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Emerging Model Systems in Plant Biology: Poplar (*Populus*) as a Model Forest Tree

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ABSTRACT

Forest trees have tremendous economic and ecological value, as well as unique biological properties of basic scientific interest. The inherent difficulties of experimenting on very large long-lived organisms motivates the development of a model system for forest trees. *Populus* (poplars, cottonwoods, aspens) has several advantages as a model system, including rapid growth, prolific sexual reproduction, ease of cloning, small genome, facile transgenesis, and tight coupling between physiological traits and biomass productivity. A combination of genetics and physiology is being used to understand the detailed mechanisms of forest tree growth and development.

Key words: Forest genetics; Tree physiology; Genomics

WHY IS A MODEL SYSTEM NEEDED FOR FOREST TREES?

The economic and ecological importance of forest trees provide the impetus for developing model systems to study tree biology. Wood is one of the most valuable commodities in the industrialized world, exceeding the weight of all other structural materials combined. More than half of the world's annual wood harvest is used as fuel, primarily in less-developed countries. In nature, forest trees are the principal form of terrestrial biomass. Wild forests provide irreplaceable environmental benefits such as carbon sequestration, watershed protection, and habitat for endangered wildlife. Trees themselves are intrinsically interesting, because they represent the pinnacle of evolutionary refinement in the competition among plants for access to sunlight, thereby dominating many of the Earth's most awe-inspiring landscapes.

Although many aspects of tree biology are common to all plants, and hence can be studied in very tractable model species such as *Arabidopsis*, some unique facets of tree anatomy and physiology must be investigated in trees themselves. A large number of these unique characters are related to their perennial growth habit. Extensive formation of secondary xylem (wood) is perhaps the most obvious, but other characters include leaf and flower phenology, seasonal reallocation of nutrients, cold hardiness, iterative development of a complex crown form, and juvenile-mature phase change. The great lifespan and large size of forest trees pose special challenges to biologists but are fundamental to understanding the role of trees in natural and managed ecosystems.

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The experimental power of genetics, including molecular biology and genomics, has now permeated every discipline in biology. Clearly, an ideal model forest tree must have the potential to be manipulated using standard genetic approaches, such as mutagenesis and the creation of transgenic plants. Despite the fact that other forest trees have been suggested as model systems, including *Salix* (willow), *Eucalyptus,* and *Pinus* (pine), for the reasons discussed later *Populus* has led the way in making use of genetic methods to study tree structure and function.

The knowledge gained from model forest tree systems will be used in the protection of forest ecosystems and in practical tree breeding. By defining, quantifying, and understanding tree physiological processes and morphological structures we hope to mathematically model, predict, and conceptualize tree growth and development. In contrast to agronomic crops, relatively few physiologically based growth models have been developed for trees, largely because of a lack of fundamental physiological information and the difficulty of working with complex perennial plants (Amaro and Tomé 1999; Ford 1985). Just as in forest genetics, poplars have been adopted by tree physiologists as a model system.

The interplay between poplar genetics and physiology, at scales as large as the landscape and as small as a molecule, is the subject we now explore in greater depth. Ultimately, we wish to trace tree physiology and anatomy to the level of individual genes, understanding and manipulating their interactions with each other and the environment.

POPULUS TAXONOMY, DISTRIBUTION, AND GENERAL CHARACTERISTICS

The genus *Populus* L. is a member of the Salicaceae, which, together with the Flacourtiaceae and 29 other families, have been placed under the Malpighiales in the recent cladistic analysis of the angiosperms (The Angiosperm Phylogeny Group 1998). A genus of deciduous trees (rarely semievergreen), it comprises aspens, poplars, and cottonwoods, having a wide natural distribution in the Northern Hemisphere and a small representation in tropical Africa. Various classifications have been suggested, the most recent recognizing 29 species that are grouped under six separate sections (Eckenwalder 1996). Many of these species have extensive distribution ranges, some spanning entire continents (for example, *P. tremuloides, P. tremula*), and only few being geographically confined (for instance, *P. ilicifolia, P. monticola*). For simplicity we will use the term poplar throughout this chapter, unless we draw attention to a specific subgroup.

Poplars are dioecious, wind-pollinated, and produce large amounts of pollen and small, cotton-tufted seed that is dispersed by wind and water in early summer. Capable of rapidly invading disturbed sites, many species occupy habitats in the dynamic environment of riverine floodplains, where they form a key component of riparian forests (Braatne and others 1996). Others, such as the aspens, commonly colonize upland areas after intense, stand-replacing fires (Burns and Honkala 1990). All poplars also have the capacity to reproduce asexually, mostly by sprouting from the root collar of killed trees or from abscised or broken branches that become embedded in the soil. Aspen and white poplars propagate through sucker shoots that arise from horizontal roots, often after a fire, typically leading to clonal stands that may cover several hectares. Recurrent fires can maintain genets of such clones for hundreds of years.

Rapid growth is the hallmark of poplars. It derives from a growth system that begins with the elongation of a preformed shoot from its bud and continues to initiate and expand shoot segments and leaves throughout the growing season. The wood is diffuse-porous, light in weight, and yet capable of building trees of 40 m in height in less than 20 years. Several of these features have made poplars attractive to humans since ancient times. Today, poplar is cultivated worldwide in plantations for pulp and paper, veneer, excelsior (packing material), engineered wood products (for example, oriented strand board), lumber, and energy. Grown at a commercial scale under intensive culture for 6 to 8-year rotations, production rates with hybrid poplar can be as high as 17–30 Mg/ha/y of dry woody biomass (Zsuffa and others 1996), comparable to the biomass produced by row crops such as corn. Historically, poplar has been widely used in windbreaks and for erosion control. Most recently, poplars have been proven effective in the phytoremediation of environmental toxins (Flathman and Lanza 1998) and as bioindicators for ozone pollution in the environment (Jepsen 1994).

STRENGTHS OF *POPULUS* AS A MODEL SYSTEM

Well-Established Collaboration among Poplar Biologists

Extensive collaboration between poplar geneticists, physiologists, and pathologists has set a solid scientific foundation for joint efforts in the future. Shared genetic materials, genetically informative two- and three-generation pedigrees, DNA-based genetic markers, common field measurement protocols, clonal plantation trials supported by industry/government/academic partnerships, and funding from multiagency grants have established a poplar research network of proven productivity. Working groups under the aegis of the International Poplar Commission (IPC) of the Food and Agriculture Organization (FAO) of the United Nations and of the International Union of Forestry Research Organizations (IUFRO), as well as several university/industrial research cooperatives, help to coordinate the work and the exchange of information. Two books synthesizing a large body of data on poplar biology have been published recently, reviewing and solidifying the status of poplar as a model forest tree (Klopfenstein and others 1997; Stettler and others 1996).

Abundant Genetic Variation in Natural Populations

Adaptations to the diverse conditions inherent in large distribution ranges, as well as prominent genetic polymorphisms in local populations, offer poplar researchers a rich source of variation in tree morphology, anatomy, physiology, phenology, and response to biotic and abiotic stress. Much of this variation is under moderate to strong genetic control (Farmer 1996). Extensive natural populations remain for many species, especially in North America.

Ease of Sexual Propagation and Interspecific Hybridization

Poplars are bred in the greenhouse on detached female branches with pollen that can be stored for several years. Each pollination can yield hundreds of seeds within 4–8 weeks. Seeds germinate within 24 h and give rise to 1- to 2-m tall seedlings by the end of the same year. Few, if any, trees can match such efficiency. Poplar species within the same section, and many of the species from different sections, can be hybridized. Because all members of the genus are diploid (2n = 38), hybrids are fertile and can generate F2 and backcross progenies that segregate for a wide range of traits. F1 hybrids often show heterosis in growth and associated characteristics that make them attractive for commercial use (Zsuffa and others 1996).

Physiologic Responses to Environmental Variables Are Rapid and Pronounced

As a consequence of the rapid juvenile growth of poplars, it is possible to measure short-term responses to biotic (for example, disease) and abiotic (for example, drought, elevated CO_2, ozone) factors. Opportunities are being explored for using this rapid growth as a means of carbon sequestration, particularly in Europe. The short turnaround time for physiological studies makes it possible to use molecular genetic approaches requiring very large sample sizes (hundreds or thousands of physiological measurements). High-throughput poplar physiology has led to the genetic mapping of quantitative trait loci (QTLs) controlling morphological (Bradshaw and Stettler 1995), phenological (Frewen and others 2000), and pathological (Cervera and others 1996; Newcombe and Bradshaw 1996; Newcombe and others 1996; Villar and others 1996) traits. Candidate genes for some of these QTLs have been identified (Frewen and others 2000).

Close Coupling of Physiological Traits and Biomass Productivity

Critical components of productivity in *Populus* have been examined from a physiological perspective (Ceulemans 1990). Both structural and functional components were identified at different organizational levels: the individual leaf, the branch, and the whole tree. Among the process-related components, stomatal morphology and behavior (Tschaplinski and others 1994), leaf morphology and leaf growth physiology (Ridge and others 1986), leaf and whole tree photosynthesis (Isebrands and others 1988), and root development (Friend and others 1991) showed the most significant genetic variation. Key anatomic characters include those that determine whole tree leaf area and its duration, such as leaf demography (Chen and others 1994; Hinckley and others 1989), leaf size and distribution (Dunlap and others 1992), branch size and distribution (Ceulemans and others 1990), and leaf and branch orientation (Isebrands and Michael 1986). A favorable combination of several leaf physiological and whole tree and canopy structural traits explains the superior growth of selected hybrid poplar clones. QTLs for many of these traits have been mapped genetically (Bradshaw and Stettler 1995; Wu and others 1997), but the genes controlling these traits remain to be identified and characterized.

Well-Characterized Molecular Physiology

The large physical size of poplars, coupled with the well-understood movement of carbon assimilates (Vogelmann and others 1982) and cotransported systemic wound signals between source and sink leaves (Davis and others 1991; Clarke and others

1998; Constabel and Ryan 1998) is an advantage over small plant models such as *Arabidopsis* in studies involving systemic signal movement. Induced resistance to insect herbivory is acquired systemically and is phenocopied in part by mechanical injury (Havill and Raffa 1999). This positions poplar as an excellent model for synthesizing ecologic and molecular perspectives of induced resistance to insect herbivory.

Dramatic Patterns of Nitrogen Allocation, Use, and Storage

Riparian ecosystems receive nutrient inputs episodically, which may help explain why poplar tissues store high levels of nitrogen in the form of vegetative storage proteins for subsequent use (Coleman and others 1994; Lawrence and others 1997). We have observed healthy poplar leaves with nitrogen concentrations exceeding 8% of dry weight (J. Cooke, K. Brown, J. Davis, unpublished), compared with the 1–1.5% maximum levels found even in fertilized conifer needles. Nitrogen levels fluctuate dynamically among organs within the same tree (roots, stem, and leaves) in concordance with seasonal rhythms of active growth and dormancy. The stem anatomy of poplar trees is particularly suited to studying the role of phloem-transmissible substances such as glutamine in regulating nitrogen allocation, because phloem can be specifically perturbed by girdling, whereas xylem transport remains intact (for example, Sauter and Neumann 1994).

Physiological Process Models for Poplar Growth and Development

Large databases of anatomical, physiological, and silvicultural traits are available for a modest number of *Populus* hybrids and clones. Several physiologically based growth and productivity models have been developed from these data. The models include basic information on carbon uptake and allocation in poplar, as well as components and parameters of leaf display and crown structure. Most of the models simulate carbon uptake, carbon allocation, growth, and/or light interception in poplar and incorporate some specific parameters of leaf display, position in the tree, and branch structure (Chen and others 1994; Host and others 1996; Isebrands and others 1996). Data on the physiological and structural growth determinants at the leaf, branch, and whole tree level indicate that differences in clonal productivity can be incorporated into the ideotype concept developed for poplar tree breeding under short rotation intensive culture (Dickmann 1985; Dickmann and Keathley 1996).

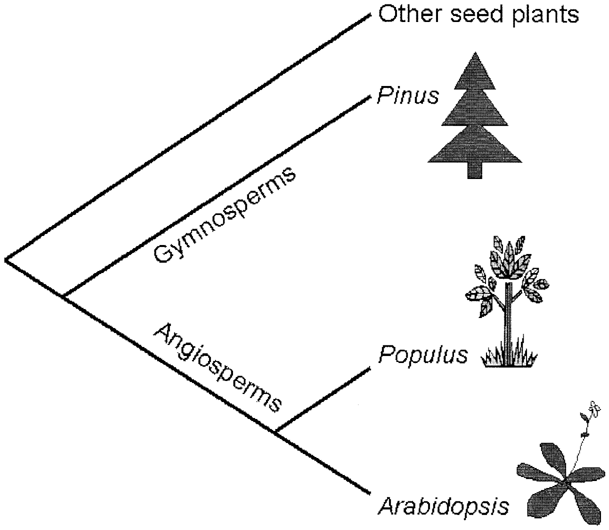

Figure 1. Phylogeny of *Populus.*

Cloning of Individual Tree Genotypes

The ease with which most materials can be vegetatively propagated is one of poplar's premier assets. Cloning captures genetic variation and allows it to be replicated in space and time in separate experiments. Cloning "freezes" genetic variation in hybrids and permits the side-by-side growth of multiple generations of a pedigree. Cloning permits the growth of abnormal plants under field conditions that in the competitive environment of a seedling population would be impossible. Cloning also allows destructive sampling for physiological studies, the sharing of materials among laboratories, and the buildup of cumulative knowledge on selected genotypes.

Closely Related to Other Angiosperm Model Plants

Unlike the pines and other gymnosperms, poplars diverged relatively recently from other angiosperms, such as *Arabidopsis,* which serve as models for integrating genetics into the study of plant biology (Figure 1).

Small Genome Size

The haploid genome size of *Populus* is 550 million base pairs (bp) (Bradshaw and Stettler 1993), only 4 times larger than the genome of the model plant *Arabidopsis,* and 40 times smaller than the genomes of conifers such as loblolly pine. The small poplar genome simplifies gene cloning, Southern blotting, and other standard molecular genetics techniques. The physical/genetic distance ratio for poplar is close to 200 kb/centiMorgan, almost identical to *Arabidopsis,* making *Populus* an attractive target for map-based (positional) cloning of genes.

Basic Molecular Genetics Toolkit

Linkage maps of the poplar genome have been made with a variety of marker types, including allozymes, restriction fragment length polymorphisms, STS/CAPs, random amplified polymorphic DNA, amplified fragment length polymorphisms, and microsatellites (Bradshaw and others 1994; Cervera and others 1996; Frewen and others 2000; Liu and Furnier 1993). Bacterial artificial chromosome (BAC) genomic libraries containing 10 haploid poplar genome equivalents (50,000 clones) have been constructed for *P. deltoides* (W. Boerjan, personal communication) and for *P. trichocarpa* (J. Vrebalov, personal communication). These libraries have been arrayed into microtiter plates and replicated for sharing with other researchers. Expressed sequence tag (EST) databases of modest size are in the public domain (Sterky and others 1998).

Facile Transformation and Regeneration To Create Transgenic Poplars

The ease of creating transgenic *Populus* is unmatched by any other forest tree. Some poplars, such as the hybrid aspen (*P. alba* × *tremula*) clone 717-1B4, can be genetically transformed with *Agrobacterium* and regenerated efficiently into intact transgenic trees within 6–10 months (Jouanin and others 1993). Transgenesis is the "gold standard" for demonstration of gene function and so is crucial for basic research into physiologic processes.

Obviously, there are also potential commercial applications for transgenic poplars. The ease with which genes are cloned and transgenic trees are produced in *Populus,* set against the backdrop of a poplar EST database of wood-forming genes (Sterky and others 1998), has created a fertile scientific field for understanding and manipulating wood composition in dramatic ways (Hu and others 1999). Progress is likely to accelerate in this important research area as the tools of genomics, biochemistry, and cell biology are collectively brought to bear on manipulating wood quality and other important physiological or morphological traits.

WEAKNESSES OF *POPULUS* AS A MODEL SYSTEM

Modest Commercial Importance

Poplar is grown commercially on a wide geographic scale and many wild populations, especially aspen in the boreal forest, are harvested by industry. However, from a corporate viewpoint, poplars are much less important than pines and other softwoods or eucalypts. It can be difficult to persuade companies to invest in (or lobby for) poplar research, either as a model tree, or as a component of fiber/energy plantations. For a variety of historical, political, and economic reasons, most of the forest products industry still relies on harvests from natural or extensively managed stands and has yet to turn to the intensive agricultural paradigm for wood production for which poplar is ideally suited.

Long Generation Interval

Many willows and some eucalypts can be induced to flower within a year or two from seed. Most poplars will not flower earlier than 4 years of age, and many will take twice that long. The long generation interval is an impediment to practical breeding and selection and the development of informative pedigrees. It limits the applicability of conventional experimental genetic techniques such as induced mutagenesis, because producing homozygotes from heterozygous mutants is impracticably slow.

Outcrossing Mating System

Poplars are dioecious, so self-pollinations cannot be done (with a very few hermaphroditic exceptions). Classical genetic tools such as inbred lines cannot be produced rapidly enough to be useful.

Large Physical Size of Trees

There is no such thing as a "small experiment" when one works with poplars! Beyond one-year of age the physiologically active crown is above human reach, requiring towers or remote sensing.

Lack of Well-Planned Breeding Programs

The advantages of cloning have been pointed out, but there is a hidden disadvantage. Most poplar breeding programs have relied on more-or-less random interspecific hybridization to produce large progeny arrays, followed by very intense clonal selection to identify the best clones out of perhaps 10,000 seedlings. Although demonstrably effective at producing remarkable genetic gains in short order, the scarcity of well-planned, long-term, sustained breeding programs and associated breeding materials hampers genetic studies. For example, multigeneration pedigrees are the basis for genetic mapping experiments designed to identify QTLs affecting important tree phenotypes, yet few such pedigrees exist.

WHAT IS NEEDED TO INCREASE THE VALUE OF *POPULUS* AS A MODEL SYSTEM?

Long-Term Support for Germplasm Collection and Maintenance

Nothing is more destructive to sustained progress on long-lived plants than inconsistently funding basic genetic infrastructure such as breeding, maintenance of living clone banks, and annual measurement of experimental plantations. Unfortunately, these routine but important activities are difficult to justify to most funding agencies and usually are jettisoned at the first dip in finances. A solution to this problem might be to establish at least one public genetic stock center on each continent with an active interest in poplar research. This would promote the centuries-old practice of exchanging pollen, seed, and cuttings from genetic stocks with value for research and commercial use. The poplar research community's tradition of accumulating and integrating knowledge about shared pedigrees would be encouraged.

Shorter Generation Interval; Early or Inducible Flowering

Delayed flowering represents a lost opportunity for tree geneticists to provide suitable pedigrees for physiologic studies and is currently the single most limiting factor in the conventional genetic improvement of forest trees. The development of methods for inducible early flowering, either by chemical/physical treatment or genetic engineering, will make it possible to decrease the generation interval for purposes of breeding, while also preventing excessive flowering from siphoning photosynthate away from wood formation in production plantations. Although some progress has been made in this area with poplars (Weigel and Nilsson 1995), much remains to be done (Rottman and others 2000).

Complete Genomics Toolkit

Probably no other group of organisms will benefit more from the development of genomics tools than forest trees, when conventional genetic approaches are so limited. Because most of the interesting traits in trees are inherited quantitatively, a dense genetic map composed of 2500–5000 genetic markers that identify homologous loci in all *Populus* species and have a high information content, such as microsatellites or single-nucleotide polymorphisms (SNPs), should be produced specifically for QTL mapping.

The genome of *Populus* is small enough to invite the construction of a complete physical map of overlapping BACs. The physical map could be tied to the genetic map either by mapping genetic markers onto the physical map or by developing genetic markers directly from BAC sequences with known physical positions. The physical and genetic maps would serve several important purposes: as a starting point for map-based gene cloning, as a means to determine the extent of collinearity between the *Populus* genome and that of the completely sequenced genome of *Arabidopsis* (thus making effective use of the growing knowledge of *Arabidopsis* genome structure and function), and as a scaffold for the eventual sequencing of the entire *Populus* genome.

Until the *Populus* genome is completely sequenced, perhaps in the next 5–10 years, a comprehensive EST database will provide a source of SNPs and become the foundation for microarray development and use for studying global patterns of gene expression. EST microarrays will allow poplar researchers to couple physiological measurements with gene expression profiles, illuminating the genes, biochemical pathways, and cellular processes that are affected by a given environmental perturbation or transgene.

Steve Strauss (personal communication) has suggested an ambitious strategy for discovering genes with novel functions in poplar using generating 50,000 dominant gain-of-function mutants in transgenic poplars by activation tagging (Walden and others 1994), an insertional mutagenesis strategy more suitable for trees with outcrossing mating systems than traditional mutagenesis approaches yielding mostly recessive mutants. The incorporation of such tools will be important if we are to carry out forward and reverse genetics with equal facility in forest trees.

SUMMARY

Poplars have been, and remain, a widely accepted model in forest tree biology. The integration of poplar genetics with physiology has been central to improving our understanding of tree growth and development from the molecular level to the landscape scale. Advancing poplar genomics toward parity with other plant model systems would bring substantial benefits to the study of unique aspects of tree form and function.

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