Commentary

Poplar genomics comes of age

The value of poplars as model species for molecular tree biology has been steadily growing for the past decade (Bradshaw *et al.*, 2000; Wullschleger *et al.*, 2002; Brunner *et al.*, 2004). Starting with the creation of DNA marker maps (Bradshaw *et al.*, 1994; Wu *et al.*, 2000; Cervera *et al.*, 2001; Yin *et al.*, 2004) and transgenic trees (Jouanin *et al.*, 1993; Peña & Seguin 2001; Strauss *et al.*, 2001), it has more recently accelerated with the production of a large number of expressed sequence tags (ESTs) (Sterky *et al.*, 1998; Bhalerao *et al.*, 2003; Kohler *et al.*, 2003; Déjardin *et al.*, 2004; Ranjan *et al.*, 2004) and analysis of thousands of genes on DNA microarrays (Hertzberg *et al.*, 2001; Kohler *et al.*, 2003; Andersson *et al.*, 2004). The imminent release of the genome sequence (Joint Genome Institute, USA Department of Energy: http://www.jgi.doe.gov/) is taking poplar genomics one large step further by providing a catalog of all genes and, most importantly, of their regulatory environments. Indeed, many biologists expect that variation in gene regulation, whether natural or induced, will be more important for controlling developmental, adaptive and economic traits than variation in protein coding sequences (Doebley & Lukwens, 1998; Carroll, 2000; Frary *et al.*, 2000).

'A tree species is being dissected with a level of genetic precision that seemed a distant dream just a few years ago'

Most of the papers published to date have been 'first looks' at problems – first maps, first descriptions of EST expression patterns, first transgenic populations. But several of the papers in this issue show that the field has begun to move beyond the exploratory, descriptive phase, into detailed studies of diverse biological phenomena and technologies. Studies range across the field, covering topics as diverse as heavy metal tolerance and metallothionein genes (Kohler *et al.*, pp. 83–93) and cryopreservation as an effective means of preserving large numbers of mutant lines (Tsai & Hubscher, pp. 73–81). Perhaps most excitingly, several of the papers make use of the preliminary (unannotated, unorganized) poplar genome sequence that has been available on the internet for several months (Kohler *et al.*; Yin *et al.*, pp. 95–105; Brunner & Nilsson, pp. 43–51; Joshi *et al.*, pp. 53–61), showing that the age of genome sequence-based poplar genomics has truly begun.

Developmental processes

Wood is a complex and highly variable tissue, whose formation is developmentally and environmentally regulated. Although cellulose and lignin biosynthesis have been intensely investigated in poplar over the last decade, the precise molecular mechanism of their biosynthetic process in poplars is still not well understood (Boerjan *et al.*, 2003; Rogers & Campbell, pp. 17–30; Joshi *et al.*). Tension wood can frequently be observed on one side of stems or branches that have been displaced by wind or a load of some kind. Its formation is associated with increased growth rate and the development of fibers with high cellulose content (Pilate *et al.*, pp. 63–72). Fifteen poplar cDNA encoding fasciclin-like arabinogalactan proteins (PopFLAs) were characterized by Lafarguette *et al.* (pp. 107–121). Ten PopFLAs were specifically expressed in tension wood.

Brunner & Nilsson discuss new genomic approaches for understanding the control of flowering and its onset in poplars. They pointed out how many aspects of the control of flowering are different in large perennial plants from that in annuals such as *Arabidopsis*. Competence for flowering, and timing of initiation, has long-term temporal (years) and complex spatial (crown and shoot position) dimensions that are very poorly understood. Numerous genes have been identified in the large MADS-box gene family that participate in control of the timing of flowering, as well as determine floral organ structures. In a large phylogenetic analysis of poplar MADS-box genes, they found that the much studied *FLC* (flowering locus C) clade of MADS-box genes appears to be entirely absent in poplar. This gene, whose expression delays flowering, takes part in epigenetic control of flowering time in *Arabidopsis*. Stratification, a cold treatment that accelerates flowering, represses *FLC* expression. Overexpression of a poplar form of *FLC* was therefore expected to be a powerful means for engineering sterility for gene containment in poplar via postponement of reproductive maturity. However, it looks as though poplar has other things in mind; it has taken a different evolutionary path in the regulation of its flowering than has *Arabidopsis* and the rest of the Brassicaceae. Poplar bioengineers may therefore also need to chart a different path.

Adaptation to biotic and abiotic stresses

Infection by rust fungi has a devastating impact on poplar plantations worldwide. Although some resistance loci have been genetically identified, no disease resistance genes have yet been cloned despite the significant advances in this

field overall (Talbot, 2003). A number of genes have likely evolved in the host to defend against pathogens, and many of these resistance genes may be clustered together on chromosomes. In an attempt to elucidate the molecular mechanisms of *Melampsora* rust resistance in *P. trichocarpa*, Lescot *et al.* (2004) and Yin *et al.* have mapped two resistance loci, *MXC3* and *MER*, and intensively characterized the flanking genomic sequence for the *MXC3* locus and the level of linkage disequilibrium in natural populations. The *MER* gene appears to be embedded in such a cluster, making it difficult to identify the particular resistance gene of interest from among the many closely linked candidates. On the other hand, the *MXC3* gene may well exist outside of such clusters, thereby facilitating functional characterization.

Poplars are also challenged by many other biotic stresses, including herbivores (Arnold *et al.*, pp. 157–164) and viruses. Smith *et al.* (pp. 123–136) provide a detailed microarray analysis of changes in the poplar transcriptome in response to abrasion and inoculation with the poplar mosaic virus. Making use of the microarray capabilities of the poplar molecular biology group in Umeå, Sweden, they hybridized arrays with more than 10 000 gene targets represented. When abrasion or virus inoculation was considered, more than 2000 targets were elevated in expression and 740 were decreased in expression. When virus effects were considered independently of inoculation, 600 genes showed an increase of expression. Of the genes that exhibited the largest increase in transcript abundance in response to viral infection, seven were predicted to encode metallothioneins. They used cluster analysis to identify sets of genes with similar expression patterns and found many groups with distinct expression spectra. For example, 25 genes were identified that were important to lignification and cell wall development, key means for plant defense.

In an effort to understand processes which are related to heavy metal sequestration, Kohler *et al.* identified six genes and their flanking regulatory sequences in the poplar genome sequence that were similar to known metallothionein genes. Metallothioneins are thought to be important to heavy metal sequestration, but are also likely to have additional roles in development and stress tolerance (see for example Guo *et al.*, 2003). They used reverse transcription PCR to show that the genes had widely varying tissue-level patterns of expression, supporting the hypothesis that metallothionein genes have a diversity of physiological functions. Yeast has been a useful tool for modelling likely plant responses in heavy metal tolerance (Clemens & Simm, 2003). Kohler *et al.* found that one gene, when overexpressed in transgenic yeast, markedly elevated the ability of yeast to tolerate toxic concentrations of cadmium. This gene may therefore be a useful tool for identification of natural variants, or genetic engineering, to increase heavy metal tolerance and bioremediation capacity in poplars.

Metabolism

Jing *et al.* (pp. 137–145) produced eight independent transgenic events with an overexpressed glutamate synthase gene from pine. They tested the transgenic poplars in a field trial in Spain for three years and found that the transgenics were an average of 41% taller than controls, had normal lignin and polysaccharide contents, but had higher nitrogen concentrations in their stems. They suggested that the results were promising as a means to bolster productivity of poplar plantations, but that more studies were needed to understand productivity–nutrition relationships, and impacts on final plantation yield. This kind of work might also provide a new way to engineer trees to elevate nitrogen uptake from the environment for aid in biofiltration. Poplars are often used near to farms, along streams in cities and rural areas, and for treatment of municipal wastes.

Defence against herbivory requires complex metabolic adjustments (Gatehouse, 2002). It is approached in this issue by Arnold *et al.* (2004) through examination of the influence of source-to-sink carbohydrate flow on the development of constitutive and inducible levels of phenylpropenoids in hybrid poplar foliage. They wished to determine whether secondary metabolic processes in plant modules can be inhibited in a predictable manner by events such as herbivory and the development of new leaves or reproductive structures which alter the path of phloem-borne resources. They found that high and inducible sink strength in developing poplar leaves provides resources for phenolic biosynthesis and, as a result, argue that restriction or re-direction of carbohydrates can affect foliar quality for herbivores. Sink strength and the vascular architecture of plants, which confers upon them a modular nature, can determine the direction and magnitude of defense responses in trees.

Carbon fluxes in trees are strikingly altered by the development of ectomycorrhizal symbioses (Nehls *et al.*, 2001; Nehls, 2003). Grunze *et al.* (pp. 147–155) studied five putative monosaccharide transporter genes isolated from ectomycorrhizas between poplar and the fungus *Amanita muscaria* that may provide new means for dissecting carbon transfer. Three highly expressed genes were studied in depth: two had reduced expression upon infection; and one had markedly increased expression upon inoculation. The latter gene had a very high basal expression level in non-inoculated poplar roots. They argue that its expression pattern suggests that plants actively compete with fungi for hexoses even while infected, and this gene may provide an important part of the mechanism whereby sugar export in response to nutrient supply by the fungal partner is regulated.

Perspectives

The poplar research highlighted in this issue gives substance to the promise of poplar genomics that has been touted by

many, including ourselves. It is not a hoax. It is not a scam. A tree species is being dissected with a level of genetic precision that seemed a distant dream just a few years ago. And unlike studies of *Arabidopsis*, because of the extensive wild populations and diverse uses of poplars, the implications of research for ecology, conservation, breeding, and biotechnology will often be direct.

Steven H. Strauss[1]* and Francis M. Martin[2]

[1]Department of Forest Science, Oregon State University, Corvallis, OR 97331–5751, USA. [2]UMR INRA/UHP 1136 Interactions Arbres/Micro-organismes centre INRA de Nancy, 54 280 Champenoux, France.
(*Author for correspondence: tel +1541 7376578; fax +1541 7371393; email Steve.Strauss@oregonstate.edu)

References

Andersson A, Keskitalo J, Sjodin A, Bhalerao R, Sterky F, Wissel K, Tandre K, Aspeborg H, Moyle R, Ohmiya Y, Bhalerao R, Brunner A, Gustafsson P, Karlsson J, Lundeberg J, Nilsson O, Sandberg G, Strauss S, Sundberg B, Uhlen M, Jansson S, Nilsson P. 2004. A transcriptional timetable of autumn senescence. *Genome Biology* **5**: R24.

Arnold TM, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. *New Phytologist* **164**: 157–164.

Bhalerao R, Keskitalo J, Sterky F, Erlandsson R, Björkbacka H, Birve SJ, Karlsson J, Gardeström P, Gustafsson P, Lundeberg J, Jansson S. 2003. Gene expression in autumn leaves. *Plant Physiology* **131**: 430–442.

Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. *Annual Review of Plant Biology* **54**: 519–546.

Bradshaw HD, Ceulemans R, Davis J, Stettler R. 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. *Journal of Plant Growth Regulation* **19**: 306–313.

Bradshaw HD Jr, Villar M, Watson BD, Otto KG, Stewart S, Stettler RF. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. *Theoretical Applied Genetics* **89**: 167–178.

Brunner AM, Busov VB, Strauss SH. 2004. Poplar genome sequence: functional genomics in an ecologically dominant plant species. *Trends in Plant Sciences* **9**: 49–56.

Brunner AM, Nilsson O. 2004. Revisiting tree maturation and floral initiation in the poplar functional genomics era. *New Phytologist* **164**: 43–51.

Carroll SB. 2000. Endless forms: The evolution of gene regulation and morphological diversity. *Cell* **101**: 577–580.

Cervera MT, Storme V, Ivens B, Gusmao J, Liu BH, Hostyn V, Van Slycken J, Van Montagu M, Boerjan W. 2001. Dense genetic maps of three *Populus* species (*Populus deltoides*, *P. nigra*, and *P. trichocarpa*) based on AFLP and microsatellite markers. *Genetics* **158**: 787–809.

Clemens S, Simm C. 2003. *Schizosaccharomyces pombe* as a model for metal homeostasis in plant cells: the phytochelatin-dependent pathway is the main cadmium detoxification mechanism. *New Phytologist* **159**: 323–330.

Déjardin A, Leple JC, Lesage-Descauses MC, Costa G, Pilate G. 2004. Expressed sequence tags from poplar wood tissues – A comparative analysis from multiple libraries. *Plant Biology* **6**: 55–64.

Doebley J, Lukens L. 1998. Transcriptional regulation and the evolution of plant form. *Plant Cell* **10**: 1075–1082.

Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert K, Tanksley SD. 2000. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit. *Science* **289**: 85–87.

Gatehouse JA. 2002. Plant resistance towards insect herbivores: a dynamic interaction. *New Phytologist* **156**: 145–169.

Grunze N, Willmann M, Nehls U. 2004. The impact of ectomycorrhiza formation on monosaccharide transporter gene expression in poplar roots. *New Phytologist* **164**: 147–155.

Guo W-J, Bundithya W, Goldsbrough PB. 2003. Characterization of the *Arabidopsis* metallothienein gene family: tissue-specific expression and induction during senescence and in response to copper. *New Phytologist* **159**: 369–381.

Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, Blomqvist K, Bhalerao R, Uhlen M, Teeri TT, Lundeberg J, Sundberg B, Nilsson P, Sandberg G. 2001. A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences, USA* **98**: 14732–14737.

Jing ZP, Gallardo F, Pascual MB, Sampalo R, Romero J, Torres de Navarra A, Cánovas FM. 2004. Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase. *New Phytologist* **164**: 137–145.

Joshi CP, Bhandari S, Ranjan P, Kalluri UC, Liang X, Fujino T, Samuga A. 2004. Genomics of cellulose biosynthesis in poplars. *New Phytologist* **164**: 53–61.

Jouanin L, Brasileiro ACM, Leplé JC, Pilate G, Cornu D. 1993. Genetic transformation: a short review of methods and their applications, results and perspectives for forest trees. *Annals of Forest Sciences* **50**: 325–336.

Kohler A, Blaudez D, Chalot M, Martin F. 2004. Cloning and expression of multiple metallothioneins from hybrid poplar. *New Phytologist* **164**: 83–93.

Kohler A, Delaruelle C, Martin D, Encelot N, Martin F. 2003. The poplar root transcriptome: analysis of 7000 expressed sequence tags. *FEBS Letters* **542**: 37–41.

Lafarguette F, Leplé J-C, Déjardin A, Laurans F, Costa G, Lesage-Descauses M-C, Pilate G. 2004. Poplar genes encoding fasciclin-like arabinogalactan proteins are highly expressed in tension wood. *New Phytologist* **164**: 107–121.

Lescot M, Rombauts S, Zhang J, Aubourg S, Mathé C, Jansson S, Rouzé P, Boerjan W. 2004. Annotation of a 95-kb *Populus deltoides* genomic sequence reveals a disease resistance gene 2 cluster and novel class-I and class-II transposable elements. *Theoretical Applied Genetics* **109**: 310–322.

Nehls U. 2003. Ectomycorrhizal development and function – transcriptome analysis. *New Phytologist* **159**: 5–7.

Nehls U, Mikolajewski S, Magel E, Hampp R. 2001. Carbohydrate metabolism in ectomycorrhizas: gene expression, monosaccharide transport and metabolic control. *New Phytologist* **150**: 533–541.

Peña L, Seguin A. 2001. Recent advances in the genetic transformation of trees. *Trends in Biotechnology* **19**: 500–506.

Pilate G, Déjardin A, Laurans F, Leplé J-C. 2004. Tension wood as a model for functional genomics of wood formation. *New Phytologist* **164**: 63–72.

Ranjan P, Kao YY, Jiang H, Joshi CP, Harding SA, Tsai CJ. 2004. Suppression subtractive hybridization – mediated transcriptome analysis from multiple tissues of aspen (*Populus tremuloides*) altered in phenylpropanoid metabolism. *Planta* **doi**: 10.1007/s00425–004–1291–9.

Rogers LA, Campbell MM. 2004. The genetic control of lignin deposition during plant growth and development. *New Phytologist* **164**: 17–30.

Smith CM, Rodriguez-Buey M, Karlsson J, Campbell MM. 2004. The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. *New Phytologist* **164**: 123–136.

Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, Holmberg A, Amini B, Bhalerao R, Larsson M, Villarroel R, Van Montagu M, Sandberg G, Olsson O, Teeri TT, Boerjan W, Gustafsson P, Uhlen M, Sundberg B, Lundeberg J. 1998. Gene discovery in the wood-forming tissues of poplar: analysis of 5692 expressed sequence tags. *Proceedings of the National Academy of Sciences, USA* **95**: 3330–13335.

Strauss SH, DiFazio SP, Meilan R. 2001. Genetically modified poplars in context. *Forest Chronicle* **77**: 1–9.

Talbot NJ. 2003. Functional genomics of plant–pathogen interactions. *New Phytologist* **159**: 1–4.

Tsai C-J, Hubscher SL. 2004. Cryopreservation in *Populus* functional genomics. *New Phytologist* **164**: 73–81.

Wu RL, Han HF, Fang JJ, Li L, Li ML, Zeng ZB. 2000. An integrated genetic map of *Populus deltoides* based on amplified fragment length polymorphisms. *Theoretical Applied Genetics* **100**: 1249–1256.

Wullschleger SD, Jansson S, Taylor G. 2002. Genomics and forest biology: *Populus* emerges as the perennial favorite. *Plant Cell* **14**: 2651–2655.

Yin T-M, DiFazio SP, Gunter LE, Jawdy SS, Boerjan W, Tuskan GA. 2004. Genetic and physical mapping of *Melampsora* rust resistance genes in *Populus* and characterization of linkage disequilibrium and flanking genomic sequence. *New Phytologist* **164**: 95–105.

Yin TM, DiFazio SP, Gunter LE, Riemenschneider D, Tuskan GA. 2004. Large-scale heterospecific segregation distortion in *Populus* revealed by a dense genetic map. *Theoretical Applied Genetics* **doi**: 10.1007/500122-004-1653-5.

Key words: DNA marker maps, DNA microarrays, ESTs, functional genomics, molecular tree biology, *Populus (*poplar), transgenic trees.

Hyphal fusion to plant species connections – giant mycelia and community nutrient flow

Hyphal fusion (anastomosis) is a ubiquitous phenomenon in filamentous fungi and it is widely assumed that vegetative hyphal fusion is important for intrahyphal communication, translocation of water and nutrients and general homeostasis within a colony (Glass *et al.*, 2004). In arbuscular mycorrhizal (AM) fungi, H-type hyphal fusions in root-internal mycelia were documented by Gallaud in 1905 (Smith & Smith, 1997) and frequent hyphal anastomoses in the presymbiotic growth phase were reported by Mosse (1959). These early observations have been confirmed and extended by Manuela Giovannetti and coworkers in Pisa and it appears that AM fungi resemble other filamentous fungi in the ability of hyphae to fuse. In this issue (pp. 175–181), Giovannetti *et al.* present beautiful micrographs of anastomosing root-external mycelia of a *Glomus mosseae* isolate, revealing that anastomoses can also form between individual mycelia originating from different plant species. This could potentially result in the formation of large mycelia and thereby have significant consequences for nutrient foraging by the mycotroph and for nutrient cycling in plant communities.

Fusions between individual mycelia

Giovannetti *et al.* show that anastomoses form readily in two-dimensional membrane 'sandwiches' with 30–80 cm hyphae cm^{-2} where the chance for interhyphal contact must be high. However, it seems likely that they could also form in soil, which typically contains 1500 cm cm^{-3} (see Olsson *et al.*, 2002). An important prerequisite for fusion of individual mycelia in soil is that they intermingle and that they originate from propagules of the same AM fungal isolate (Giovannetti *et al.*, 2003). The community structure of AM fungi in different ecosystems is not well understood, but is likely to be characterised by the dominance of sporulating and nonsporulating genotypes in disturbed and undisturbed systems, respectively. Accordingly, the conditions for fusion to occur will be different in these contrasting systems. In disturbed systems, fusion of individual mycelia originating from dispersed propagules could result in rapid built-up of large mycelia. The probability for mycelia meeting will depend on the initial density of infective propagules and on the distance of spread of the root-external mycelium. Such spread can be considerable in experiments with semisterile soil (Jakobsen *et al.*, 1992; Jansa *et al.*, 2003) while recent work by Rosendahl and Stukenbrock (2004) indicates that mycelia of, for example, *Glomus mosseae* may be rather small in undisturbed soil. Such limited mycelium sizes were also indicated in a field study of P uptake into pea plants, which grew equally well in symbiosis with the native AM fungi in untreated soil and with an inoculant *G. caledonium* in fumigated soil (I. Jakobsen, unpublished). Surprisingly, uptake of ^{32}P from hyphal in-growth cores by the native community was only 10% of the uptake by the inoculant fungus. This suggests that fungal spread was considerably depressed by components present in untreated, but not fumigated, soil. Occurrence and importance of anastomosis in soil could be investigated by simultaneous labelling with ^{32}P and ^{33}P of neighbouring mycorrhizal plants separated by a root-free zone. The experiment should use one AM fungal isolate and a root-free zone containing natural soil at different zone width.

The widespread occurrence of an unidentified AM fungus along 30 m transects in undisturbed plots was suggested to reflect either individual mycelia or large mycelia of at least 10 m width (Rosendahl & Stukenbrock, 2004). An attractive hypothesis is that anastomosis could have played a role in the formation of such large mycelia. Host specificity would probably be an interactive component in the formation of large mycelia from the fusion of individual ones. Specificity in root colonisation is not usually obvious in single host–fungus pairs, but appears to be common in the field situation resulting in a selection pressure of plants on AM fungal communities (Vandenkoornhuyse *et al.*, 2003; Gollotte *et al.*, 2004). The latter reported from a field study that monocultures of two grass species shared only one of the 10 AM fungal genotypes detected in the roots in total. This implies