some 45 000 genes [20]. Significant sequence resources in the form of expressed sequence tags are available for numerous angiosperm and coniferous trees (<http://plantta.tigr.org/>), with the largest conifer resource being >78 000 transcript assemblies for *Pinus taeda* (loblolly pine). Notably, these resources are being expanded through resequencing of alleles in support of association genetics studies (see below). Examples of additional resources for forest genomics include microarray resources [21], proteomics [22,23], gene tagging and mutant collections [24,25], and ecotilling [26]. For several angiosperm (e.g. Ref. [27]) and coniferous species (e.g. Ref. [28]), transformation systems have been established that enable assessment of gene function using various strategies, including misexpression, knock down using RNAi or synthetic miRNAs, or introduction of mutations into the amino acid sequence. Although *Populus* and *Pinus* have the deepest genomic resources, other angiosperm and coniferous species are accumulating a critical mass of sequence and other resources, and also benefit from gene discovery and comparative genomics that leverage resources in model annual plants and trees.

The extension of genomics to questions of forest tree evolution, population genetics, and landscape-scale ecology is reflected in the quickly developing field of forest population genomics [18]. Central to forest population genomics is the need to identify the genes regulating adaptive traits that determine the ability to grow in specific environments, and identify allelic variants of those genes that play causative roles in determining phenotypic differences. Association genetics can be used to accomplish this, and the large, unstructured populations of forest trees are in many respects ideal for this approach [16]. Currently, association genetic studies in trees require a survey sequencing of alleles of candidate genes within a population to identify single nucleotide polymorphisms (SNPs) that define unique gene alleles. SNP genotypes and phenotypes are then measured for individuals sampled from the population, enabling testing for statistical association between SNP genotypes and phenotypes (Figure 1). Furthermore, linkage disequilibrium decays rapidly within a few hundred base pairs in both pine [1] and aspen [2]. As a result, a SNP with significant association with a phenotypic trait is likely to be close to or in the gene influencing the phenotype. This allows knowledge of gene function to be considered in understanding the genetic mechanisms regulating the trait being evaluated. SNP markers in close proximity to and in linkage disequilibrium with the actual polymorphism influencing the phenotype are predictive not just in pedigrees, and can be used in natural populations to define crucial allelic

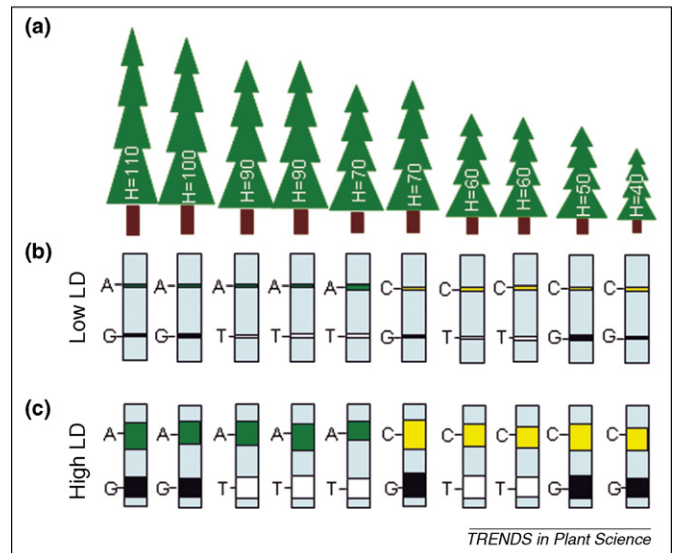


Figure 1. A simplified illustration of an association genetic approach for identifying allelic variation influencing a phenotypic trait under scenarios of either low or high linkage disequilibrium. (a) Phenotypic traits of interest are measured for unrelated trees taken from a population. As an example, height values at a specific age are shown for each tree. (b) Single nucleotide polymorphism (SNP) genotypes are next determined, typically by first discovering SNPs in a modest sample of trees followed by genotyping of the entire population sample for selected SNPs. SNP genotypes are illustrated for two loci on a single chromosome under a scenario with low linkage disequilibrium. For the top SNP (A versus C), trees carrying the A SNP have an average height of 92, whereas trees carrying the C SNP have an average height of only 56, consistent with a significant association between the SNP genotype and height phenotype. For the second SNP (G versus T), trees carrying the G SNP have an average height of 74, and trees carrying the T SNP also have an average height of 74, indicating that the SNP genotype has no association with the height phenotype. Low linkage disequilibrium would typically result in a small chromosomal region (e.g. a few hundred base pairs) flanking the SNP to be commonly shared among individuals (illustrated by colored boxes). (c) The same SNP genotypes but under a scenario of high linkage disequilibrium. In this scenario, a relatively large chromosomal region (e.g. a few hundred kilobase pairs) surrounding each SNP is common among individuals, and is illustrated by colored boxes.

variation underlying adaptive differences among divergent populations. Population substructure can complicate association genetic studies and lead to false positives. For example, the residents of San Francisco represent a highly structured population, including recent immigrants from different parts of the world. A common allelic variant of aldehyde dehydrogenase found in Asians might be found to associate not only with sensitivity to alcohol, but also with other attributes common to people of Asian descent in the population of San Francisco such as complexion, hair color, or eye shape. The latter associations are obvious artifacts of population substructure. Small but significant population substructure has been noted for pine [1] and aspen [2], which should be considered in sampling and statistical models in future studies but should not be a major impediment. In sum, association genetic technology holds promise for a variety of applications, ranging from breeding to conservation efforts in natural populations, and its feasibility has been directly demonstrated recently through the identification of positive associations between allelic variation and wood property phenotypes in loblolly pine [29].

New drivers in forest biotechnology – management and conservation of natural forest

Even forests perceived of as ‘natural’ by the general public have typically experienced significant impacts by human

actions, and conservation and management efforts might be required to mitigate these impacts and ensure long-term viability. In general, forest populations are impacted by introduced pathogens, habitat fragmentation, air pollution, introduction of non-local seed sources, and other direct or indirect human actions. The impact of climate change could potentially result in migration or extinction of forest tree species, and might preclude a passive preservationist approach.

Successful conservation and management efforts ultimately rely on basic information about the genetic makeup of populations. Forest population genomics could play a vital role in more precisely assessing populations, including description of the allelic variation for genes underlying adaptive traits, and providing markers that are predictive of adaptive potential [18]. This information is central to understanding and conserving differences among populations adapted to different environmental conditions, and could be used as an aid in the practical work of identifying populations as evolutionarily significant units in conservation plans. Plans for successful conservation using *in situ* or *ex situ* collections ultimately aim at capturing essential allelic variation at genes underlying adaptive traits. Likewise, plans for restoration or translocations ultimately seek to introduce trees with the ability to grow and reproduce in a specific environment, and capture of allelic diversity for genes underlying adaptive traits to allow for future evolution. Predictive markers identifying allelic variation for genes underlying adaptive traits would be a major new tool for forest conservation and management.

New drivers in forest biotechnology – carbon sequestration and biofuels

Forests buffer the greenhouse gas CO₂ through carbon sequestration in woody tissues. In addition, trees are a potential source of net-zero carbon emission lignocellulosic biofuels. In general, forest trees have not been directly bred to optimize either carbon sequestration or biofuels production, and major gains could potentially be realized. These insights are driving major research initiatives aimed at understanding the genes and mechanisms responsible for traits related to carbon sequestration and biofuels production, and are increasing investments in forest genomics.

Genomics research relevant to both carbon sequestration and biofuels applications would reveal the genes regulating carbon partitioning to woody tissues, wood formation and wood properties. Desirable traits for improved biofuels tree feedstocks would include increasing relative carbon partitioning to above ground woody tissues, and increasing cellulose availability for enzymatic digestion to release its component, fermentable sugars [30]. Desirable traits for carbon sequestration would include increasing resistance of wood to decay, and increasing carbon partitioning to woody tissues. Genomic approaches have been used to identify the genes expressed during wood formation in poplar [31], and the major challenge is now to understand the collective function of the genes and mechanisms underlying wood formation. The biochemical pathways underlying lignin and cellulose biosynthesis are increasingly well understood, and lignin content can be significantly

altered through direct modification of the expression of genes in the lignin biosynthetic pathway [32]. Although major determinants of wood qualities, little is known about patterning of woody tissues or the differentiation of cell types. The interplay among these different levels of regulation are illustrated by Class I KNOX homeobox transcription factors, which regulate tissue-level development and directly regulate the expression of multiple genes involved in cell wall biosynthesis and lignification [33,34]. This observation could point to a means of altering suites of genes that have co-evolved to affect secondary growth phenotypes. In addition, the observation that overlapping mechanisms regulate the shoot apical meristem and secondary growth [35] provide the means to use comparative genomic approaches to better understand the regulatory mechanisms underlying wood formation.

With regards to more immediate and direct genomic applications, association genetic strategies can assist selection and breeding efforts, and could provide the most reliable means of accelerating tree improvement. Understanding the genetic mechanisms underlying adaptive traits (including insect and disease resistance, drought tolerance, and tolerance of sub-optimum sites and saline soils) would assist in planting appropriate genotypes for local environments, which is crucial to successful plantation forestry. Similarly, advanced domestication and enhanced agronomic traits will only be successful if introgressed into genotypes adapted to local environmental conditions. The deployment of appropriate genotypes is made less certain by climate change, but an understanding of allelic variation underlying adaptive traits can allow for predictive approaches to translocating genotypes in advance of environmental changes. Transgenic approaches could further tailor genotypes, such as the introduction of transgenes encoding cellulase or novel enzymes that could enhance the pretreatment of tree feedstocks for biofuels applications [30]. Genomic approaches such as a microarray analysis or metabolomic analysis can provide rapid insight into the secondary effects of such novel traits on overall growth and fitness.

Future prospects

Perhaps the most important and scientifically challenging role of forest genomics and biotechnology will be understanding and monitoring complex ecosystem processes at landscape-scales [36]. For example, genomic approaches for monitoring soil microbial communities could become an important tool in understanding the effects of biomass removal for biofuels, or enhancing durable below-ground carbon sequestration. The genome of *Laccaria bicolor* has recently been sequenced (<http://genome.jgi-psf.org/Lacbi1/>), which will enable functional genomic studies of how this fungus forms beneficial mycorrhizal associations with the host, *Populus*, supported by fully sequenced genomes of both the host and symbiont. *Melampsora larici-populina*, poplar leaf rust fungus, is also being sequenced (<http://www.jgi.doe.gov/sequencing/why/CSP2006/poplarrust.html>), allowing functional genomics to be applied to host–pathogen interactions and landscape-scale forest pathology [37].

It should be recognized that, just like other technologies, genomics is not a panacea. Genomics research must be

integrated with other scientific disciplines, and development of genomics-based applications will only be successful when integrated with breeding, conservation and other applied disciplines. The biggest challenges are likely to be outside of the research realm, and the application of forest genomics will be heavily influenced by political policies and public opinion. The planting of willow as an energy crop in Sweden serves as a cautionary tale: this was largely unsuccessful because of inconsistent public policies, opportunistic behavior spurred by generous government subsidies not directly tied to economic markets, and poor forestry practices by farmers unfamiliar with tree crops [38]. Apprehension of transgenic forestry [13] underscores the general need for public education and discourse about application of genetic technologies to forests. Although researchers can present various technological options, ultimately the decisions about whether and how to use the technology will be decided in the realm of public debate.

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