12th *New Phytologist* Symposium

Functional genomics of environmental adaptation in *Populus*

GATLINBURG, TENNESSEE, USA

10–13 October 2004

PROGRAM & ABSTRACTS

New Phytologist

Program & Abstracts

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Functional Genomics of environmental adaptation in *Populus*

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Park Vista Resort Hotel, Gatlinburg, Tennessee, USA

Organizing Committee

Stephen DiFazio (*Oak Ridge National Laboratory, USA)*
Malcolm Campbell (*University of Toronto, Canada)*
Gerald Tuskan (*Oak Ridge National Laboratory, USA)*
Francis Martin (*INRA-Nancy, France)*
Richard Norby (*Oak Ridge National Laboratory, USA)*
Holly Slater (New Phytologist, *Lancaster, UK)*

Acknowledgments

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Abstracts compiled and edited by Holly Slater

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Dicussion Groups

Blue	Green	Red	Yellow
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Benjamin Babst	Sally Aitken	Gery Allan	Danas Baniulis
Erin Beneski	Rishikesh Bhalerao	John Bassman	Suchita Bhandari
Wout Boerjan	Brian Boyle	Marie-Béatrice Bogeat-Triboulot	Kim Brown
Victor Busov	Toby Bradshaw	Amy Brunner	Steven Brunsfeld
Sarah Covert	John Carlson	Christopher Cole	Fanny Casado
Quentin Cronk	Chris Cullis	Marie Connett	Janice Cooke
Chris Dervinis	Rebecca Doerge	John Davis	Jonathan Cumming
Steve DiFazio	Sébastien Duplessis	Silvia Fluch	Ryan Cunningham
Arun Goyal	Fernando Gallardo	Fernando Guerra	Scott Harding
David Hall	Steve Hanley	Jean-François Hausman	Sara Jawdy
Pär Ingvarsson	Anne Jambois	Berthold Heinze	Véronique Jorge
David Karnosky	Kevin Kosola	Udaya Kalluri	Christian Lexer
Edward Kirby	Tatsuya Kushida	Jaakko Kangasjärvi	Nicolas Marron
Mark Mallott	Huimin Man	Richard Lindroth	Francis Martin
Romain Monclus	Michele Morgante	Thomas Mitchell-Olds	Uwe Nehls
Michael Muratet	Matt Olson	Rich Norby	Neil Nelson
David Neale	Eric Ottow	Kimberly Norris-Caneda	Gary Peter
Andrea Polle	Heather Robertson	Colin Orians	Ryan Phillipe
Michael Purugganan	Antje Rohde	Christophe Plomion	Gilles Pilate
Anne Rae	Andreas Sjödin	Gustavo Ramirez	Jeanne Romero-Severson
Steven Ralph	Holly Slater	Jenny Renault	Pierre Rouzé
Tom Whitham	Ramesh Thakur	Nat Street	Wei Tang
Kimberly Winkeler	Chung-Jui Tsai	Helen Tai	Gail Taylor
Tongming Yin	Jerry Tuskan	Ming-Xiu Wang	Tim Tschaplinski
	Chunsheng Zhang	Claire Williams	María Victoria Garcia

Alphabetical list in back of abstract book

Questions for Discussion Session I, Monday, October 11, 2004

We hope that these questions will stimulate productive discussions that will help the group explore approaches to studying adaptive molecular variation in poplar and other species. By adaptive variation we are explicitly referring to genetically-based variation in natural populations. This does not preclude the use of well-defined genetic materials (e.g., pedigrees, transgenics, and mutants) to gain insight into mechanisms, but the ultimate goal is to understand molecular bases of adaptation in natural populations.

1. Describe the perfect model species for studying molecular bases of adaptation. This can be a theoretical organism. Some characteristics that you might want to take into account:
    a. Mating system
    b. Biogeography
    c. Genome size
    d. Population structure
    e. Evolutionary history
    f. Size of the genus and interfertility of species
    g. Ecological characteristics
    h. Size
    i. Generation time

2. How does poplar compare to the perfect model species? Where is it deficient? Where does it stand out in relation to other possible models such as *Arabidopsis*, *Mimulus*, or pine?

3. Can adaptive variation be studied directly in natural populations, or is it necessary to begin with highly defined physiological or developmental mechanisms in laboratory settings? To what extent can highly controlled laboratory studies be extrapolated to provide information about adaptation in natural populations? Can this information then be extended to communities and ecosystems?

4. What are the most appropriate traits to study? What are the relative advantages and disadvantages for studying adaptation to biotic vs. abiotic stress?

5. How well defined are the molecular and physiological mechanisms that control important adaptive developmental traits? Do we have sufficient information about the genes that control and modulate these physiological mechanisms to allow productive research on adaptive molecular polymorphisms in natural populations?

6. What are the relative advantages and disadvantages of different sources of biological material for studying molecular bases of adaptation? Pedigrees or wild populations? Pure species or hybrids?

7. How should candidate genes be chosen? How much relative emphasis should be placed on information from heterologous model organisms versus information from the target organism? What is acceptable evidence for causative associations between traits and polymorphisms? Is complementation of mutants required, or is there an accumulation of lesser evidence that would provide adequate certainty? How can transcriptomic, metabolomic, and proteomic studies enhance our understanding of, and confidence about candidate genes?

8. How should sampling be allocated for accurate estimates of population parameters such as linkage disequilibrium, nucleotide diversity, and population structure? In what situations are population-wide transects required versus intensive sampling of individual populations? How should sampling be allocated to accurately represent/detect
    a. nucleotide diversity,
    b. Linkage Disequilibrium
    c. Selection
    d. Population structure

9. What are the major scientific challenges for studying molecular bases of adaptation? Where are the greatest information gaps?

Questions for Discussion Session II, Wednesday, October 13, 2004

Please continue with unfinished questions from the previous session if necessary.

1. Where are the most critical information gaps in studying molecular bases of adaptation in *Populus*? Please include considerations of:
    a. Genomic resources
    b. Population genetics
    c. Physiology
    d. Ecology

2. Develop a research plan for studying molecular bases of adaptation in *Populus*. Include considerations of
    a. Research questions/traits to be studied
    b. Selection of study populations
    c. Sampling schemes
    d. Tool development/methodologies
    e. Analytical approaches

3. What are the prospects for leveraging the Populus genome sequence to enhance our fundamental understanding of plant adaptation and the organization and functioning of ecosystems?

Speaker Abstracts

Session I: Molecular Bases of Adaptation & Evolution in Plants
Chairperson: Toby Bradshaw

S1. Functional and adaptive significance of natural variation in relatives of *Arabidopsis*

MITCHELL-OLDS, THOMAS
Max Planck Institute of Chemical Ecology, Hans-Knoll Strasse 8, 07745 Jena, Germany

What evolutionary factors influence insect resistance in natural plant populations? We identified the MAM2 gene, an enzyme-encoding locus responsible for biosynthesis of glucosinolates, biologically active secondary compounds which provide defense against generalist insect herbivores. Fine mapping reveals that MAM2 constitutes an insect resistance QTL, caused by variation in glucosinolate profiles conferred by allelic polymorphism at this locus. A sequence survey of randomly chosen accessions indicates that the MAM2 locus is highly variable among *A. thaliana* ecotypes. Furthermore, statistical methods of molecular population genetics suggest that MAM2 is subject to balancing selection. This may be caused by ecological trade-offs, i.e., by contrasting physiological effects of glucosinolates on generalist vs. specialist insects. We then examined a large, undisturbed population of perennial *Arabidopsis lyrata*. Data from microsatellites and nuclear loci show high levels of molecular variation compatible with equilibrium neutral models. Quantitative genetic variation for morphological and phenological traits also shows very high levels of genetic variance. Little heritable variation for resistance to mustard specialists was found, whereas plant resistance to a generalist herbivore showed highly significant genetic variation. This result corresponds with patterns of insect herbivory in undisturbed natural populations, where specialist insect herbivores cause greater damage than generalists.

S2. Molecular population genetics of *Arabidopsis* inflorescence development

PURRUGANAN, MICHAEL.
Department of Genetics, North Carolina State University, Campus Box 7614, Raleigh, NC 27695-7614, USA

The timing of the onset of flowering is a major life history transition in flowering plants. Flowering time is sensitive to climactic signals, including day length (photoperiod) and prolonged cold treatment (vernalization), which serve as ecological cues to ensure that reproductive effort occurs in optimal seasonal environments. These climactic signals vary systematically with latitude, and evolutionary adaptation to these ecological cues would be expected to lead to latitudinal clines in the timing of flowering. The model genetic plant *Arabidopsis thaliana* is a wild weed distributed over a wide latitudinal range in Eurasia and North Africa, and latitude-dependent variation in flowering time might be expected to be associated with molecular polymorphisms in genes that regulate the flowering time transition in response to environmental cues. We will discuss the role that two genes, FRI and FLC, play in establishing a latitudinal cline in flowering time in this species.

S3. Identifying determinants of expression level polymorphism

DOERGE, REBECCA.
Department of Statistics, Purdue University, West Lafayette, IN 47907-2067, USA

In a current experiment sponsored by the National Science Foundation we are studying the expression level polymorphisms of quantitative trait loci (QTL) that affect the disease resistance pathways in *Arabidopsis*. Using Affymetrix GeneChip technology gene expression measurements are viewed as quantitative traits and mapped on to the known genetic map of *Arabidopsis*. The

originating parental crosses have been chosen to maximize statistically significant differential expression, and then used to initiate a recombinant inbred population. QTL analysis and gene expression analysis are used in a coordinated manner for the purpose of uncovering regulatory regions or, expression level polymorphisms (ELPs), of the *Arabidopsis* genes that control quantitative trait loci. An update on this work will be presented along with statistical analyses and simulation results that investigate the many statistical issues that are known to surround both QTL analysis and microarray gene expression analyses.

Session II: Patterns of Adaptive Variation in Tree Populations
Chairperson: Claire Williams

S4. Population and Evolutionary Genomics of Adaptation in Forest Trees

NEALE, DAVID B.[1,2], ERSOZ, ELHAN.[2], KRUTOVSKY, KONSTANTIN.[1], GONZALEZ-MARTINEZ, SANTIAGO[3] & WHEELER, NICHOLAS C[4]
[1]Institute of Forest Genetics, USDA Forest Service, [2]Department of Plant Science, University of California, Davis, [3]Centro de Investigacion Forestal, CIFOR-INIA, Madrid, Spain and [4]Department of Forestry, North Carolina State University

Population and evolutionary genomic studies in forest trees is leading to an understanding of the molecular basis of adaptation. The association genetics approach is used to discover relationships between naturally occurring allelic variation and phenotypic variation for a suite of adaptive and economic traits. This approach has been pioneered in human genetics and is leading to the discovery of genetic variation causing complex and common disease. To begin, direct DNA sequencing of candidate genes in a small sample of pine megagametophytes provides measures on nucleotide diversity, haplotype (allele) diversity and linkage disequilibrium. Conifers appear to have intermediate levels of nucleotide diversity but significant haplotype diversity in part due to very low linkage disequilibrium. These factors combine to increase power of a candidate gene association genetics approach. This approach has now been used to show associations between allelic diversity in candidate genes involved in adaptive traits such as drought tolerance and cold hardiness and phenotypic diversity for these complex adaptive traits. It will soon be possible to perform genetic selection for desirable adaptive and economic traits directly on DNA sequence and thus fully realizing the potential of marker-based breeding and conservation.

S5. Naturally occurring nucleotide diversity in candidate genes for forest tree adaptation: magnitude, distribution and association with quantitative trait variation

PLOMION, CHRISTOPHE., POT, DAVID., DERORY, JEREMY., EVENO, EMMANUELLE., GARNIER-GERE, PAULINE & KREMER, ANTOINE
UMR BIOGECO 1202, INRA Equipe de Génétique, 33612 Cestas, France

Growth, development, productivity but also abundance and distribution of long-lived organisms such as forest trees, are continuously challenged by abiotic stresses and may also be greatly affected by climatic changes in the near future. If they have to survive without migration and colonization of new habitats, these species will have to adapt to changing environmental conditions. However, because of a predicted increasing rate of global change, there is a risk of reduced potential for short-term survival and long-term adaptation compared to annual plants with much higher reproduction rate. One first question is therefore whether the present structure and amount of genetic diversity in forest tree species is sufficient to allow adaptation to future stressful conditions. The genomic toolkits are discovering remarkable variation in gene/protein expression. One second question is therefore if, how and why this expressional/physiological variation matters, i.e. affects the function and fitness of an organism in natural populations. This presentation will review the program we are developing at INRA, to identify mutations of adaptive significance based on nucleotide diversity pattern analysis, and validate the functionally important

SNPs in associating nucleotide diversity with the phenotypic variation of adaptive traits (drought stress resistance in pine and bud phenology in oak).

S6. Sequence variation and linkage disequilibrium in *Populus*

MORGANTE, MICHELE & ZAINA, GIUSI
Dipartimento di Scienze Agrarie e Ambientali, Universita' di Udine, Via delle Scienze 208, I-33100 Udine, Italy

Association studies, that make use of historical recombinants rather than *ad hoc* created ones, allow a shorter time to trait mapping, because no mapping population needs to be created, and higher resolution than traditional map-based strategies because of a larger number of meiotic recombination events. Thus, association strategies would result very effective in outcrossing long-lived plants, where the long generation times and the difficulties in obtaining segregating populations make other mapping strategies difficult. The recent availability of the *Populus trichocarpa* genomic sequence makes poplar an attractive system to test association approaches to trait mapping in tree species. Data on nucleotide polymorphisms and linkage disequilibrium levels are not available in *Populus* species, yet they are necessary to derive guidelines for the design of candidate gene association studies and to obtain inferences on the past population history. We surveyed sequence diversity at 31 loci from 12 different genotypes of *Populus nigra*, representing a total of 24 independent chromosomes sampled. Through direct sequencing of amplification products, followed by sequencing of selected cloned PCR products, we examined the frequency and the nature of polymorphisms among genotypes, we derived SNP haplotypes and analysed linkage disequilibrium (LD) within and between loci. We observed a low nucleotide variability in *Populus nigra*, if compared to other plant species, but comparable to that previously described for other forest trees. We observed a low but significant LD extending over a few kb distances. We will discuss the implications of these results for the design of an association study aimed at identifying genes involved in the determination of phenological traits.

S7. Adaptation to cold in forest tree populations

AITKEN, SALLY N.[1], HOWE, GLENN T.[2], NEALE, DAVID B.[3], JERMSTAD, KATHLEEN D.[3], CRONK, QUENTIN.[4], & GILCHRIST, ERIN[4].
[1]Department of Forest Sciences, University of British Columbia, 3041-2424 Main Mall, Vancouver, BC V6T 1Z4 ; [2] Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, OR 97331-5752; [3]Institute of Forest Genetics, Pacific Southwest Research Station, USDA, 2480 Carson Rd., Placerville, CA 95667, USA and Department of Environmental Horticulture, University of California, Davis, CA, 95616, USA;[4]University of British Columbia Botanical Garden and Centre for Plant Research, 6804 SW Marine Drive, Vancouver, B.C., V6T 1Z4

Populations of temperate forest tree species typically show little differentiation for selectively neutral genetic markers (low F_{st}) but strong differentiation for some traits related to synchronization with local climate including phenology and cold hardiness (high Q_{st}). This makes these traits ideal for the study of genomic architecture of local adaptation. Strong genetic tradeoffs between growth rate and cold hardiness exist among, but not within, populations. The timing of growth cessation appears to be under stronger selection than the timing of growth initiation. High levels of variation in cold hardiness in fall and spring are largely a function of the timing of acclimation and deacclimation. Little variation exists within species for cold hardiness during active growth or in maximum hardiness in winter. QTL studies indicate cold adaptation traits are under the control of many genes with small effects. QTL-by-environment interactions are common. Association studies of candidate genes using large numbers of individuals from multiple environments offer potential for developing ecologically relevant, selectively non-neutral markers. Such markers could be used to understand the genetic basis of local adaptation, prioritize populations for conservation, and predict the ability of populations to adapt to a changing climate. Peripheral, disjunct populations may provide phenotypic extremes and unique

nucleotide sequences for such studies, but may also have higher linkage disequilibrium than core populations.

S8. Community and ecosystem genetics of *Populus*: a consequence of extended phenotypes

WHITHAM, TOM
Department of Biological Sciences & the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011, USA.

The heritable genetic variation within individual species, especially dominant species such as *Populus* spp., has community and ecosystem consequences. These consequences represent extended phenotypes, i.e., the effects of genes at levels higher than the population. Extended phenotypes can be traced from the individuals possessing the trait, to the community, and to ecosystem processes such as leaf litter decomposition and N mineralization. Common garden experiments show a heritable component to community structure and species richness. Furthermore, feedback loops suggest that extended phenotypes feed back to affect the survival of tree genotypes possessing those traits. Documenting community heritability and feedback loops are essential first steps in demonstrating community evolution. These findings have important basic and applied implications. For example, the loss of genetic diversity in a dominant tree can result in the loss of community members that are dependent upon specific tree genotypes for their survival. The community genetics of *Populus* represents a model system for developing the field of community and ecosystem genetics.

Session III: The Poplar Molecular Toolbox

Chairperson: Berthold Heinze

S9. A structural overview of the *Populus* genome

DIFAZIO, STEPHEN P.[1], YIN, TONG-MING.[1], PUTNAM, NIK.[3], CHEN, GWO-LIANG.[2], LOCASCIO, PHILIP F.[2], DUBCHAK, INNA[4], STERCK, LIEVEN[5], ROKHSAR, DAN.[3], LARIMER, FRANK.[2], & TUSKAN, GERALD A.[1]
[1]Environmental Sciences Division and [2]Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA; [3]DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598; [4]Lawrence Berkeley National Laboratory, Berkeley, CA 94720;[5]Ghent University, Technologie park 927, B-9052 GENT, Belgium.

The international effort to sequence and characterize the *Populus* genome is a pioneering event for woody perennial plant research. The genome was sequenced to 8.1X depth by the Joint Genome Institute of DOE, supplemented by end-sequencing of a 10X BAC library by Genome Canada. Assembly was accomplished using the JAZZ shotgun sequence assembly program, coupled with physical mapping data derived from BAC fingerprinting and genetic mapping using 688 microsatellite loci. The assembled genome consists of 19 map-based scaffolds (corresponding to all *Populus* chromosomes) containing approximately 307 megabases (Mb) of genome sequence. Physical distance was directly proportional to genetic distance, but with substantial variation across the genome. An additional 177 Mb of sequence is contained in nearly 22,000 unmapped sequence scaffolds, many of which consist of repetitive, noncoding DNA. We have characterized repeat composition of the genome using several independent methods, including an assessment of the frequency of 16mers in raw sequence reads, and all-vs-all blast searches of sequence scaffolds followed by clustering and multiple sequence alignment of repetitive elements. We have identified over 1000 putative transposable elements and over 25,000 uncharacterized repeat elements, comprising approximately 25% of the assembled genome. Repeat occurrence is inversely proportional to the size of sequence scaffolds. In addition, approximately 27% of the raw sequence reads remain unassembled, and repeat composition is higher than that of assembled reads. Map-based methods will be used to

assemble the vast majority of euchromatic DNA, thus circumventing the difficulties posed by repetitive DNA for assembly of whole-genome shotgun sequence.

S10. Towards annotation of the poplar genome

STERCK, LIEVEN *, ROMBAUTS, STEPHANE *, DEGROEVE, SVEN, VAN DE PEER, YVES & ROUZÉ, PIERRE
*Bioinformatics & Evolutionary Biology, VIB Department of Plant Systems Biology & INRA-associated Laboratory, Ghent University, Technologie park 927, B-9052 GENT, Belgium. *contributed equally.*

The genome sequencing of the female *Populus trichocarpa* clone 'Nisqually-1'is now in its finishing state, under the coordination of the International *Populus* Genome Consortium. Next to other teams at JGI and ORNL, our team has taken up the task to provide a comprehensive gene models and to contribute to annotation of the entire genome.

We developed a poplar-specific gene prediction system, based on the Eugene platform initially developed for *Arabidopsis*. Eugene is a DAG integrative system which allows to plug-in and weight *a priori* all information available for such a task, i.e. both *ab initio* gene prediction component software and outputs of database comparisons to proteins, ESTs/cDNAs and genome sequences (especially *Arabidopsis thaliana* in this case).

Eugene gene prediction operates on full length chromosome sequences where large repeats such as transposable elements have been masked, to avoid annotating such elements as genes. Large repeats were retrieved and the most widely represented transposons in the poplar genome were annotated.

The *ab initio* prediction components from Eugene, coding potential, splice sites and translation initiation sites, were all specifically trained for poplar on a carefully checked gene data set using documented genes from poplar and knowledge from closely related species. A novel SVM-based software, called SpliceMachine, was developed and used for splice site prediction.

Eugene, trained as described and using database information, predicts about 50,000 protein-encoding genes on the current version of the *Populus trichocarpa* genome sequence.

The apparent occurrence of recent genome duplication within poplar, posing a potential problem for proper assembly, was also investigated. Within the publicly available EST data collection of different plant species, we identified paralogous EST sequences after which the time of their duplication was inferred by estimating the number of synonymous substitutions. This way, we found evidence for a large-scale gene, most likely an entire genome duplication event that occurred about 8 million year ago.

S11. Molecular analysis of dormancy – Integrating genetics and genomics to understand environmental adaptation

BHALERAO, RISHIKESH P.
Umeå Plant Science Center, S-901 87 Umeå, Sweden

The wood forming tissue in plants, the vascular cambium cycles between active and dormant state. The transition of the cambium from active to dormant state is regulated by environmental and hormonal signals. We have taken a genomics approach to unravel the genetic networks underlying the transition of the cambium from active to dormant state in poplar. Our results indicate that during transition to dormancy, there is reduction in the complexity of the cambial transcriptome. Global transcript profiling indicates activation of 707 genes in the dormant cambium. These genes include those involved in cold tolerance, starch breakdown and glyoxalate cycle. We have also used poplar clones with differential timing of growth cessation in

order to identify the underlying changes in the genes expression patterns. The results indicate not only temporal differences between the two clones but also differential response at the level of activation and repression of several genes. This data is now being used to identify candidate genes involved growth cessation and cold hardiness.

S12. Transformation and adaptation: power in forward and reverse

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[1] School of Forest Resources and Environmental Science, Michigan Technological University Houghton, MI 49931-1295, USA. [2] Department of Forest Science, Oregon State University, Corvallis, OR 97331-5752, USA.

Plant transformation and regeneration systems have become indispensable parts of functional gene discovery and characterization over the last two decades. However, adoption of transformation methods in studies of plant adaptation to natural environments has been slow. This is a result of poor genomic knowledge and inefficient transformation systems in species of ecological significance, and legal difficulties in conducting ecologically meaningful field tests of genetically modified organisms. In trees, where long generation cycles, high background polymorphism, large sizes, and outcrossing systems of mating make production of near isogenic lines and large experiments difficult, transformation is an attractive alternative for making direct linkages between genes and adaptively significant phenotypes. We outline the capabilities, challenges, and prospects for transformation to become a significant tool for studying the molecular ecogenetics and ecophysiology of adaptation to environment in trees. Focusing on poplars as model species, we describe how two transformation-centered approaches can provide insights into the genes that control adaptive traits. Activation tagging is a forward (phenotype to gene) genetic screen that, when coupled with field testing, appears capable of identifying large numbers of genes that control traits of adaptive significance. Reverse (gene to phenotype) approaches include RNAi-based suppression and induced suppression or over-expression. They provide the means to rigorously test the function of any genes identified in model organisms or via microarray or association genetic analyses. The availability of the poplar genome sequence, and its large EST databanks, facile transformation, and rapid growth, enable reverse transgenic approaches to be used to test virtually any hypothesis of gene function in poplars.

S13. Transcriptomic adaptation to mutations

ROHDE, ANTJE.[1], CHRISTENSEN, JØRGEN H.[1], ANDERSSON, SARA.[2], STORME, VERONIQUE.[1], MORREEL, KRIS.[1], GOEMINNE, GEERT.[1], RALPH, JOHN.[3], MESSENS, ERIC.[1], SUNDBERG, BJØRN[2] & BOERJAN, WOUT.[1]
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Lignin is an aromatic heteropolymer that is mainly deposited in secondary thickened cells. Although the biosynthetic pathway of the monolignols has been well described, only fragmentary knowledge exists on its regulation and its interaction with other metabolic processes and plant development. How these interactions can be revealed with the advent of genomic tools will be illustrated with two examples. In poplar, we have compared wild type and transgenic plants down-regulated for *CAD* and *COMT*, using 13k poplar microarrays, and show that a discrete set of genes is altered in expression. In *Arabidopsis*, we have compared mutants defective in two members of the *PAL* gene family by cDNA-AFLP and microarrays. These mutants have no clear visible phenotypic alterations, yet combined transcriptome and metabolite profiling demonstrates that the functions of these apparently redundant genes can be resolved by thorough molecular phenotyping. We show that mutations in single *PAL* genes do not only affect the expression levels of particular genes within phenylpropanoid biosynthesis, but also in other metabolic pathways, revealing interactions at the transcript and metabolite levels. Together, these data demonstrate how plants adapt to a single mutation by altering their transcriptome.

S14. Functional genomics in *Populus*: towards understanding plant–herbivore interactions

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Plants respond to insect herbivory with a combination of direct (e.g. protease inhibitors, condensed tannins) and indirect defenses (e.g. phenolic and terpenoid volatiles that attract natural enemies of herbivores). To understand the inventory and regulation of defense mechanisms in poplar, the Treenomix project has developed standard, normalized, and full-length cDNA libraries from a range of tissues (xylem, phloem, bark, foliage, roots and cultured cells) at different developmental stages, as well as from trees exposed to biotic stress (insect herbivory), chemical elicitors, nutrient depletion or mechanical wounding. Thus far, we have obtained 100,000 3' and 25,000 5' expressed sequence tags (ESTs). Using this sequence resource we have developed a cDNA microarray containing ~15,000 unique cDNAs, including ~5,500 cDNAs derived from insect-treated libraries. This array has been utilized to examine transcript profiles of leaves from *Populus trichocarpa x deltoides* exposed to continuous feeding by the defoliating insect *Malacosoma disstria* (forest tent caterpillar) over a 48 hour time-course, compared to leaves treated with mechanical wounding, *M. disstria* regurgitant, several chemical elicitors, rust infection, as well as untreated control leaves. A summary of this data will be presented, with a focus on transcriptional regulation of defense mechanisms.

Session IV: Molecular Bases of Adaptation in Poplar
Chairperson: Quentin Cronk

S15. Global environmental change, poplar gene expression and links to quantitative genetics

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Over half of all global carbon held in vegetation is in forests but the consequences of global environmental change for adaptation of forest ecosystems remain uncertain. Limited information is available on the genetic variation associated with plant response to the major global change, that of rising CO_2 or in identifying genomic regions controlling plant traits that might be sensitive to elevated CO_2. There is a pressing need to understand more about long-term adaptation and genetic change in future CO_2 environments.

Adaptive traits that are relevant to plant productivity and ecological characteristics that determine survival, fitness and interaction with pests and pathogens should be the focus of attention and in trees, phenotypic plasticity is found in a number of important traits that determine plant productivity. To our knowledge, few studies of QTL identification in elevated CO_2 have been published. Using poplar, we have identified ninety-two QTL for twenty six traits of plants in ambient CO_2 and 79 QTL for plants in elevated CO_2. The results suggest that although many QTL

mapped in common in ambient and elevated CO_2, there was differential genetic control for a number of traits in these two CO_2 conditions, including for leaf development and quality, canopy and root architecture and canopy senescence. The significance of these finding will be considered and similar QTL analysis in *Arabidopsis thaliana* grown in ambient and elevated CO_2 compared to those in *Populus*.

In a second approach, we have utilised the large developing genomic resource in *Populus,* undertaking global gene expression analysis using poplar microarrays when the parents of our mapping pedigree were exposed to elevated CO_2, ozone and drought. These gene expression studies provide clues to the nature of tree adaptation to a changing environment.

S16. *Populus euphratica* EST sequencing and microarrays – what can we learn from a stress-tolerant species?

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To identify genes involved in stress responses and tolerance in the salt tolerant *P. euphratica,* large scale EST sequencing was combined with microarray analysis. In total 13838 sequences were obtained from 17 normalized and stress enriched subtracted libraries. A uni-gene set of 7706 ESTs was reamplied and spotted onto glass microarrays together with spiking and negative controls and 1300 birch genes with a putative stress related function. The microarrays were used for three major stress experiments; NaCl, drought and ozone, and for characterization of trees grown in the desert Ein Avdat valley in Israel. Unexpectedly, the number of stress regulated genes is much lower than would have been indicated by corresponding experiments performed in *Arabidopsis thaliana*. Analysis of the expression data indicate that desert grown trees uses synthesis of compatible solutes, maintenance of internal K^+ and Ca^{2+} levels and relief from oxidative stress as defense mechanisms. The signal intensity from the birch genes on the array is comparable to the *Populus* genes when using a *Populus* derived probe. Vice versa, hybridizing with a birch-derived probe also gives equal signal strength from the *Populus* genes, thus suggesting that *Populus* arrays might be used in a wider area of tree research.

S17. Characterization of the early response of poplar to rust infection using expression profiling

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Foliar rust diseases causes by *Melampsora* spp. have the potential to be very damaging to poplar plantations. The most effective method of reducing the risk is to select varieties that are rust tolerant. Plants respond to microbial attack by activating an array of inducible defense responses. Poplar molecular responses to *Melampsora* spp. infection are however poorly understood. To characterize the response of *Populus trichocarpa x deltoides* cv. Beaupré to virulent (98AG31) and avirulent (93ID6) strains of *M. larici-populina* and identify genes that may play a role in resistance, expression profiling of plants have been carried out. Approximately 200 (4.3%) of the ~ 4600 genes monitored showed reproducible and significant (*t*-test $P>0.05$) expression level changes in at least one of the interactions. In the incompatible interaction, defense- and stress-

response genes (e.g., PR-2, PR-3, PR-5, PR-10) showed an increase in transcripts which peaked 48h post-contact. Decreased levels of transcripts encoding primary metabolism, photosynthesis and photorespiration enzymes was observed in both interactions. Expression profiling technologies, in combination with other tools, such as RNAi, will have a substantial impact on our understanding of poplar-rust interactions and defense signaling pathways.
This research was supported by the European Commission (contract No.QLK5-CT-2002-00953-POPYOMICS)

S18. The wood underground: a glimpse of the interactions between trees and ectomycorrhizal fungi

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Regulated gene expression is an important mechanism for controlling ectomycorrhizal symbiosis development. Our research programme is aimed to elucidate the coordination between development of mycorrhiza and the differential gene expression in both partners. We analyzed RNA levels from sequential samples of symbiotic tissues of *Eucalyptus globulus bicostata or Populus tremula x alba,* and the basidiomycete *Pisolithus microcarpus* progressing through ectomycorrhiza development using cDNA arrays. We derived groups of coordinately expressed genes using hierarchical and non hierarchical clustering algorithms. Major temporal patterns of induction/repression were observed with distinct groups of early-, middle-, and late-transcriptionally responsive genes to symbiosis formation. At earliest stages, the differentially expressed fungal genes included cell wall symbiosis-regulated proteins, hydrophobins and mannoproteins, whereas transcripts coding for defense-related proteins were up-regulated in plant tissues. Middle- and late-transcriptionally responsive genes coded enzymes of glycolysis, tricarboxylic acid cycle and amino acid biosynthesis, as well as protein synthesis, auxin metabolism and signal transduction components. These investigations showed that changes in morphology associated with mycorrhizal development were accompanied by changes in transcript patterns, but no ectomycorrhiza-specific genes were detected. Further studies are now needed to (1) identify signalling molecules cordinating these processes and (2) investigate the expression of the regulated genes in environmental samples.

Poster Abstracts

P1. fAFLPs in cottonwoods: arthropod communities track plant genetic composition across hybrid zones

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Cottonwoods are dominant riparian trees that form habitat for numerous arthropod species. Cottonwoods also form hybrid zones consisting of different cross types, which appear to attract different assemblages of arthropods. We analyzed 40 trees for genetic similarity using fluorescent amplified fragment length polymorphisms (fAFLPs). Eighteen of these trees were analyzed to test the hypothesis that trees with similar genetic compositions also have similar arthropod compositions. A Bray-Curtis similarity matrix was calculated for the leaf-modifying arthropod community and regressed on the Euclidean distance matrix of 1116 fAFLP markers, with a Mantel test. Trees with similar genetic compositions had similar arthropod compositions (Mantel r = -0.1495; p = 0.048). We performed the same test on 13 trees from the hybrid zone, eliminating pure narrowleaf trees, and obtained a similar result (Mantel r = -0.3488; p = 0.004). Our results suggest that plant hybrid zones may play an important role in adaptive deme formation and evolution in leaf-modifying arthropod communities.

P2. Herbivory and jasmonic acid increase carbon partitioning to roots in *Populus*: can herbivory induce enhanced tolerance?

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Herbivory can result in remobilization of storage reserves to increase shoot re-growth over weeks, but less studied are changes in resource partitioning that happen within days of herbivory. Using Carbon-11 ($t_{1/2}$: 20.4 mins.), we tested whether carbon partitioning is altered in *Populus* in response to herbivory and jasmonic acid (JA), which is involved in defense induction. By 12hrs, herbivory and JA led to an increase [^{11}C]-photosynthate export rate from the treated leaf. Partitioning of ^{11}C became more basipetal, increasing inputs to the lower stem and roots. Transport and partitioning were similarly affected systemically, in leaves not directly treated. We hypothesize that the increased partitioning of carbon to the lower stem and roots following induction by herbivory and JA may be an important part of plant tolerance to herbivory, and possibly other stresses. An increase in photosynthate partitioning to stem and roots could allow plants to bolster their tolerance, possibly by increasing root growth or nutrient uptake to replace nutrients lost to herbivores and by temporarily augmenting carbohydrate stores in the stem and roots. A small change in partitioning after the initial attack could make a large difference in the ability of a defoliated plant to re-grow once herbivory has declined.

Research was supported by a Laboratory Directed Research and Development grant awarded by Brookhaven National Laboratory, by the U.S. DOE OBER (contract DE-ACO2-98CH10886), and by a grant from the Andrew Mellon Foundation (to CMO).

P3. Expression studies of a membrane anchored endo 1,4-glucanase gene from aspen

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Cellulose synthesis in plants involves -1,4 glucan chain initiation, elongation and termination. Earlier evidence suggests that a membrane-anchored cellulase encoded by a Korrigan (KOR)

gene is essential for cellulose biosynthesis in plants. We have isolated for the first time a full-length KOR cDNA by screening an aspen xylem cDNA library. The aspen KOR gene encodes for a membrane-anchored member of the cellulase family, a feature that is highly conserved between monocot and dicot plants. Sequence analysis of the KOR gene from aspen showed that the predicted protein shares high identity with *Arabidiopsis* KOR (82%). Moreover, gene expression studies using in situ hybridization showed higher expression level during the synthesis of secondary cell walls as compared to primary cell wall formation. The KOR expression patterns in aspen tension wood tissues were similar to the secondary wall associated CesA gene expression. Further molecular genetic analysis will clarify the functional importance of KOR in cellulose synthesis of aspen trees.

P4. Physiological and transcriptomal responses of *Populus euphratica* to drought

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Populus euphratica is a poplar species famous for its ability to cope with high salinity. Moreover, its distribution area comprises deserts with very hot and dry summers. All species of genus *Populus* are known to be rather drought sensitive and *P. euphratica* may grow in such dry areas due to its phreatophytic habit. In this experiment, we studied the ecophysiological and transcriptomal responses of *P. euphratica* to an increasing drought stress. The drought stress was applied for 6 weeks. Growth and physiological parameters were recorded at different stress intensities and root and leaf tissues were harvested for transcriptome analysis. To identify the molecular mechanisms of stress tolerance in *P. euphratica* we have sequenced ESTs from several normalized control cDNA libraries and stress enriched subtracted libraries. From this selection of 14000 ESTs about 8100 ESTs were re-amplified and spotted onto DNA microarrays. The DNA microarrays were used in a drought experiment, in a salt experiment and in comparison of natural sites. In common with the other *P. euphratica* microarray experiments, very few genes (~50) were regulated by drought. An EST database with the *P. euphratica* ESTs including annotative attributes can be viewed at http://sputnik.btk.fi.

P5. A functional genomics approach to identifying regulators of wood formation in forest trees

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Wood formation is an exquisitely dynamic process that is modulated by a variety of developmental and environmental cues. We are using a functional genomics approach to identify and characterize regulators that influence morphological and biochemical wood properties in two forest tree taxa, poplar and white spruce. To this end, we have selected as candidates for functional analyses a number of genes from forest tree EST collections that we hypothesize are regulators of wood formation. Most of these candidate genes encode putative transcription factors or other components of signal transduction networks. As a means to further define the role that the products of these genes play in wood formation and carbon partitioning, a suite of transgenic poplar and spruce trees is being created that misexpress each of the candidate genes.

In tandem with the transgenic experiments, we are also conducting manipulative physiology experiments with wildtype trees to explore how wood formation is governed by environmental factors, and specifically to examine how the candidate genes respond to these factors. Transcript profiling using microarrays will play an integral role in assessing how these candidate genes and environmental conditions act to modulate wood formation.

P6. Interacting genomes

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Gene expression is altered in both plant and pathogen upon interaction with each other. We propose a genomics approach to identify fungal and plant genes that are up- or down-regulated in interactions involving trees and biotrophic rust. Gene expression will be monitored during compatible, incompatible and non-host interactions resulting from the infection of different poplar clones with *Melampsora* species. Random sequencing of cDNA libraries generated from infected leaves and sequencing of subtracted libraries are used to identify *Melampsora* specific EST. cDNA arrays will be generated with a unique gene set and used to compare well define plant-pathogen interactions. Monitoring of poplar gene expression will be performed with cDNA arrays generated in collaboration with the Arborea project. A more complete analysis could be performed with Affymetrix gene chip arrays that should be available shortly following the release of the annotated poplar genome sequence.

P7. Within-canopy leaf plasticity and canopy architectural contrasts in hybrid *Populus*: links to productivity.

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Growers of short-rotation *Populus* have a stake in selecting the ideal clone, or ideotype, for maximizing productivity in their silvicultural setting. often, plant structural features are common selection points for an ideotype. Our objective in this study was to analyze the leaf morphology and canopy architecture of two clones similar in above-ground standing biomass and yet contrasting in physical form. Research was conducted on 3- , 4- and 5- year-old stands of hybrid *Populus* growing in Eastern Washington State. The two clones were a *P.deltoides* x *P. trichocarpa* hybrid (TD) and a *P.deltoides* x *P. nigra* hybrid (DN). Clones were found to have significant differences in leaf angle, number of leaves, and within-canopy leaf size distributions, and yet remarkably similar vertical leaf area distribution. Using a simple model, leaf angle distribution was found to contribute most highly to simulations of maximum potential carbon gain. Based on the various age classes studied for this work, the trajectory of leaf area production is well established by year four. However, if one were to elucidate suites of genes linked to particular architectural traits (leaf angle, leaf size, branching frequency and angle), this could potentially assist in more rapid phenotyping of clones.

P8. Tradeoffs between growth and secondary metabolites during environmental stress in *Salix*

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Water and nutrient deficits are common in temperate forests and negatively affect tree growth by restricting nitrogen acquisition and assimilation. In response to nitrogen deficiency, the allocation of carbon into phenolic sinks increases. Condensed tannins (CT) and phenolic glycosides (PG) are carbon-rich secondary metabolites that constitute large defense sinks in willow and closely related species. They are derived from the flavonoid and salicylate branches of the phenylpropanoid pathway. CT and PG concentration and presence are considered diagnostic of fitness allocation in *Salix* and *Populus* species. Carbon allocation into CT and PG may involve a potential long-term cost to growth. Willow species that exhibit a range of CT-PG phenotypes were subjected to stress by manipulating hydroponically nitrogen availability. The effects on CT-PG allocation and growth were analyzed using physiological and chemical data. Metabolic profiling and microarray experiments showed differential responses to nitrogen deprivation among phenotypes. Analysis of these results may reveal differential nitrogen effects on the interface of primary and secondary metabolism that are informative with respect to stress tolerance in *Salix*.

P9. Microsatellite variation in aspens (*P. tremuloides*)

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I assayed 192 aspens (P. tremuloides) from 11 populations in northern,central, and southern Wisconsin for 16 microsatellite loci. These loci represent 2, 3, 4, and 6 bp repeat units and are located on at least 13 of the 19 Populus linkage groups. Capillary electrophoresis using an Applied Biosystems 3700 provided better than 0.5 base resolution, and revealed a total of 132 alleles. All loci were polymorphic, with a mean of 8.25 alleles/locus, though the number of alleles per locus was more highly variable (2-20) than reported in other studies, which have tested much smaller numbers of loci and individuals. Contrary to several other reports on microsatellite variability, allele frequencies were tightly clustered around the modal allele frequency, and the genetic diversity (measured as alleles per locus or as expected heterozygosity) were not related to either the repeat unit size or to the number of repeats. Expected and observed heterozygosities were high (0.46 and 0.41, respectively), and the level of inbreeding seemed high (0.09) considering the high level of gene flow reflected in the slight differentiation among populations (Fst = 0.05), though not as high as reported in some other studies of aspens from the Great Lakes region or from Alberta, based on isozymes and microsatellites.

P10. Transcription factor and cell signalling genes from *Pinus radiata* and *Eucalyptus grandis* produce enduring and potentially useful phenotypes in eastern cottonwood (*Populus deltoides*)

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ArborGen is applying the principles of high-throughput functional genomics to trees to test genes that may affect wood quality and tree architectural traits. Demonstration of gene function in commercially important tree species is a key requirement for developing improved tree products based on gene transfer. *Populus deltoides* (Eastern cottonwood) is being used as a tree

functional testing platform following development of efficient transformation protocols to introduce large numbers of gene constructs into this tree species. Starting with the contigs and full-length sequences derived by ArborGen from large databases of ESTs isolated from *Pinus radiata* and *Eucalyptus grandis* by Genesis and ArborGen, systematic elucidation of the function of candidate genes selected by ArborGen is in progress. In this poster we describe phenotypes of putative transcription factor and signalling genes selected for ectopic expression under the control of constitutive and vascular-preferred promoters that have also been placed into multi-year field tests in cottonwood. Early screening of transgenic tissue cultures, glasshouse plantlets and plants being acclimated for field planting has identified lines with phenotypes suggesting potentially valuable uses. These results will be used to identify transcription factor and signalling genes that are expected to affect key commercial traits in plantation forestry.

P11. Rapid adaptive variation in flax

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The environmental induction of heritable changes in flax is a well-described system in which genomic alterations occur in response to specific, but loosely defined, environments. The relatively stable, genetically altered plants resulting from growth in these environments are termed genotrophs, which differ from one another and their progenitor line in genomic, morphological and biochemical characters. Genomic changes have been identified using RFLPs, RAPDs, representational difference analysis, and by the characterization of a complex insertion. Each of these regions that have been tested are also polymorphic in natural flax populations. The appearance of the insertion has been followed during the growth of plants under a range of growth conditions. Under some conditions it becomes homozygous before flowering and is always transmitted to all the progeny when stable genotrophs arise. Under other conditions it is never transmitted to the next generation. Therefore it appears the presence of the insertion under some conditions has the potential to be adaptive and selected, even though it is not trivial to demonstrate adaptive responses to a fluctuating environment. Some of the components included in the complex insertion also highlight polymorphisms elsewhere in the genome suggesting that these fragments are active in far-reaching, rapid stress-related genome restructuring.

P12. Integrated laboratory management system for sequencing and genotyping

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As the Oak Ridge National Laboratory Plant Genomics group expands its genotyping and sequencing activities, an integrated laboratory information management system has become imperative to coordinate millions of dollars in laboratory equipment, dozens of software packages, and hundreds of gigabytes of data. To ensure efficiency and accuracy, a central datastore universally accessible from anywhere in the laboratory will be created to collect and store all relevant data, and a pipeline for automating data processing tasks will be developed. The datastore will be located on a Linux server running Apache and MySQL. Inventory of reagents will be tracked using RFID technology. A user-friendly GUI will be developed in Java to permit seamless interaction with the datastore and pipeline. A data pipeline for EST sequencing and alignment has been produced which takes chromatograms from an ABI 3730 sequencer through a sequencing process using Phred, LUCY, RepeatMasker, and StackPack. Eventually, this will be incorporated into an overall laboratory pipeline built around a MySQL database which will track each experiment, the materials involved and their current inventory, raw data produced, and results obtained. This system will free the Plant Genomics from mundane data management tasks while ensuring greater speed, accuracy, and interpretability.

P13. Genomics and tension wood formation: toward an understanding of *Populus* adaptation to mechanical stress

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In response to a mechanical stress, trees are able to reorientate their axes thanks to the differentiation of a special wood named tension wood. In poplar, tension wood fibres are mainly characterized by an additional secondary layer, named G-layer mostly constituted of highly crystalline cellulose microfibrils orientated almost parallel to the fibre axis. These specific features account for the particular mechanical properties of tension wood.
In order to understand the mechanisms underlying G-layer formation, different cDNA libraries have been prepared from cambium, developing and mature xylem sampled in wood area with or without tension wood. 10,062 EST were sequenced from these libraries and annotated. Genes specifically or preferentially expressed during tension wood formation were identified from EST distribution in the different libraries. In addition, a poplar microarray was used to determine global gene expression pattern during tension wood formation.
Among other genes, many fasciclin-like arabinogalactan proteins (PopFLAs) appeared highly expressed in the differentiating xylem, once secondary cell wall synthesis occurred. Ten of these PopFLAs were specifically expressed in tension wood. Further immunocytochemical studies indicated that some PopFLAs accumulated at the inner side of the G layer suggesting a specific function for these proteins in the building of the G layer.

P14. Effect of zinc on glutathione metabolism in *Populus deltoides x P. nigra* (*P. x euramericana*) I-214 clone

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The release of heavy metals in the biosphere has become a widely diffuse problem. Data concerning the effects of heavy metals on plant metabolism, response mechanisms and adaptation to this stress are limited, particularly in woody species. Transitions metals as Zn and Cu are essential micronutrients for many physiological processes, but they became toxic at elevated levels. Plants express Cys-containing metal binding ligands including metallothioneins (MTs) and phytochelatins (PCs). PCs are enzymatically synthesized from the tripeptide glutathione (γ-Glu-Cys-Gly). Heavy metals cause oxidative damages, and transitions metals and oxygen metabolism are intimately linked in all the redox mechanisms of cells. As an antioxidant and PCs precursor, glutathione and its metabolism play an important role in plant response and adaptation to natural stresses.
Poplar (*Populus deltoides* x *P. nigra*) was chosen as a model system to investigate the relationship between glutathione metabolism and Zn excess. For this purpose plants of I-214 clone were grown in hydroponic systems supplied with different concentrations of Zn (1 µM, control; 1, 5 and 10 mM). The determinations have concerned: Zn accumulation; Cys, γ -EC (γ -Glu-Cys) and glutathione (reduced and oxidized form) contents, GR (glutathione reductase) and γ -ECS (γ-glutamylcysteine synthetase) expressions. Results showed that modifications of glutathione metabolism at biochemical and molecular levels are involved in poplar responses to high concentrations of Zn.

P15. Gene expression analysis and photosynthetic activity during leaf senescence in *Populus nigra*

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In order to use photosynthesis values for the quick evaluation of the physiological state of plants under stress, we want to investigate the correlation of changes in the gene expression with photosynthetic parameters. Therefore different age classes of leafs of one-year old root suckers of Black Poplar were used for analysis.
Leaves show characteristic changes in the maximal rate of net photosynthesis - measured as the assimilation of carbon dioxide under saturating light conditions (A_{sat}) – during their development. Generally the photosynthetic rate of young leaves increases until they have reached their full size and leaf thickness. This is followed by a period of constant A_{sat} in the mature leave and finally by a more or less rapid decrease in A_{sat} in senescing leaves. The absolute levels of the maximum rate of photosynthesis and the speed of leaf development and senescence are dependent on the genetic background of the individual plant, as well as environmental factors.
A cDNA chip holding 15.000 Poplar ESTs from different sources being available in house (http://www.picme.at) was used for the analysis of the gene expression patterns. Individual ESTs which originated from various tissue libraries were spotted in duplicates on commercially available glass slides.
For this experiment we selected young and mature leaves of four age stages from root suckers of identical age of one single old poplar tree. A_{sat} of 6 leaves form each of the tree fully expanded age classes was measured.
Two hours after measurement leaves were harvested directly into liquid nitrogen. Leaf material within the age classes from the different plantlets were pooled for RNA extraction. Differences in gene expression were established by comparing each age class with each other, resulting in 12 comparative hybridizations. Dye swap experiments were conducted for data verification. A statistical analysis of differentially expressed genes during leaf senescence in correlation to photosynthetic changes will be presented.

P16. Characterization of transgenic hybrid poplar overexpressing glutamine synthetase

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Glutamine synthetase (GS, EC 6.3.1.2) is a key enzyme in the synthesis and recycling of nitrogen compounds, since GS catalyses the incorporation of ammonium into glutamine, a precursor of all nitrogen compounds in plants. We introduced a pine GS gene in the hybrid *Populus tremula X P. alba* 7171 B4 clone (Gallardo *et al* Planta 210:19-26, 1999) to study its function in transgenic trees. Transgenic poplar produced a new GS holoenzyme that was associated with increased GS activity, protein and chlorophyll content in leaf, higher vegetative growth, and enhanced tolerance to water stress in transgenics (Fu *et al.,* Plant Cell Environ.26:411-418, 2003; Gallardo et al. Plant Physiol. Biochem. 41:587-594; El-Khatib et al. Tree Physiol. 24:729-736). A three-year field test of GS-transgenic poplars was carried out in Andalusia (Spain). Transgenics reached an average height that was 41% greater than controls after the third year of growth. Transgene expression affected some plant features with time, including protein content, GS and ferredoxin-dependent glutamate synthase.The analysis of stem diameter, and protein contents in the bark suggest that higher levels of reserves accumulated in the stem of transgenics. Our results suggest that modification of GS expression is an adequate strategy for tree breeding in short term plantations.

P17. Nucleotide diversity and haplotype structure at *phyB2*, a putative QTL for bud phenology in *Populus tremula*

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Phytochromes have a central role in mediating photoperiodic response and a major QTL for bud phenology in *Populus* co-locates with a member of the phytochromes gene family, *phyB2*. We have studied the patterns of DNA sequence variation in *phyB2* in European aspen (*Populus tremula*) in an attempt to elucidate the genetic basis of bud phenology. We analyzed complete sequences of *phyB2* in 24 individuals from four different sites throughout Europe (France, Austria, southern and northern Sweden). There was substantial nucleotide diversity in *phyB2* (π = 0.00927), on par with several other genes in *P. tremula*. Also, Tajima D was significantly negative (D = -1.813), indicating an excess of singleton mutations. Selective constraint across the *phyB2* gene was generally high, as evidence by a ratio of non-synonymous to synonymous diversities of π_a/π_s = 0.218. The same was true when divergence from *P. trichocarpa* was analyzed, as the K_a/K_s ratio was 0.236. Surprisingly, genetic differentiation among populations was low and not significant (Fst = 0.0083). Also, a neighbor-joining tree indicated that the *phyB2* sequences are made up of two diverged sequence clusters, but they did not correspond to geographic origin of the samples.

P18. Use of ecotilling to survey natural genetic variation in *Populus trichocarpa*

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Relatively little is known about how genetic diversity contributes to the considerable variation in phenotypic characteristics that mark different populations of dioecious and highly heterozygous species such as Populus trichocarpa. In order to address this question, we have established a reference collection of P. trichocarpa at the University of British Columbia that includes trees from more than 140 different populations spanning a range of geographic and ecological environments. The individuals in this collection exhibit great phenotypic variation even when grown under nearly uniform conditions. We have optimised a variation of TILLING (Ecotilling) to survey natural variation in this P. trichocarpa collection using a mismatch-specific endonuclease to detect DNA polymorphisms in these trees. In our pilot Ecotilling study we examined 10 different genes in individuals from 48 different populations. We analysed both within-tree variation (i.e. heterozygosity) and variation between our sample populations and the reference tree 383-2499[1] (Nisqually-1) whose genome has been sequenced. Our study reveals that there are considerable differences in the amount and type of variation between the different genes, and that for some genes allelic variation is correlated with geographic distribution. We also have preliminary evidence that Ecotilling can provide information about conserved non-coding domains of genes which may be involved in regulation of gene expression.

P19. *In vitro* plant regeneration, acclimatization and identity confirmation by RAPD of aspen hybrid (*Populus tremuloides* X *Populus tremula*) clones

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To reduce time of forest tree breeding it is important to develop methods for vegetative propagation of mature trees and mature tree quality predictions based on seedling characters. Potency of somatic embryogenesis and organogenesis of 7 clones of poplar hybrids (*P. tremuloides* × *P. tremula*) was tested in tissue culture regeneration and acclimatization

experiments. Successful regenerantion of the clones was obtained on the medium MS (Murashige and Skoog) supplemented by benzyladenine at 2.0 mg/l with highest frequency of rooting (94%) observed at low concentrations of auxin, indolilacetic acid and 20 g/l sucrose. During first adaptation step, optimal height of the plant regenerants was 2.8 - 4.5 cm (with 88.0-94% survival rate). Transfer of plant regenerants was performed during most of the vegetation period – from the beginning of May untill the end of August. The results showed that it is possible to grow plants of optimal height (1.0-1.5 m) for forest stands if the second adaptation is done at the end of June. RAPD method used to assess genetic stability of the hybrid clones did not show any polymorphism between microclones. The RAPD technique was shown to be a valuable tool for genetic stability assessment.

P20. Analysis of genes related to molecular mechanisms of copper tolerance in *Populus*

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Copper is essential for normal development of plants, however in high concentrations, it can cause oxidative stress and limit their survival. The study of genes regulating the molecular mechanisms of copper tolerance and accumulation is a basic step to develop trees useful for phytoremediation. In order to analyze the expression of that class of genes, an experiment assessed the response of ten *Populus* genotypes to copper stress. In a first stage, plants originated from rooted cuttings were grown in a hydroponical system for four weeks and treated with copper (100 μM and 1000 μM) during 72 h. Significant differences were observed among genotypes for the final root length (FRL), with genotypic variation accounting for 44.3 % of total variation. *Populus deltoides* registered the highest values for FRL and a minimal damage in aerial tissues. Thus, *P. deltoides* and (*P. trichocarpa* x *deltoides*) x *P. deltoides* were selected as contrasting candidates for a second stage. In this, these two genotypes were compared, under copper stress, in relation to the levels of transcription observed for a set of selected genes related to molecular mechanisms of heavy metals tolerance and homeostasis in plants. Results obtained at this stage will be presented and discussed.

P21. Gene expression patterns of trembling aspen trees following long-term exposure to interacting elevated CO_2 and tropospheric O_3

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Expression of a unigene set of poplar ESTs was studied over the 2002 growing season using trees of the moderately ozone (O_3)-tolerant trembling aspen (*Populus tremuloides* Michx.) clone 216 exposed to elevated CO_2 and/or O_3 for their entire 5-year life history. Under the elevated CO_2 treatment, relatively small number of genes, primarily those involved in photosynthesis were highly upregulated, whereas for O_3, a higher expression of many signaling and defense-related genes and lower expression levels of several photosynthesis and energy related genes was observed. Senescence associated genes (SAGs) and genes involved in flavanoid pathway were also upregulated under O_3, with or without CO_2 treatment. Interestingly the combined treatment of CO_2 + O_3 resulted in differential expression of several genes that were not seen with individual gas treatments. This study provides the first look into gene expression following long-term exposure of trees to interacting effects of elevated CO_2 and O_3. Our results contribute to the growing knowledge base as to how trees may respond to increasing elevated greenhouse gases and increase our understanding of gene regulation under oxidative stress. Patterns of gene-specific regulation in this study were highly correlated with previously published physiological responses of aspen clone 216.

P22. The SwAsp collection: a germplasm resource for ecological and evolutionary functional genomics

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To establish a better understanding how adaptation occurs in natural populations of forest trees, a collection of aspen (*Populus tremula*) clones have been established. The collection originates from clones of individuals from an eastern and western locality at six different latitudes in Sweden. A total of 116 individuals were cloned from the twelve different sites and four clones from each individual were planted in a randomized block design at two sites; one northern (Sävar) and one southern (Ekebo) site. Furthermore, additional clones are available for controlled experiments in climate chambers and greenhouses. Phenotypic traits such as growth, bud set, chlorophyll degradation, autumn coloration are scored continuously; *Venturia* and *Malmpsora* infection and herbivory damage are scored once per season. All individuals will be scanned for variation at approximately 120 microsatellite loci. In a preliminary test, one third of the microsatellite loci scored showed variation among 8 individuals. We are also in the process of developing candidate SNPs collected from a large EST dataset.

P23. Functional genomics of phenolics regulation in *Populus*

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The regulation of leaf condensed tannins (CT) and salycilate-derived phenolic glycosides (PG) in natural cottonwood backcrosses was investigated using traditional analysis of leaf phenolics coupled with metabolic profiling and cDNA microarray hybridization. Seven backcross lines of *Populus fremontii* and *P. angustifolia* exhibiting growth/CT-PG phenotypes ranging from fast/low to slow/high were further characterized by metabolic profiling. The lines were separable on the basis of principal component analysis of their primary metabolite profiles, with higher and lower CT lines tending to segregate according to their underlying metabolism. Levels of amino acids and the Krebs cycle intermediate fumarate were greatly reduced in the high CT-PG line compared to the other lines. Three lines exhibiting differential CT-PG responses to nitrogen starvation were chosen for basal and stress-induced gene expression analysis by microarray. Lines RM5 and 1012 substantially increased their phenolic levels during stress, but at greatly differing overall metabolic costs based on key metabolite levels and gene expression changes. Line 18 mounted a weak phenolics response and exhibited reduced expression of genes directly associated with photosynthetic light harvesting and carbon fixation. Our system appears to bear promise for identification and future testing of candidate genes regulating CT-PG metabolism under stress and non-stress conditions.

P24. Molecular population genetics of inducible herbivore resistance genes in *Populus tremula*

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Plants have evolved several mechanisms to defend themselves against attack by herbivores, including secondary metabolites that deter or poison potential herbivores. However, plants also have the ability to actively defend themselves by inducible defense mechanisms. A common such defense mechanism is the production of protease inhibitors that inhibit the activity of proteolytic enzymes, resulting in starvation of feeding herbivores. Here I use molecular population genetics

methods to study the evolution of six wound-induced protease inhibitor genes in European aspen (*Populus tremula*). These genes show high levels of segregating amino acid polymorphisms and also evidence for rapid divergence between closely relates species, suggesting that positive selection may act on these genes. Models of coevolutionary "arms races" and selectively maintained hypervariability are used to characterize the population dynamics of inducible protease inhibitor genes in *P. tremula.*

P25. Auxins and hypaphorine, the indol alkaloid from the ectomycorrhizal fungus *Pisolithus microcarpus,* alter gene expression in poplar roots

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Ectomycorrhizal fungi release several active metabolites, including auxins and derivatives, in the rhizosphere. These compounds induce striking morphological and biochemical changes in root tissues of the host-plant and they likely contribute to the ectomycorrhiza development. Hypaphorine, an indole-3-acetic acid (IAA) antogonist, released by *Pisolithus* species, controls elongation rate of root hairs and induces a transitory root hair tip swelling [Ditengou *et al.*, 2001], suggesting that this indolic compound might contribute to the lack of root hairs in ectomycorrhiza. Here, we have analyzed the effect of 500 µM hypaphorine and 5 µM IAA on gene expression in poplar roots, using a poplar microarray (*i.e.*, a Unigene set of 4608 cDNAs of *Populus trichocarpa x deltoides*). Incubation in the presence of hypaphorine for 24h lead to the down-regulation of two poplar genes, an hydroxyproline–rich extensin-like protein (x 3-fold), and the hypothetical protein R73F09 (x 2-fold). The alkaloid induced the upregulation of a methionine synthetase (x 3-fold). On the other hand, IAA induced the up-regulation of these three genes, together with two aquaporins, a GTPase ß subunit and a nitrate-induced gene, whereas it repressed the metallothioneins PtdMT1,2,4. The changes in the concentration of extensin transcripts was confirmed by northern blotting and RT-PCR. The present data suggest that ectomycorrhizal fungi are able to trigger changes in root hormone metabolism through the release of active metabolites. The inhibition of root hair elongation by hypaphorine may be the consequence of the observed decrease in transcripts of extensin, a cell wall protein. Whether the hypaphorine-regulated extensin is located in root hair cell walls is currently investigated. *Acknowledgments:* Annegret Kohler was supported by an INRA postdoctoral fellowship. The present investigation was supported by grants from the INRA (Programmes '*Functional Genomics of Poplar*' and *LIGNOME*) and the European Commission INCO-DC program (contract number: ERBIC18CT-98319).

P26. Expression analysis of genes in *Populus* roots induced by auxin

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Due to its many advantageous characteristics, such as a small sequenced genome, genetic variation in natural populations, and ease of vegetative propagation, *Populus* is widely becoming accepted as the model species among trees. In addition, DOE has chosen hybrid poplar as the model bioenergy feedstock tree. Due to the growing importance of the *Populus* species, genetic and genomic resources are becoming increasingly available and are leading to a greater understanding of the functionality of the *Populus* genome. We are using these resources to further characterize the genetic controls of root growth in poplar so that these mechanisms may eventually be manipulated to improve carbon sequestration ability in belowground sinks. Because auxin is known to play an important role in lateral root growth, the expression of genes induced by auxin is being analyzed using a sequenced subtracted cDNA library from poplar roots exogenously treated with auxin. The libraries are being amplified with rolling circle amplification technology and sequenced on an ABI 3700 DNA analyzer. Preliminary data analysis indicates

that there is not strong enrichment in the libraries for auxin regulated genes. However, many poplar ESTs not currently present in the publicly available *Populus* EST library have been discovered. In addition, many of the new ESTs are homologous to sequences in *Arabidopsis* EST databases.

P27. Alignment of poplar linkage maps through SSR markers genetic mapping

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Several linkage maps have been produced for genus *Populus*. One of them, constructed at INRA Orléans have been based on 91 F_1 progeny derived from an interspecific cross between two *P. deltoides* and *P. trichocarpa* clones, using double pseudo-testcross mapping strategy and different types of markers (AFLPs, RAPDs, RFLPs, SSRs, STSs, including genes with known function). RFLPs, SSRs and STSs mapped allowed only very low alignment with the poplar maps published. Additionally, these maps were not saturated.
Interspecific F_1 progeny used has been increased to 342 F_1 genotypes to improved recombination frequency estimations. As a part of the POPYOMICS European project and in order to improved the map alignment and saturation, we used publicly available SSR primer sequence corresponding to markers already mapped. Fluorescent genotyping using M13 tailed primer technique and ABI PRISM sequencer has been used. Two to 5 markers by linkage groups were chosen to cover the 19 linkage groups defined previously corresponding to a total of more than 100 SSR primers. Additionally, 16 SSR markers were developed mining poplar EST databases (so-called EST-SSR markers). This new linkage map will be used in comparative genetics and QTL mapping and EST based alignment with poplar genome sequence.

P28. An integrated functional genomics consortium to increase carbon sequestration in poplars: the POPGENICS Consortium (POPulus GENomics to Increase Carbon Sequestration)

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Because elevated [CO_2] stimulates poplar growth, we hypothesize that comparison of poplar gene expression under ambient and elevated [CO_2] will allow us to identify and isolate genes for superior growth and improved carbon sequestration. To test this novel hypothesis, we will utilize: (a) parallel QTL and EST/gene expression studies to identify and isolate candidate genes for rapid growth; and (b) develop superior clones of (*Populus deltoides* x *P. trichocarpa)* x *P. deltoides* through engineering selected candidate genes to increase carbon sequestration and reduced flowering to enhance carbon allocation to stems and roots to increase carbon storage. We developed a multidisciplinary, multinational Consortium of scientists in a functional genomics approach to developing poplars with increased capacity for carbon sequestration and ability to cope with increasing [CO_2]. These poplar clones will be capable of growing at unprecedented rates and able to allocate more carbon belowground to roots and associated mycorrhizae, thus improving long-term carbon sequestration into both wood and soil carbon pools. This **POP**ulus **GEN**omics to **I**ncrease **C**arbon **S**equestration (POPGENICS) Consortium utilizes state of the art poplar cDNA microarrays (POPCHIPIII) provided by INRA, France and the poplar Oligo arrays constructed at Oregon State University. The cornerstones of the project are the two poplar open-air CO_2 exposure facilities (Aspen FACE in the U.S. and POPFACE in Italy).

P29. Comparative transcriptional profiling of growing leaves and roots from *Populus trichocarpa* x *deltoides* cv 'Beaupré' in response to water-deficit stress

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The response of *Populus trichocarpa x deltoides* transcriptome to water-deficit stress was assessed using experimental conditions that closely approximate stress development in the field. For dehydration treatment, 2-month-old cutting plantlets were grown for 19 days under restricted irrigation to reach 35% of the transpiration rate of fully watered plants. At this stage, leaf water potential and shoot growth were not affected. In contrast, stomatal conductance and photosynthesis of the fast growing leaf F3 were reduced by 90 and 60%, respectively. Osmotic adjustment was detected in this leaf – full turgor osmotic pressure was 20% higher than in control plant –. Root growth was stimulated although relative water content was decreased. No osmotic adjustment was observed. RNA was used in hybridizations against a poplar 4,608 cDNA microarray. The transcript level of 182 (4%) genes expressed in leaves were regulated. Genes encoding aquaporins, metallothionein PtdMT3, peroxidase, SOD and histones were up-regulated, whereas RUBISCO small subunit and a PSII 5 kDa were down-regulated. In roots, 10% of the genes showed an increased expression including ribosomal proteins, the wound-induced protein POP3, SOD and HSP70. In contrast, genes coding for several aquaporins, PtdMT1 and PtdMT2 were down-regulated. It remains to determine by knock-out experiments whether the potential function of these gene products has an adaptative role in water-deficit.

P30. Tools for sampling roots of known origin, age, and root order from mixed stands.

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How can we quickly sample roots in a mixed stand and identify their tree of origin? How can we determine both the age and root order of a sampled root? Field research on QTL's for root traits and on the effects of root age, root order, and rhizosphere on root gene expression will require answers to these questions. Air excavation is a useful method for rapidly tracing roots out from the trunk of individual trees in mixed stands, and provides the simplest method for rapidly sampling multiple root orders. Fine root recovery by air excavation and by hydropneumatic elutriation of soil cores was similar. Sampling from removable minirhizotrons or windowed rhizotrons provides a method for sampling roots of known age. Laparascopic vascular clip applicators can be used to tag individual roots of known age which are observed on a removable minirhizotron. Air excavation of these tagged roots would allow determination of their tree of origin, age and root order.

P31. Research on cellular pathways peculiar to trees in the pathway database: INOH

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Knowledge about cellular pathways peculiar to trees (e.g., wood formation) was extracted from scientific articles and stored in the INOH pathway database system (http://www.inoh.org/). Subsequently, the examples of their practical use in this system were investigated. Their possible contribution to progress in the field of molecular biology in tree physiology was estimated.

Curated information includes that of molecules such as proteins, chemicals, and the like, environmental factors, the type of organ, tissue, and cell and growth stage. Each biological event is represented by the pathway graph in which genes, gene products, chemicals, environmental factors, and physicochemical reactions (e.g., phosphorylation) are described by nodes and the relations (e.g., activate) among them are described by edges. Already curated pathways include the "circadian clock regulation," "inhibition of hypocotyls elongation," "lignin biosynthesis," and "cell cycle regulation." These pathways comprise 42 sub-pathways, such as the "coniferyl alcohol export to cell wall." The organisms include five species, such as *Populus tremuloides*. With this system, on the pathway graph, it is possible to visually understand the function of genes, which are of interest to experimental researchers. Further, a comparison and analysis of the pathways under various conditions (e.g., by organisms) is expected.

P32. Defence response in poplar: a combined approach of transcript profiling and functional analysis

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With their long life cycle, trees must have developed distinct defense mechanisms allowing long term survival. Poplar is a fast growing species found world-wide and is economically important. Unfortunately poplar is susceptible to several forest pests including poplar leaf rust caused by *Melampsora* species. To improve our understanding on molecular defense mechanisms in trees, we initiated a genomic approach based on the identification of key elements in stress signalling, combined with functional analysis. This approach will allow us to study the concerted action of candidate genes that are involved in tree defense and to understand their interactions. Genes encoding for transcription factors (WRKY and TGA) as well as genes belonging to the signalling cascade (MAP kinase, NPR1) linked with those transcription factors have been selected. Experiments on gain of function and loss of function of those candidate genes have been undertaken. We will present the molecular analysis of candidate genes, phylogenetic relationship and, preliminary results on transcript profiling and functional analysis.

P33. Towards the molecular genetic analysis of a barrier to gene flow between two ecologically divergent *Populus* species, *P. alba* and *P. tremula*.

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Barriers to gene flow play a central role in many topics of evolutionary genetics. Species barriers between ecologically divergent forest trees are particularly interesting because the genes conferring ecological character differences are expected to segregate in hybrids. Hence, hybrid populations offer a powerful tool for the genetic analysis of adaptively important traits. Perhaps the biggest impediment to unlocking the potential of interspecific populations lies in the difficulty of generating multi-generation crosses in long-lived species. Natural hybrid zones offer an attractive alternative: the increased genetic variability and admixture linkage disequilibrium (admixture LD) present in hybrid populations can be utilized for association studies. This approach has been remarkably successful for the study of disease-related genes in admixed human populations. The same methodology is applicable to long-lived trees, but evaluating its potential requires a study system with a favourable setting, i.e., extensive interspecific gene flow, pronounced interspecific character differences, and good genomic resources. We have launched a research program to study the potential of admixture LD – based studies in hybrid zones of

Populus alba and *P. tremula* in Europe. First results based on nuclear microsatellites and plastid DNA markers indicate that hybrid zones among the two species will be suitable for this purpose.

P34. Genetics and ecology of chemical defense in aspen (*Populus tremuloides*)

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Trembling aspen (*Populus tremuloides*) is a dominant species of early-successional forests over much of North America. It is a primary food plant for over 100 species of herbivores, and can experience massive defoliation over extensive areas during outbreaks by insects such as forest tent caterpillars and gypsy moths. Aspen exhibits striking phenotypic variation in secondary chemistry (phenolic glycosides and tannins), as a consequence of genetic, environmental, G x E, and developmental factors. Genetic variation in chemistry at the population level is related to a trade-off between growth and defense, and expresses itself as differential resistance to herbivores (invertebrate and vertebrate). Phenolic glycosides provide the main line of defense against many insect herbivores, explaining 65-98% of the variation in insect performance (survival, growth, reproduction) and much of the variation in defoliation rates. Aspen chemistry can influence herbivore (e.g., forest tent caterpillar, gypsy moth) performance not only directly (bottom-up effects) but also indirectly, via impacts on insect natural enemies (top-down effects). Finally, genetic, environmental, and G x E variation in aspen chemistry influence leaf litter decomposition and nutrient cycling. Differential secondary chemistry may contribute to a geographic mosaic of community assemblages and ecosystem function in landscapes dominated by aspen clones.

P35. Physiological and molecular studies of GS1a transgenic poplar: possible mechanisms for enhanced nitrogen utilization efficiency, regulation of growth, and stress tolerance

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Improved growth of transgenic poplar expressing the pine cytosolic glutamine synthetase (GS1a) prompts us to examine the central role of glutamine in overall nitrogen metabolism and in the regulation of plant growth. Growth under low nitrate was significantly increased in transgenics and total GS activity was 60% higher. At both low and high nitrate, free glutamine levels in transgenic leaves were higher than in controls. ^{15}N-enrichment experiments revealed that transgenic poplar have enhanced nitrogen assimilation efficiency, especially under conditions of low nitrogen availability. The role of glutamate/glutamine ratio in moderating nitrogen/carbon metabolism and the possible function of glutamine in promoting plant growth has been assessed using RT-PCR and real-time PCR. We have shown that transcription of the α-subunit of anthranilate synthase, a key enzyme in the initial reactions leading to IAA synthesis, is enhanced in leaves of transgenics. Furthermore, under water stress, transgenic poplar showed enhanced sustained photosynthetic electron transport capacity. Our data also suggest that transgenic lines have increased photorespiratory activity which is an effective protective energy sink to light-harvesting capacity. Preliminary mircoarray analysis has shown that under water stress, transcription of photosynthesis-related enzymes and proteins were significantly up regulated in GS1a transgenics.

P36. Heterosis and genotype x environment interaction of two poplar pedigrees grown in three contrasting environments

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The extent of hybrid vigor (heterosis) and the genotype x environment (GxE) interaction are important cornerstones in the formulation of a forest tree breeding program. When heterosis is strong, hybridization is the most effective means to improve productivity. Strong GxE interaction makes it difficult for breeders to select superior genotypes for a broad context of environments. Nevertheless, GxE interaction may be evident in two different ways: (1) the genotypic ranking may differ in different environments; and (2) the real difference between genotypes may vary in magnitude between environments without changing the ranking.

Two hybrid poplar pedigrees resulting from controlled crosses sharing the same female parent, *Populus deltoides* Marshall x *P. nigra* L. and *P. deltoides* x *P. trichocarpa* T.&G., were grown in three contrasting environments, i.e. northern Italy, central France and southern England. Juvenile growth traits, height, circumference, and stem volume, were measured on one-year-old shoots to examine the heterosis of hybrids and the GxE interaction. The performance of both pedigrees significantly differed between them as well as among the sites. A pronounced heterosis was observed and a highly significant GxE interaction was found for all traits across the sites. However, the genotypic ranking was relatively stable between sites.

P37. Adaptation to drought in *Populus*: QTL analysis of productivity and carbon isotope discrimination.

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In poplars, a high productivity is associated with a high water demand. Extension of poplar cultivation, from bottomland to upland where water availability is more irregular, needs hybrids for which productivity is affected in a lower extend by water limitation. Such trait can be appreciated by water-use efficiency (WUE), defined, at the whole plant level, as the ratio of biomass gain to water loss. At leaf level, this parameter can be indirectly estimated *via* the carbon isotope discrimination (Δ).
With the aim of dissecting genetic factors controlling productivity and drought adaptation, we have selected a *P. deltoides* x *P. trichocarpa* 336 F_1 progeny (about 720 molecular markers located on two linkage genetic maps). The experiment was done in nursery on five randomized complete blocks with one year old cuttings. Genetic control of productivity, specific leaf area, Δ and leaf carbon and nitrogen contents was studied in 2003, through QTL analysis, from the whole F_1 progeny. Preliminary results, taken on a sampling of this F_1 progeny, showed high variability and high broad-sense heritability for all parameters. Few minor QTLs inherited from *P. deltoides* parent are controlling variability of productivity in height and specific leaf area.

P38. Poplar aquaporins: what a difference a symbiont makes!

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Under natural conditions poplar tree roots are infected by certain soil fungi, together forming a specialized organ, the ectomycorrhiza. This tight association increases the competitiveness of the plant in a nutrient-limited environment and is able to increase plant survival under water stress. Since water chanels (aquaporins) regulate the water flux into and out of the cell, they might play an important role in enabling plants to persist under water stress in symbiosis.
We isolated genes of seven putative water chanel proteins from a poplar ectomycorrhizal cDNA library. Four out of these genes are preferentially expressed in roots. Mycorrhiza formation resulted in a strong increase in transcript level in roots for three genes, two of which (PttPIP2.3 and PttPIP2.5) are the most prominently expressed transporters. When expressed in *X. laevis* oocytes, the corresponding proteins of both genes are able to transport water and PttPIP2.5 reveals the highest water transport capacity measured in oocytes so far. Together these data indicate that the water transport capacity of root cells is increased in mycorrhized plants. This suggestion was confirmed by root hydraulic conductance measurements on intact plants.

P39. Population genetic patterns of candidate genes for seasonal dormancy in balsam poplar

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Intraspecific divergence in life history traits is commonly observed along latitudinal gradients in plants. Understanding the population genetics of genes associated with these traits will provide insight into adaptation and patterns of range expansion. Balsam poplar (*Populus balsamifera*) is a common forest tree throughout the boreal region of North America. More importantly, it also occurs in isolated stands in the otherwise treeless Alaskan Arctic. Previous work demonstrated balsam poplar displays a striking genetically determined latitudinal cline across its range in the timing of seasonal dormancy (e.g. bud break, cessation of growth, bud set, etc.). Our work aims to compare the population structure of candidate genes associated with seasonal dormancy to that of genes not associated with dormancy to shed light on whether these candidates are responsible for adaptation and range expansion of this high latitude plant species. Here, we report on preliminary analyses of population genetic structure of the abscisic acid insensitivity 1B (*ABI1B*) gene, a candidate for control of bud set and bud flush, to the structure of one genes that is putatively unassociated with seasonal dormancy; alcohol dehydrogenase (*Adh*).

P40. Primary characterization of the putative Na^+/H^+ antiporter *POPeu;NhaD* and its role in salt resistance

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Desertification and increasing salinity are among the world's most limiting factors for plant productivity. *Populus euphratica,* a highly salt resistant tree species, has been used for reforestation on saline soils. When exposed to excess NaCl, *P. euphratica* accumulates high sodium concentrations in leaves and develops pronounced leaf succulence, due to an increase in cell number and cell size. Using X-ray microanalysis (EDX), we found that the apoplastic space

and not the vacuole is the main site for Na^+ accumulation. A key role in protecting the cytoplasm from toxic Na^+ concentrations play Na^+/H^+ antiporters located in membranes. Searching the POPEST database, we found a sequence with high homology to the *NhaD* Na^+/H^+ antiporter gene family so far characterized only in *Vibrio cholerae* and called it *POPeu;NhaD*. Expression analysis indicated that *P. euphratica* is able to maintain *POPeu;NhaD* transcript levels similar to those under control conditions while they are strongly decreased in *Populus* x *canescens*, a salt-sensitive poplar. To address the further function of *POPeu;NhaD* in plants, *Arabidopsis thaliana* knock-out mutants have been studied.

P41. Structure and expression profile of the Pi transporters *PHO1, Pht1* and *Pht2* gene families in *Populus* indicates a broad role in Pi homeostasis

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Inorganic phosphate (Pi) is a limiting factor of tree growth in forest ecosystems. Trees have evolved a series of metabolic and development adaptations (i.e., high affinity Pi transporters, ectomycorrhizas) aimed at increasing the acquisition of this vital but poorly available nutrient from the soil, as well as to sustain growth and survival under low P availability. Our knowledge of Pi transport and homeostasis in *Arabidopsis* has been extended in recent years by the cloning of several gene families encoding high and low affinity H+-Pi cotransporters (*Pht1 & Pht2*), and Rcm1 mammalian-related Pi transporters (*PHO1*). To address the role of these transporters in Pi homeostasis in poplar, we identified *Pht1*, *Pht2* and *PHO1* homologs in the JGI *P. trichocarpa* genomic sequence. These homologs have then been cloned, sequenced and their expression characterized in *P. trichocarpa x deltoides* cv. Beaupré. We used RT-PCR to examine *Pht1*, *Pht2* and *PHO1* expression in poplar tissues. They displayed differential gene expression patterns, which may be associated with the diverse roles and functions these transporters play in the import and export of Pi; *e.g.*, root uptake, loading of Pi to the xylem. Analysis of *Pht1*, *Pht2*, and *pho* mutants will help to clarify the role of these genes in Pi homeostasis.

P42. Protein expression during wood formation

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Wood is one of our most important natural resources. Surprisingly, we know hardly anything about the details of the process of wood formation. The aim of this work was to describe the main proteins expressed in wood forming tissue of a conifer species (*Pinus pinaster* Ait). Using high resolution 2-DE with linear pH gradient ranging from 4 to 7, a total of 1039 spots were detected. Out of the 240 spots analyzed by MS/MS, 71.7% were identified, 12.9% presented no homology in the databases, and 15.4% corresponded to protein mixtures. Out of the 57 spots analyzed by MALDI MS, only 15.8% were identified. Most of the 184 identified proteins play a role in either defense (20.1%), carbohydrates (16.3%,) and amino acid (14.7%) metabolisms, genes and proteins expression (13.6%), cytoskeleton (7.6%), cell wall biosynthesis (5.4%), secondary (4.9%) and primary (4.4%) metabolisms. A summary of the identified proteins, their putative functions, and behavior in different types of wood are presented. This information was introduced into the PROTICdb database and is accessible at http://cbi.labri.fr/outils/protic/ProticDB.php. Finally, the average protein amount was compared with their respective transcript abundance as quantified through EST counting in a cDNA-library constructed with mRNAs extracted from wood forming tissue.

P43. Inactivation of an arabinogalactan protein gene mediated by short interfering RNA results in alternation in cell wall properties

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Short interfering RNA (siRNA) holds great promise as a tool for identifying function of novel genes produced by genomics research projects. Here, we report efficient inhibition of an arabinogalactan protein gene *PtaAGP6* (GenBank # AF101785) by a chemically synthesized siRNA in cultured cells of loblolly pine (*Pinus taeda* L.). Stable gene silencing was obtained and confirmed by northern blot and siRNA analysis. Suppression of 83-87% *PtaAGP6* transcripts was achieved on the target site. The phenotypes including cell wall collapse, thinner in cell wall thickness, and lower cellulose content in silenced cell lines were examined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and cellulose analysis. Our results demonstrate for the first time the functionality of siRNA in silencing endogenous arabinogalactan protein gene in loblolly pine. These results support that siRNA is a valuable tool for targeted gene knockdown and for functional identification of novel genes.

P44. Quantitative expression of Kunitz-type protease inhibitors in *Malacosoma disstria*-attacked poplar: a microarray and real-time PCR approach

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Plants have evolved a variety of defense responses to protect themselves against pests. These mechanisms can be constitutive, or induced upon pathogen or herbivore attack. We have utilized the Treenomix 3,317-clone cDNA microarray to examine expression changes in response to 24hrs of *Malacosoma disstria* (Forest Tent Caterpillar) feeding in *Populus trichocarpa x deltoides*. Protease inhibitor (PI) genes were among the most strongly upregulated elements on the array, warranting further study. PIs accumulate to high levels in many plants in response to wounding and are posited to inhibit digestive enzymes in the insect's gut, thereby decreasing the nutritional value of the plant tissue. *In silico* mining of the Treenomix poplar EST database, combined with targeted sequencing, has revealed >20 full-length PIs not previously identified in poplar. Phylogenetic analysis indicates that these poplar PIs, similar to the Kunitz family of trypsin inhibitors, fall into four distinct subfamilies. To identify defensive PIs, we examined transcript levels of multiple members of each subfamily in response to *M. disstria* attack, mechanical wounding or regurgitant application, along with constitutive expression using real-time PCR. Although most PIs respond to mechanical wounding with or without regurgitant, the response to insect feeding and the temporal pattern differed among PIs.

P45. Branching Out Early: The initiation of Sylleptic Branching in *Populus*

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It has been shown that the production of late season (sylleptic) branches in *Populus* is usually linked to high yielding genotypes and successful adaptation to changing environmental conditions. Number, position and initiation of sylleptic branches on the main stem were analyzed in parental material, *Populus trichocarpa* (clone 93-968) x *Populus deltoides* (clone 111-129), and selected F_2 genotypes from family 331 (POP1 from the POPYOMICS project); both 0-3 month old and 1 year old saplings were used. The parents of the cross exhibited extremes of the phenotype. *P. trichocarpa* initiated many sylleptic branches from an early stage of development whereas *P. deltoides* did not show any signs of sylleptic production until very late in the year if at all. QTL

mapping of number and position of sylleptic branches identified a common QTL. Initiation of sylleptics was found to be related to distance from shoot apical meristem (SAM) in *P. trichocarpa*; removal of the SAM effectively removed control of sylleptic branching. This and other evidence suggests that sylleptic branching may be under hormonal control; however removal of SAM from *P. deltoides* did not initiate sylleptics, merely re-growth of the SAM so other factors may be involved. Our latest findings on transcript and hormone profiling in these contrasting species will be presented.

P46. Nitrogen availability affects wood formation in poplar

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Populus species and their hybrids exhibit strong phenotypic plasticity in response to nitrogen fertilization. In the first phase of this project, we carried out differential display in conjunction with macroarray analyses to identify putative N-responsive genes whose identities might point to cellular and molecular processes that are altered in response to nitrogen availability. These analyses revealed that genes involved in lignin biosynthesis are differentially expressed in response to nitrogen availability. These observations led us to examine the wood properties of trees subjected to high versus low levels of nitrogen in more detail. Pyrolysis molecular beam-mass spectrometry, klason analysis, and histochemistical tests all demonstrated that lignin deposition is altered under high nitrogen conditions. Microscopy studies revealed further differences in wood structure under high nitrogen versus low or adequate nitrogen conditions. Currently, we are using Q-PCR to examine the effect of nitrogen availability on the expression of other genes in the lignin biosynthetic pathway. The results to date suggest that partitioning of carbon resources into cell wall components is altered in response to nitrogen availability.

P47. Elucidating QTL for growth and development in elevated CO_2

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Limited information is available on the genetic variation associated with plant response to elevated CO_2 ($[eCO_2]$) or in identifying genomic regions controlling plant traits that might be sensitive to $[eCO_2]$. Here, QTL for leaf, stem and root growth and development were determined in a hybrid pedigree of *Populus trichocarpa* T. & G. and *P. deltoides* Marsh following a season-long exposure to either current day ambient carbon dioxide ($[aCO_2]$) or $[eCO_2]$ at 550 µl l^{-1}. Leaf and root growth and development differed between the parents such that the female *P. trichocarpa* parent showed greater response to elevated CO_2. In the F_2 generation, the traits leaf width to length ratio, petiole length, mid season leaf area, epidermal cell area, stem volume, primary and secondary root density all increased significantly in $[eCO_2]$, whilst specific leaf area (SLA), leaf plasticity and elasticity, senescence index, leaf cell number, stomatal density (abaxial and adaxial), and stomatal index (adaxial) declined. Ninety-two QTL were mapped for the twenty six traits of plants in $[aCO_2]$ whilst 79 QTL were mapped for plants in $[eCO_2]$. The results suggest that although many QTL mapped in common in ambient and elevated CO_2, there was differential genetic control for a number of traits in these two CO_2 conditions, including for leaf development, canopy and root architecture and canopy senescence. This study provides new information on developmental adaptations in future CO_2 conditions. Contrasting developmental response to $[eCO_2]$ has a large genetic component and may be the basis of long-term plant adaptation and evolution to future CO_2 environments.

P48. Using the POPYOMICS experimental network across contrasting European climates to identify robust QTL for adaptive traits.

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Quantitative Trait Loci (QTL) analysis can be used to understand the genetic control of complex traits, but QTL mapping experiments usually give poor resolution of position, and verification of the presence of QTL is rare making further study difficult. Increased competence of QTL mapping can be achieved in four ways; by repeating experiments over several years, repeating across different environments, comparison across different pedigrees or even different species, and the study of highly correlated traits. Adaptive traits such as those related to phenology and biomass can be particularly difficult to map due to small additive effects and susceptibility to environment, but many such traits are easy to score and highly correlated, therefore are ideal for this four stage approach to QTL verification.

A hybrid poplar pedigree (*Populus trichocarpa* T. & G. x *P. deltoides* Marsh) has been established in three environments across Europe, within the project POPYOMICS. Work has been carried out to assess traits highly correlated to phenology and biomass (Rae et al 2004). QTL have been mapped for stem, leaf and cell traits over a number of years using both single and multi-trait analysis to give improved mapping ability.

Rae, A.M., Robinson, K.M., Street, N.R., & Taylor, G. (2004). Morphological and Physiological Traits Influencing Biomass Productivity in Short Rotation Coppice Poplar. *Canadian Journal of Forest Research* 34, 1488-1498.

P49. The Treenomix project: A multifaceted approach to insect herbivory in spruce and poplar

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Scientists at the University of British Columbia, Vancouver, Canada, are leading a 3.5-year forestry genome project (Treenomix, http://www.treenomix.com) to develop and provide access to genomics resources for species of spruce, a conifer, and poplar, an angiosperm model for tree biology. Research of the Treenomix project targets two areas important for forestry: Forest Health and Wood/Fibre Formation. In poplar and spruce, we have completed to date much of our original goal of sequencing 200,000 3'-ESTs and 50,000 5'-ESTs, as well as 10,000 full-length (FL) cDNAs from a diverse collection of standard, normalized, and FL-cDNA libraries. This EST and FL-cDNA collection is the basis for gene- and protein expression profiling, genetic marker development and mapping, phylogenetic comparisons, functional gene discovery and biochemical gene characterization. One application of these resources has been to examine the response in *Picea sitchensis* (Sitka spruce) to herbivory by the stem-boring insect *Pissodes strobi* (white pine weevil) using a multifaceted approach. This study consisted of transcript profiling using a ~16,000 unigene cDNA microarray, protein profiling using 2-D gel electrophoresis and

LC-MS, and metabolite profiling using GC-MS. Candidate genes identified using these approaches have also been incorporated into a SNP genotyping program of *P. sitchensis* provenances to identify resistant genotypes for future tree breeding programs.

P50. Characterization of the poplar cytokinin response regulator gene family

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Cytokinin affects different physiological processes including cell division and differentiation, nutrient metabolism, apical dominance, senescence, and sink/source relationships. Cytokinin signal transduction involves a phosphoryl group transfer from a sensor histidine kinase to a response regulator. Based on homology with *Arabidopsis* response regulators sequences, we have found 22 poplar members of this family (10 type A and 12 type B). As their *Arabidopsis* counterparts, they exhibit the conserved D-D-K residues at the receiver domain, an output domain, and (only present in the type B) a conserved DNA-binding domain. Because there are no biological data regarding the pattern of expression of these genes in poplar, we determined their level of expression during development and in the presence of cytokinin. Seven type A response regulators were differentially expressed in roots, stems and leaves, while three were undetectable in these experimental conditions. They also showed different modes of regulation after cytokinin treatment, with transcript levels for five type A genes increasing, while one type A decreased. Functional testing of these response regulators will be carried out in transgenic poplars.

P51. *Populus* chemistry: the mechanistic link to extended phenotypes

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Naturally occurring hybrid zones allow examination of *Populus* genetic influences at the community and ecosystem levels. Genes largely determine the defensive chemical content of the trees, leading to extensive effects at the community and landscape levels. In a series of experiments in the field and in common gardens comprised of a range of natural and synthetic crosses of the cottonwoods *P. fremontii* and *P. angustifolia*, we examined foliar chemical contents to understand how they varied ontogenetically, clonally, and among cross-types. Leaf concentration of the main anti-herbivore chemicals, salicortin, HCH-salicortin and condensed tannins, varies in response to all three factors. Not only are the foliar concentrations of these chemicals predictive of arthropod communities, but they also affect keystone herbivores (e.g. beetles and elk) and ecosystem engineers (beaver), as well as terrestrial and aquatic nutrient cycling. Finally, these data provide key mechanistic support for the concept that genes affect levels higher than the population ("extended phenotypes"), potentially leading to community evolution.

P52. Low nonfreezing temperatures induce proteomic and physiological changes in poplar

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The effects of cold acclimation on primary metabolism in actively growing poplar (*Populus tremula* L. x *P. tremuloides* Michaux) were studied. Three-month-old poplar plants were exposed to chilling stress (4°C) and compared to plant material kept at a control temperature (23°C). The freezing tolerance of the adult leaves increased from –5.7°C for the control plants to –9.8°C after 14 days of exposure to 4°C. During acclimation, the evolution of soluble carbohydrate contents was followed in the leaves. Sucrose, glucose, fructose and trehalose accumulated rapidly under chilling conditions while raffinose content increased after one week at 4°C. Proteomic analyses by bidimensional electrophoresis performed during this stage revealed that a large number of proteins showed a higher expression while much less disappeared or showed a lower abundance. MALDI-TOF-MS analyses enabled ca. thirty spots to be proposed for candidate proteins. Among the accumulating or appearing proteins proposed, about a third presented similarities with chaperone-like proteins (heat-shock proteins, chaperonins). In addition, dehydrins and other late embryogenic abundant proteins, i.e. stress-responsive proteins, detoxifying enzymes, proteins involved in stress signalling and transduction pathways were also activated or neo-synthesised. Finally, cold exposure induced a decrease in the proteins involved in the cell wall production or energy production.

P53. Proteomic analysis of response to drought in *Populus euphratica*

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Important environmental factors such as temperature, salinity or drought are limiting productivity of crops and distribution of vegetation. Plant exposure to abiotic factors induces morphological, physiological and molecular changes. In the present work, effects of drought on protein metabolism were studied in *Populus euphratica*. Analyses were performed on fully expanded leaves. Control conditions corresponded to a relative soil humidity (RSH) of about 25%. Proteomic analyses by bidimensional electrophoresis were carried out on two different 'stress times' corresponding to a RSH of 10% and 5%. Plants were then rewatered, and after 4 days at a RSH of 25%, samples were collected. These different harvesting points were compared with plants maintained in control conditions, at the same moment. Analyses were carried out on 24 cm gels of pH 4 to 7 and 6 to 9, which were silver stained. For further analyses and identification, DiGE (fluorescence difference gel electrophoresis) technique was used. Analyses revealed that the expression of a high number of proteins was either up-regulated or down-regulated during the treatment. Mass spectrometric analyses allowed identifying different spots, e.g. Chaperonins, HSPs, Malate dehydrogenase, Kinases, CDC proteins. These results will be furthermore discussed in correlation with the physiological stress status of plants.

P54. Functional genomics of bud development and dormancy in poplar

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The seasonal cycle of growth and dormancy is a distinct character of perennial plants. The transitions of meristems into and out of dormancy are of fundamental importance to plant productivity and survival of adverse environmental conditions. Irrespective of its great importance, the process of bud development and dormancy is poorly described at the molecular level.

We follow two strategies to decipher the transcriptome during bud development and dormancy. First, the gene expression in apical buds during the whole developmental process of dormancy induction, dormancy, and dormancy release was monitored using cDNA-AFLP transcript profiling. 483 fragments with interesting expression patterns along the process were sequenced and clustered into groups. Based on homology-derived function assignment, biochemical as well as signal transduction pathways are reconstructed. Second, we conducted microarray experiments (in collaboration with UPSC) during dormancy induction in apical buds of transgenic poplar up- or downregulating the ABI3 gene, a gene that is necessary for correct bud set in autumn (Rohde et al., 2002). All cDNA-AFLP fragments and differentially expressed genes from the microarray are also placed on the poplar genome sequence to find co-localization with QTLs controlling the natural variation in dormancy-related traits (bud set, bud flush).

Rohde, A., Prinsen, E., De Rycke, R., Engler, G., Van Montagu, M., Boerjan, W. PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. Plant Cell 14, 1885-1901 (2002).

P55. Isolation and expression of a host response gene family encoding thaumatin-like proteins in incompatible poplar-rust fungus interactions

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Foliar rust caused by *Melampsora larici-populina (Mlp)* is the most damaging disease affecting hybrid poplar plantations in Europe. Leaves of *Populus trichocarpa x deltoides* cv. Beaupré accumulate several pathogenesis-related (PR) proteins during infection by the incompatible *Mlp* strain 93ID6 (pathotype 3-4). The transcripts showing the highest up-regulation 48h post-contact were coding for PR-5 proteins, a family of proteins that are induced by different pathogens in many plants and share significant sequence similarity with thaumatin. We have characterized four thaumatin-like protein (*PtdTLP*) transcripts in *P. trichocarpa x deltoides*. The corresponding genes have been identified in the JGI *P. trichocarpa* genomic sequence. We have used cDNA microarrays and RT-PCR to examine *PtdTLP* expression in poplar tissues. They displayed differential gene expression patterns, which may be associated with the diverse roles and functions PtdTLPs play in order to cope with particular environmental (pathogens, wounding, drought stress) and developmental (e.g., root development and leaf senescence) cues. Further studies will help to clarify the role of *TLP* genes in defense reactions and poplar development, and to select poplar varieties that are rust tolerant.

P56. Comparative analysis of gene expression induced by safeners and abiotic stresses in *Populus*

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Safeners are compounds that selectively protect crops against herbicide damage. Safeners act by enhancing the metabolism of herbicides in three phases; viz., oxidation, conjugation, and sequestering. Safener-inducible Expressed Sequence Tags (ESTs) were generated using the Suppressive Subtractive Hybridization (SSH) technique. A set of 1,344 EST clones were analyzed using contig and BlastX analysis, resulting in 745 to 804 hits, respectively, with a redundancy of 69%. Putative functions of over half of the clones have not been identified. Genes that are involved in all three phases of herbicide detoxification were found in the library, indicating that the SSH strategy was effective in identifying safener-inducible ESTs. The EST clones were used to construct a cDNA microarray. In order to identify unique safener-inducible genes that are not induced by other environmental stresses, we have compared safener-inducible genes with various environmental stress-inducible genes. Results will be presented on using safener-induced ESTs and cDNA microarrays to identify homologous genes that are induced by safener treatment in hybrid poplar. The microarrays and results for chilling, wind, and water stress can be applied in a broad range of studies of stress response in *Populus*, as well as in annotating the Poplar Genome.

P57. *Populus* genotypes differ in infection and systemic spread of *Poplar mosaic virus* (PopMV).

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The spatial and temporal dynamics of *Poplar mosaic virus* (PopMV) infection were investigated using four different genotypes of *Populus*. Four *Populus* clones were vegetatively propagated under sterile conditions, and used as hosts for PopMV infection in controlled growth-room experiments. The presence of PopMV RNA was assessed using reverse transcription polymerase chain reaction. The presence of infectious PopMV particles was assessed by the ability of extracts from *Populus* leaves to generate PopMV symptoms on the propagation host, *Nicotiana megalosiphon*. Neither PopMV RNA nor infectious particles accumulated to detectable levels in one of the four clones (CT6009), suggesting that this clone had pre-formed resistance to PopMV. PopMV RNA and infectious particles were restricted to the inoculated leaf in two of the clones (AP37 and NM6) suggesting that these clones either restricted the rate of virus accumulation or the spread of PopMV itself. In contrast, PopMV systemically infected one clone (52-226), suggesting that this host was either fully susceptible, or had delayed resistance. The significance of these findings is discussed from both applied and basic science perspectives.

P58. The response of the *Populus* transcriptome to wounding and subsequent infection by a viral pathogen.

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The *Populus*-Poplar Mosaic Virus (PopMV) pathosystem is the best characterised of all forest tree-virus interactions. The details of the host response to this virus are completely unknown. The transcript abundance for approximately 10000 *Populus* genes was simultaneously interrogated using spotted cDNA microarrays. Relative transcript abundance was compared for RNA extracted from *Populus* leaves that were untreated, mock-inoculated leaves that were wounded by leaf abrasion, and inoculated leaves that were abraded and then infected by virus. Statistical analysis of the microarray data identified suites of genes that exhibited increased or decreased transcript abundance in response to wounding, systemic PopMV infection or both together. Genes implicated in programmed cell death, and cell wall reinforcement were a major feature of the wound response; whereas, genes encoding metallothionein-like proteins, and proteins implicated in cell wall remodelling were a major feature of the PopMV response. The identification of wound- and PopMV-regulated genes opens the door for future studies aimed at testing specific hypotheses related to the mechanisms used by forest trees to contend with stress.

P59. Gene regulation in leaves of free-growing aspen trees

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Plants acclimations to their environment are often investigated in greenhouse experiments with a few genes coupled to specific simulated stress factors. Here, a genomic and multivariate statistic approach was chosen to analyze plants acclimations to their natural environment. Microarrays were used to measure mRNA levels in leaves collected from a free-growing aspen tree (*Populus tremula*) during the whole growing season (which in Umeå is about 1/6 to 1/10). The aim was to analyze the relationship between a) gene expression, b) weather and c) date of sampling, ultimately to define all the regulons in Populus leaves.
First, samples from eleven days, similar according to measured weather parameters and evenly spread over the growing season, were selected to pinpoint developmental changes and disregard weather-dependent changes. We have been able to separate the season into three phases. During the first month in the spring, developmental factors were the main determinants of gene expression. In the autumn, senescence regulates gene expression, but in the middle-phase, gene expression was mainly dependent on environmental factors.
Secondly, samples in the middle-phase showing large variation in weather parameters were studied, which revealed important environmental factors for both known genes like ELIP, and new putative stress adaptation genes.

P60. Microarray, QTL and ELP: can we use them in poplar to identify adaptation to drought?

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There is ever-increasing interest in the growth of Poplar as a source of bioenergy. This requires high-yield clones that can be grown intensively as short rotation coppice. Drought is the most important abiotic stress limiting yield, and future expansion of Poplar-culture will require the development of high-yielding clones that can be grown in an increasingly variable range of environmental and cultural conditions.

165 genotypes of an F_2 mapping pedigree (Family 331) were grown and exposed to drought. QTL were mapped for leaf and cell development traits, biomass yield traits, and traits associated with drought response and tolerance. Expression levels of previously identified candidate genes were measured in the progeny and QTL mapped from the expression data. Twenty genotypes with contrasting biomass yield were identified and a microarray experiment conducted to elucidate genetic mechanisms influencing yield.

The availability of the physical map of Poplar will allow comparison of the location of morpho-physiolocial and expression QTL, and the physical position of candidate genes to be examined. These results can direct breeding and the genetic improvement of Poplar.

P61. Polymorphisms in an alternatively spliced intron of the β-keto acyl ACP dehydratase gene of *Picea marianna*

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Genes associated with germination in black spruce were screened using differential display RT-PCR. One gene showing overexpression in 6-day germinated black spruce seedlings was identified as having high homology with the *Brassica* and *Arabidopsis* 3-keto acyl ACP dehydratase. Three cDNA clones of this gene were isolated from a 6-day germinated black spruce seedling cDNA library. The three clones were identical over the coding region but contained sequence variations in the non-coding regions. In addition, two of the clones contained an unspliced intron, which carried two single nucleotide polymorphisms. The intron was spliced out of the third cDNA clone. An examination of 3-keto acyl ACP dehydratase gene expression in dry and 6-day germinated seeds shows that alternative splicing of the transcript occurs in germinated seeds. We are currently examining differences in splicing efficiency between transcripts expressed from polymorphic alleles in heterozygotes.

P62. High efficiency inducible gene expression system based on activation of a chimaeric transcription factor in transgenic Virginia pine (*Pinus virginiana* Mill.)

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Inducible gene expression systems are needed in functional genomics of tree species. A glucocorticoid-inducible gene expression system was established in a gymnosperm species Virginia pine (*Pinus virginiana* Mill.) through *Agrobacterium tumefaciens*-mediated genetic transformation. This system is based on the chimaeric transcriptional activator GVG, which binds to the response sequence upstream of the gene of interest after activation by a chemical inducer and initiates transcription of the target gene. A fragment of *m-gfp5-ER* was inserted into an

optimized binary vector pINDEX3, and this vector was used to produce transgenic plants for testing this inducible system in Virginia pine. The results demonstrate that expression of the *m-gfp5-ER* reporter gene was tightly controlled and 0.1 µM of the glucocorticoid hormone triamcinolone was able to induce *m-gfp5-ER* expression in transgenic cells. Differential expression of *gfp* in transgenic cells induced by different concentrations of triamcinolone was observed and confirmed by northern blot analysis and by quantitative green fluorescence analyses with Laser Scanning Microscopy. In transgenic plantlets, triamcinolone was taken up efficiently by roots. The inducible gene expression presented in this study could be a very valuable alternative for functional identification of novel genes in plants, especially in pine.

P63. Development of a high-throughput platform for functional analysis studies in transgenic hybrid poplar

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We developed a high-throughput platform for the production of transgenic poplar to improve and extend our knowledge on the potential biological role of well-known transcription factors. In this poster, we will describe how functional genomic studies are applicable to forest tree species, with a specific emphasis on wood formation and defense response. The candidate genes are placed in a cassette with the maize ubiquitin promoter for ectopic over-expression. We previously showed that this promoter gives strong and uniform expression in all organs of poplar. Experiments are ongoing for the production and characterization of transgenic poplar for 23 different constructs. The selection of transgenic lines is based upon a qualitative assessment of GUS reporter gene activity, as well as transgene expression levels that are quantified by QPCR analysis. As an example we showed that GUS activity is relatively uniform from one transgenic poplar line to another. Moreover, there is a clear correlation of transgene expression between analyses made from *in vitro* material or greenhouse grown poplar trees. QPCR analysis also showed a clear correlation between *uidA* transcript quantification and GUS activity. Besides their utility in selecting lines for plant production, these experiments provide baseline data for the characterization of transgenic poplar lines.

P64. Genomic regions determining environmental adaptation of leaf development in *Populus*

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Leaf growth in the fast-growing tree *Populus x euramericana* was stimulated in response to long-term exposure to elevated CO_2 using FACE (free Air CO_2 enrichment) technology. Leaf development was stimulated through increased cell expansion and cell production and, using cDNA microarrays, candidate genes for this adaptation were revealed. Differential gene expression for cell cycle and cell wall genes and for their hormonal signals was confirmed and quantified relatively, using real-time PCR in a mapping population. Candidate genes were co-located with QTL to identify important *trans*-acting regions for leaf development and growth and as an aid to marker-assisted selection.

P65. Differential survival of cottonwood hybrids and parental species in relict populations

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Recent models predict increased frequency of severe drought for western North America. Since the Pleistocene, the drying out of the western U.S. should have resulted in the evolution of drought tolerant poplar genotypes, especially in the sky islands of Nevada where riparian areas have been reduced to a small fragment of their former distributions. Because hybridization in cottonwoods is known to generate novel genotypes as well as increased genetic and phenotypic variation, we predict that natural hybrids should exceed parental species in adaptation to extreme environmental change. To support this, we have identified nine isolated populations of cottonwood in Nevada with hybrid morphology. We believe these to be relicts of past populations which became fragmented during post-pleistocene climate change. We are conducting genetic marker (AFLP) surveys of these populations to verify/reject their putative hybrid nature. Preliminary results suggest two of these consist of single F_1 genotypes distributed as multiple clones. We are in the processes of conducting analyses on the remaining populations, all but one of which appear to consist of multiple genotypes. These populations represent potential "natural laboratories" to examine the genetic basis of hybrid adaptation, as well as its' effects on associated arthropod and fungal taxa. Our research is consistent with recent surveys showing that the current record drought is producing the same pattern of hybrid survival seen in the relicts.

P66. An integrated platform for comparative mapping and genome assembly in poplars: the perennial plant model system.

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We report a complete consensus genetic map constructed from two interspecific pedigrees sharing the same grandmother in *Populus*, *P. trichocarpa* clone 93-968. The consensus map was first integrated with 565 framework markers from the two pedigrees, including 131 SSRs. Another 496 SSRs were mapped on the consensus map as alternative markers. A heterogeneity test indicated that the recombination frequency was quite constant between the two families. Therefore, the consensus map provides reliable estimates of marker locations. Syntenic studies among the maps with SSRs in poplars showed a high level colinearity, and 12 new SSRs were put on the map using marker synteny. Altogether, this map contained 639 SSR loci with an average interval of 3.8 cM. More than half of the 672 SSRs designed from Nisqually-1 genomic sequence were mapped in our pedigree. Using sequences from primers of the mapped SSRs, 169 of the sequence scaffolds from Nisqually-1 were linked into chromosomal units representing 307 Mb of the genome. SSR markers are highly transferable between different pedigrees of different species due to high conservation of priming sites and heterozygosity. This map should therefore serve as a a platform for uniting studies on *Populus* species from throughout the genus. Furthermore, it will provide a widely used reference for comparative mapping, genome assembly, and future functional genomics studies in *Populus*.

P67. Identification and validation of single nucleotides polymorphisms in poplar using public expressed sequence tags

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Abstract: Using assembled expressed sequence tags (ESTs) from 14 different cDNA libaraies, containing 84,132 sequence reads, we identified 510 candidate single nucleotide polymorphisms (SNPs). 110 candidate SNPs were tested. 38 candidate SNPs were validated through directed sequencing of PCR products. In addition, 13 new SNPs in intron regions were validated. This analysis reveals that assembled ESTs from multiple libraries in public database may provide a rich source of comparative sequences to search for SNPs in poplar genome.

Key words: single nucleotide polymorphisms, expressed sequence tags, poplar

P68. Construction of cDNA Libraries with Leaves of Clones Susceptible and Resistant to Black Spot Disease in Poplar and Relevant Analysis of Expressed Sequence Tags

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Poplar is one kind of trees with most wide culture area in middle latitude region and high timber output , it is a mainly kind of trees cultured for industry artificial forest in China too. At present, poplar is cultured in the form of single pure stand in China, plant diseases and pests are serious in some area. And black spot disease in poplar is one of the diseases among them . The disease takes place in wide range, nearly distributes in the area where poplar has been cultured . Injured poplar has a dropping photosynthesis efficiency and fallen leaf ahead of times , the growth of the trees has been seriously influenced. With the application of molecular biology in forest genetic improvement , poplar has been used in the molecular biology study of forest as a kind of mode trees .This research takes the gene expression profile of the black spot disease in poplar as the research object, will expound the mechanism of resistance to disease from level of the genome and screen the disease-resistant candidate gene, and lay solid foundation for cloning the disease- resistant gene. It is significant to whole poplar genome project.
Expressed Sequence Tags (ESTs) are small pieces of DNA sequence (usually 300 to 500 nucleotides long) that are generated by sequencing either one or both ends of an expressed gene. Finding and separating new gene based on EST technology is not only the focus of human genome study, but also the important content of plant genome study. In order to isolate target genes associated with disease resistance, two cDNA libraries viz. L45-72 and L69-72 were constructed with ready-to-use kits and pathogen-inoculated poplar leaves of I-69 and I-45, two clones either extremely resistant or susceptible to black spot disease in poplar. Based on the libraries, expressed sequence tags have been sequenced by 3100 genetic analyzer on the large scale of random clones picked. The library of I-45 (L45-72) had a titer of 3.44×109pfu/mL and the library of I-69 (L69-72) had a titer of 3.94×109pfu/mL, with inserts larger than 500 bp accounting for 96.67% and 73.33%, respectively. We have sequenced more 20000 ESTs using the two libraries and the analysis work is in progress now. The preliminary assembly analysis of 8062 EST sequenced at the beginning has been done (trim-off+0.01, Q20) with the result that the number of effective EST is 7411, the average effective length is 535bp, the contig number is 3588 and singlet number is 3008. The result show that the EST has higher quality and lay a foundation for further analysis.

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