

Session VII

**QUANTITATIVE GENETICS AND
MOLECULAR BREEDING**

Session VII

QUANTITATIVE GENETICS AND MOLECULAR BREEDING

Chairs

David Neale
Janice Cooke
René Vaillancourt

Keynote Talks

SVII.1 ASSOCIATION GENETICS OF NATURAL GENETIC VARIATION AND COMPLEX TRAITS IN FOREST TREES

David Neale

SVII.2 THE ARBOREA PROJECT: THE PURSUIT OF GENES THAT UNDERLIE QUANTITATIVE VARIATION OF COMPLEX TRAITS IN WHITE SPRUCE

Janice E.K. Cooke*, Nathalie Isabel, Jean Beaulieu, Claude Bomal, Brian Boyle, Sébastien Caron, Nancy Dallaire, Walid El Kayal, Carmen C. Gibbs, Isabelle Giguère, Manuel Lamothe, Stéphanie Beauseigle, Frank Bedon, Annick Bertrand, Sylvie Blais, Catherine Dhont, France Gagnon, Susanne King-Jones, Caroline Levasseur, Patrick Meirmans, Betty Pelgas, Wenchun Zhou, Armand Seguin, Yves Castonguay, Philippe Rigault, Jean Bousquet, John Mackay

Oral Communications

SVII.3 ASSOCIATING GENETIC VARIATION IN LIGNIN GENES WITH PHENOTYPIC CHANGES IN *EUCALYPTUS GLOBULES*

Luke J McManus*, Saravanan Thavamanikumar, Desmond J Stackpole, Paul G Nevill, René E Vaillancourt, Gerd Bossinger

SVII.4 IMPROVING WOOD FIBRE PROPERTIES IN *PINUS RADIATA* AND EUCALYPTS THROUGH ASSOCIATION STUDIES

Reddy Thumma, Shannon Dillon, Nahida Bhuiyan, Julie George, Maureen Nolan, Wei Li, Jayaraj Gorantla, Xinguo Li, Charlie Bell, Colleen Macmillan, Simon Southerton*

SVII.5 TOWARD AN UNDERSTANDING OF NATURAL VARIATION FOR CARBON ALLOCATION AND PARTITIONING IN *POPULUS*: A GENETICAL GENOMICS APPROACH

Derek Drost*, Evandro Novaes, Brianna Miles, Qibin Yu, Luis Osorio, Christopher Dervinis, Gary Peter, Matias Kirst

Poster Communications

- SVII.6p ON OF PROVENANCE AND PROGENY TRIALS OF *CRYPTOMERIA JAPONICA* POPULATIONS IN AZORES ISLANDS
Carla Faria*, Jorge Belerique, Carina Nobrega, Leonor Penacho, M Helena Almeida
- SVII.7p DEVELOPMENT OF DA_rT MARKERS FOR ULTRA-HIGH-THROUGHPUT GENOTYPING OF EUCALYPTS
René E Vaillancourt*, Dorothy Steane, Andrzej Kilian, Alexander Myburg
- SVII.8p GENETIC ARCHITECTURE OF WOOD QUALITY RELATED TRAITS IN EUCALYPTUS
JM Gion*, A Carrouche, S Dewer, L Dolmare, JG Combes, H Bailleres, A Vaillant, N Ouniabi, A Saya, C Plomion, P Vigneron
- SVII.9p MOLECULAR MARKERS FOR PORTUGUESE PINE WOOD QUALITY
Jorge AP Paiva*, Ana Alves, Rita Costa, Maria I Carrasquinho, M João Gaspar, M Emilia Silva, José Morais, José Rodrigues, Alexandre Aguiar, José Louzada, Pedro S Fevereiro
- SVII.10p GENETIC ASSOCIATION STUDIES IN *EUCALYPTUS GLOBULUS* LABILL
Saravanan Thavamanikumar, Luke J. McManus *, Josquin F.G. Tibbits, Desmond J. Stackpole, René E. Vaillancourt, Gerd Bossinger
- SVII.11p FINE-MAPPING OF SEX-RELATED MARKER, IDENTIFICATION AND CHARACTERIZATION OF SEX-DETERMINING GENES IN ASPEN (*POPULUS TREMULA* L.)
Birte Pakull, Sandra Paasch, Torsten Markussen, Matthias Fladung*
- SVII.12p ELIMINATION OF MARKER GENES AND TARGETED INTEGRATION OF TRANSGENES VIA THE FLP/FRT-SYSTEM
Tobias Schenk, Dirk Becker, Horst Lörz, Matthias Fladung*
- SVII.13p CONSTRUCTION OF GENETIC LINKAGE MAPS IN ASPEN (*POPULUS TREMULA* L. AND *P. TREMULOIDES* MICHX.) AND THEIR USE FOR QTL MAPPING
Matthias Meyer*, Torsten Markussen, Gerhard Helle, Doris Krabel, Matthias Fladung
- SVII.14p QTL FOR MYCOSPHAERELLA LEAF DISEASE RESISTANCE AND WOOD PROPERTIES IN *EUCALYPTUS GLOBULUS*
René E Vaillancourt*, Jules S Freeman, Simon P Whittock, Brad M Potts

ASSOCIATION GENETICS OF NATURAL GENETIC VARIATION AND COMPLEX TRAITS IN FOREST TREES

David B. NEALE

Dept. Plant Sciences, University of California, Davis
dbneale@ucdavis.edu

Economic and adaptive traits in forest trees are generally controlled by many genes, often with small individual effects on phenotypes. We have used a QTL mapping approach for many years to identify such genes. The number, position and size of effects can be estimated but the individual genes remain unknown. Positional cloning of QTLs in large genomes (20Gb) is not highly tractable. Thus, we have turned to the association genetics approach to identify genes underlying QTLs. The association genetics approach is rather amenable to conifers and other forest trees due to: (1) large, random mating and unstructured populations of forest trees, (2) moderate to high levels of nucleotide diversity, (3) precise evaluation of phenotype due to clonal or family-based testing and (4) the rapid decay of linkage disequilibrium. We have used this approach to detect associations between candidate gene SNPs and phenotypes in several trait groups (wood properties, disease resistance and biotic stresses). A limitation of these studies, however, has been the small number (<100) of candidate genes in association screens. Recently we have completed resequencing of ~8000 candidate genes and will begin genotyping SNPs in large association populations. This approach approximates a gene-space scan. These discoveries can be applied to marker-based breeding and for gene resource management in domesticated or natural populations.

THE ARBOREA PROJECT: THE PURSUIT OF GENES THAT UNDERLIE QUANTITATIVE VARIATION OF COMPLEX TRAITS IN WHITE SPRUCE

Janice E.K. COOKE^{1*}, Nathalie ISABEL², Jean BEAULIEU², Claude BOMAL³, Brian BOYLE³, Sébastien CARON³, Nancy DALLAIRE³, Walid EL KAYAL¹, Carmen C. GIBBS¹, Isabelle GIGUÈRE³, Manuel LAMOTHE², Stéphanie BEAUSEIGLE³, Frank BEDON³, Annick BERTRAND⁴, Sylvie BLAIS³, Catherine DHONT⁴, France GAGNON³, Susanne KING-JONES¹, Caroline LEVASSEUR², Patrick MEIRMANS², Betty PELGAS², Wenchun ZHOU¹, Armand SEGUIN², Yves CASTONGUAY⁴, Philippe RIGAULT⁵, Jean BOUSQUET³ and John MACKAY³

¹Department of Biological Sciences, University of Alberta, Edmonton AB, CANADA T6G 2E9, ²Ressources naturelles Canada, Service canadien des forêts, Centre de foresterie des Laurentides 1055 rue du P.E.P.S., Québec QC, CANADA G1V 4C7, ³Département des sciences du bois et de la forêt, Université Laval, Québec QC, CANADA G1K 7P4, ⁴Agriculture et Agroalimentaire Canada, 2560 boulevard Hochelaga, Québec QC, CANADA G1V 2J3, ⁵Centre de Génomique de Québec, Centre de Recherche du CHUL, 2795 Boul. Laurier, Québec QC, CANADA G1V 4M7 janice.cooke@ualberta.ca

White spruce (*Picea glauca* [Moench] Voss) is a keystone species of Canada's boreal forest ecosystem, and together with other native spruce species forms the backbone of the nation's forest products industry. The overarching goal of the Arborea project is to identify genes that govern naturally occurring phenotypic variation in traits of commercial and adaptive significance in white spruce. We are focusing on timing of terminal bud formation – an adaptive trait that is a determinant of the length of the growing season, and thus influences productivity – along with a suite of wood chemical and physical property traits. To discover genes that underlie these complex traits, we have undertaken genomic-scale gene mapping approaches that combine high-throughput genotyping with large-scale gene expression profiling. We are conducting quantitative trait loci mapping of an outbred F1 cross, as well as association mapping of populations comprising either unrelated individuals or a collection of small families. Selection of thousands of candidate genes for development of gene-based single nucleotide polymorphism (SNP) molecular markers forms the cornerstone of these genomics strategies. The majority of candidate genes are being pre-selected through data-mining of a large set of microarray experiments that we have carried out on a unique collection of transgenic spruce mis-expressing genes hypothesized to play a role in wood formation, as well as a suite of physiological experiments. The data-mining is revealing not only candidates that one might predict by analogy to model systems, but also genes not previously associated with these traits. Selected candidate genes enter a SNP detection pipeline, and validated SNPs are being used to design highly multiplexed genotyping arrays for the IlluminaTM Golden Gate assay. This ultra high-throughput technology will generate thousands of genotypic data for the hundreds of individuals in each experimental population, enabling comparatively fine-scale mapping. Phenotyping of these individuals is being carried out by both conventional means and large-scale gene expression profiling by microarray analyses. These microarray gene expression data will be analyzed by expression-QTL (eQTL) and association mapping to reveal expression level polymorphisms, and the determinant structural and regulatory loci that constitute the genetic architecture underlying time of budset and wood properties in white spruce.

ASSOCIATING GENETIC VARIATION IN LIGNIN GENES WITH PHENOTYPIC CHANGES IN *EUCALYPTUS GLOBULUS*

Luke J. MCMANUS^{1*}, Saravanan THAVAMANIKUMAR¹, Desmond J. STACKPOLE², Paul G. NEVILL¹, René E. VAILLANCOURT², Gerd BOSSINGER¹

¹The University of Melbourne, School of Forest and Ecosystem Science and Cooperative Research Centre for Forestry, Creswick, Victoria 3363, Australia, ²University of Tasmania, School of Plant Science and Cooperative Research Centre for Forestry, Private Bag 55, Hobart, Tasmania 7001, Australia
lmcmamus@unimelb.edu.au

Eucalypts are an important resource for paper manufacturers in many countries but because of their long reproductive cycles, breeding *via* selection of expressed traits is slow. One means of accelerating eucalypt breeding is to utilise genetic markers that are associated with variation in desired physical traits. To identify genetic markers of this sort, we are conducting a genetic association study which aims to identify markers controlling wood basic density, lignin content and lignin syringyl/guaiacyl (S/G) ratio. A Gunns Ltd *Eucalyptus globulus* ssp. *globulus* base-population trial growing near Latrobe, Tasmania, was selected for this study. In excess of 2200 trees were cored to permit sitespecific heritability estimates for basic density, NIR-predicted lignin content, and lignin S/G ratio. DNA was isolated from cambial scrapings of more than 800 of these trees, representing 370 families and a wide geographic range for the species. A modified form of EcoTILLING was applied to all coding sequence of the lignin genes cinnamyl alcohol dehydrogenase (CAD) and cinnamoyl co-A reductase (CCR) in a subset of 72 trees to identify genetic variation for association testing. The MYB2 gene, purported to control lignin gene expression, of 20 individuals was also sequenced. Highly significant genetic variation was identified between and within subraces for all wood property traits with heritabilities ranging from 0.31 for klason lignin to 0.44 for wood basic density. All variables had low standard errors due to the scale of sampling. A total of 68 novel single nucleotide polymorphisms (SNPs), two novel SSRs and 11 novel insertion-deletion mutations (INDELs) were identified in CAD, CCR and MYB2 by EcoTILLING and/or sequencing. Of particular note were three distinct alleles adjacent to the Goldberg-Hogness box in CAD and four polymorphisms that are predicted to cause amino acid alterations in MYB2. A combination of primer extension and allele specific amplification is currently being applied to assay selected polymorphisms within the larger population. To permit analysis of data given the highly structured *E. globulus* population, eight previously characterised microsatellite markers are also being assayed. Although this project is ongoing, we expect it will provide valuable information for eucalypt breeders and facilitate breeding of trees with reduced and/or altered lignin content and composition, and modified wood basic density.

IMPROVING WOOD FIBRE PROPERTIES IN *PINUS RADIATA* AND EUCALYPTS THROUGH ASSOCIATION STUDIES

Reddy THUMMA, Shannon DILLON, Nahida BHUIYAN, Julie GEORGE, Maureen NOLAN, Wei LI, Jayaraj GORANTLA, Xinguo LI, Charlie BELL, Colleen MACMILLAN and Simon SOUTHERTON*

Ensis Genetics, the joint forces of CSIRO and SCION, PO Box E4006 Kingston ACT, 2604 Australia

Simon.Southerton@csiro.au

We are using association studies to identify allelic variation in genes that influence wood fibre development in *Pinus radiata* and *Eucalyptus nitens*, the two major forestry species grown in south-eastern Australia. In both species, microarrays have been screened to identify candidate genes for association studies. Selected genes are being sequenced to identify common single nucleotide polymorphisms (SNPs) which are being genotyped across association populations containing large numbers of unrelated trees growing on a single uniform site. For each individual in the association population, we have been compiling a database of phenotypic information relating especially to wood fibre properties. By comparing genotype with phenotype across the population, we are testing for associations between SNPs and wood properties. SNPs identified in these screens are being validated in mapping and breeding populations. In an attempt to understand the molecular basis of allelic variation we have tested allelic expression in genes linked to pulp yield in eucalypts. Using this approach and transgenic studies, we are attempting to ascertain the role of selected genes in cell wall development and wood fibre properties in pines and eucalypts.

TOWARD AN UNDERSTANDING OF NATURAL VARIATION FOR CARBON ALLOCATION AND PARTITIONING IN *POPULUS*: A GENETICAL GENOMICS APPROACH

Derek DROST^{1,2*}, Evandro NOVAES², Brianna MILES², Qibin YU², Luis OSORIO², Christopher DERVINIS², Gary PETER^{1,2,3}, Matias KIRST^{1,2,3}

¹Plant Molecular and Cellular Biology Program; ²School of Forest Resources and Conservation; ³University of Florida Genetics Institute, University of Florida, Gainesville, FL, USA 32611
ddrost1@ufl.edu

Substantial increases in anthropogenic carbon dioxide (CO₂) emissions since the pre-industrial era have prompted extensive interest in the moderation of atmospheric greenhouse gas levels by enhancing CO₂ uptake and long-term carbon sequestration by plants. Carbon allocation between source and sink organs and carbon partitioning into chemical structures are largely under genetic control, making these traits amenable to modification by breeding and genetic engineering. Although some individual gene products that control allocation and partitioning in herbaceous and woody crops have been characterized, the underlying genetic basis of natural variation for these traits remains largely unknown. We have adopted a genetical genomics approach to unravel the mechanisms regulating allocation and partitioning in woody crops. We detected quantitative trait loci (QTL) for key carbon allocation traits in a widely segregating pseudo-backcross population of *Populus deltoides* X (*P. deltoides* X *P. trichocarpa*) (N=384) grown in controlled conditions under two nitrogen regimes. Using a selective sampling approach to obtain crossover enrichment and a highly heterogeneous representative sample, we selected 100 genotypes for expression analysis of annotated genes underlying QTL intervals in the genome sequence of *P. trichocarpa*. By coupling these results, we present a powerful approach to identify specific loci, as well as more elaborate transcriptional networks and metabolic pathways, mediating carbon allocation and partitioning phenotypes. Identification of specific genes and pathways will provide candidates for targeted genetic modification to enhance carbon uptake and sequestration in plantation forest ecosystems.

PRELIMINARY EVALUATION OF PROVENANCE AND PROGENY TRIALS OF *CRYPTOMERIA JAPONICA* POPULATIONS IN AZORES ISLANDS

Carla FARIA^{1*}, Jorge BELERIQUE², Carina NOBREGA³, Leonor PENACHO³ and M^a Helena ALMEIDA¹

¹Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Centro de Estudos Florestais, Tapada da Ajuda, 1349-017 Lisboa, ²Serviço Florestal da Terceira, Rua Francisco Ornelas 12, 9701-863 Angra do Heroísmo, ³Direcção Regional dos Recursos Florestais, Rua do Contador, n° 23. 9500-050 Ponta Delgada
carlafaria@isa.utl.pt

Cryptomeria japonica specie was introduced as an ornamental tree, in the middle of the 19th century in Azores Islands, like other exotic species introduced in Europe in those days. Its good adaptation and fast growth singled sugi out for forest purposes and rapidly superseded all the other species. In 1998 *C. japonica* breeding program was launched, by local government, as part of the forest breeding Azorean program. Its main objectives are: resistance to *Armillaria* and wind breakage, wood quality and growth rate.

In order to evaluate the genetic basis of Azores sugi population and to compare it with natural populations a set of 4 provenance trials were established, a group of 26 populations are represented in all sites, 5 of them are representative of Azorean populations. Survival, growth have been the evaluated traits. So far Azorean populations showed a higher performance compared to the natural ones, pointing out to the development of a local race. Though, Kyushu origin shows no significant difference from those of the Azorean. Within the sugi breeding program 240 plus trees were selected in Azorean archipelago and submitted to 19 progeny trials to confirm their breeding value, an overview of their performance will be presented.

DEVELOPMENT OF DArT MARKERS FOR ULTRA-HIGH-THROUGHPUT GENOTYPING OF EUCALYPTS

René VAILLANCOURT^{1*}, Dorothy STEANE¹, Andrzej KILIAN², Alexander MYBURG³

¹ School of Plant Science and Cooperative Research Centre for Forestry, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia

² Diversity Arrays Technology Pty Ltd, PO Box 7141, Yarralumla, ACT 2600, Australia

³ Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

R.Vaillancourt@utas.edu.au

Diversity Arrays Technology (DArT) could provide a low cost, high-throughput genotyping tool for eucalypts (at 1000s of polymorphic loci for approximately 30 euros per sample). The technology has been shown to work well in eucalypts. The development of DArT for eucalypts will help overcome two of the main problems in molecular tree breeding: the high cost of genotyping and the low genome coverage offered by most marker systems. This project aims to develop generic DArT arrays across several eucalypt species in the anticipation that enough markers will be polymorphic to perform genome-wide fingerprinting, population genetics, linkage mapping or phylogenetics, in any eucalypt species. Two arrays totalling 13,824 DArT markers will be developed across eight species; *E. grandis*, *E. urophylla*, *E. globulus*, *E. nitens*, *E. camaldulensis*, *E. cladocalyx*, *E. pilularis* and *Corymbia variagata*. These will be tested for their repeatability and cross-species utility through screening of six mapping families, each from different species and also by completing a phylogenetic analysis of eucalypts using samples from over 80 species sequenced for ITS. In order to increase the genome coverage of commercially important species and make possible high-resolution linkage mapping and comparative mapping, a second phase of DArT development will be started in August 2007. This will aim to develop an additional array of 6,912 DArT markers for the first four species listed above using germplasm from breeding programs contributed by project partners. We seek partners to help fund this second phase. Partners would need to contribute a minimum of \$2,500 AUD and provide six DNA samples by August 2007. By including their genetic material in the process of marker discovery, partners will maximize the potential information content of the final DArT array for their own studies. Those interested in participating should contact René Vaillancourt by July 2007.

GENETIC ARCHITECTURE OF WOOD QUALITY RELATED TRAITS IN EUCALYPTUS

JM GION^{1*}, A CARROUCHE¹, S DEWER¹, L DOLMARE¹, JG COMBES², H BAILLERES², A VAILLANT¹, N OUNIABI³, A SAYA³, C PLOMION³, P VIGNERON¹

¹CIRAD, UPR39 - BIOS Department, Montpellier France, ²CIRAD, UPRXX - Department, Montpellier France, ³UR2PI, Pointe Noire, Congo, ⁴INRA, UMR1202 BIOGECO, F-33610, France
gion@cirad.fr

Wood and end-use properties are taking more and more importance in forest tree breeding programmes. Within the framework of the eucalyptus breeding programme developed in the Congo, genetic architecture of wood quality related traits has been studied in an interspecific cross. Wood quality components were analyzed in a full-sib mapping pedigree between *E. urophylla* and *E. grandis* consisting on 200 F1s. Three types of properties were investigated: anatomical characteristics (fibre length, fibre thickness...), technological properties (Longitudinal growth strain, density...) and chemical composition (lignin content). Fully saturated parental maps were first constructed, on which 47 candidate genes from a SSH xylem vs. leave library were localised. QTL analysis was then performed using the MultiQTL software under single and two-linked QTL models, based on both classical interval mapping and multiple interval mapping methods. In this poster we will present the main results of this study with emphasis on the colocation analysis between QTLs for different traits and coincidence between QTL and candidate genes.

MOLECULAR MARKERS FOR PORTUGUESE PINE WOOD QUALITY

Jorge A. P. PAIVA^{1,2*}, Ana ALVES², Rita COSTA³, Maria Isabel CARRASQUINHO³, Maria João GASPAR⁴, Maria Emília SILVA⁴, José MORAIS⁴, José RODRIGUES², Alexandre AGUIAR³, José LOUZADA⁴, Manuel Pedro Salema FEVEREIRO^{1,5}

¹Plant Cell Biotechnology Lab. Instituto de Tecnologia Química e Biológica (ITQB/UNL), Av. da República (EAN), Apartado 127, 2781-901 Oeiras (Portugal), ²Forest and Forest Products Centre, Tropical Research Institute of Portugal (ICT), ISA-DEF, Tapada Ajuda, 1349-017 Lisboa (Portugal), ³Estação Florestal Nacional (EFN/INIAP), Quinta do Marquês, 2780-159 Oeiras (Portugal), ⁴Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta dos Prados - Apartado 202, 5000-262 Vila Real (Portugal), ⁵Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1700 Lisboa, Portugal
psalema@itqb.unl.pt

Maritime pine (*Pinus pinaster* Ait.) is an important commercial species in southwest Europe. In Portugal, *P. pinaster* is one of the most important native species, covering 1Mha, is the only source of long fibre for pulp and paper and the main source for solid sawn timber industries. A tree improvement plan has been developed since the early 80s with the aim of increasing volume per hectare and stem conformation. The introduction of wood quality selection criteria is now considered an important objective of breeding programmes. Such selection is however hampered by the lack of information for wood quality traits, not only at the genetic but also at the industrial levels. Wood quality can only be defined in terms of particular end use, and may involve several traits (e.g. density, wood heterogeneity, wood chemical composition and fibre properties). Mean density is acknowledged to be the best single predictor of wood mechanical properties. Lignin, polysaccharides and extractives must also be considered, having direct consequences for production costs and final product quality.

Single nucleotide polymorphisms (SNPs) and indels are an essentially inexhaustible source of polymorphic markers for use in association studies that are based on candidate genes. Haplotypes associated with agronomic performance will provide excellent diagnostic tools for marker-assisted breeding and facilitate identification and molecular cloning of genes controlling complex agronomic characters.

The aim of this project is to use polymorphic sequences of candidate genes expressed during the synthesis of wood (e.g. CAD and PAL) to associate specific SNPs alleles with specific wood characteristics of Portuguese pine trees. We will use 184 trees from a progeny test set at Mata de Leiria, coming from the open cross of 46 plus trees maintained at Mata do Escaroupim.

The specific objectives of the project are:

- 1 Assess of genetic parameters for growth, stem and branch characteristics and selection of plus trees the progeny trial;
- 2 Assess the variability and heritability of density components and mechanical properties of the wood;
3. Assess the variability and heritability of chemical composition traits (extractives, lignin amount and quality and polysaccharides);
4. Identify the relevant haplotypes of the polymorphic sequences and chose the SNPs to be used;
5. Genotype the trees for the chosen SNPs;
6. Associate specific alleles with specific wood characteristics.

GENETIC ASSOCIATION STUDIES IN *EUCALYPTUS GLOBULUS* LABILL.

Saravanan THAVAMANIKUMAR¹, Luke J. MCMANUS^{1*}, Josquin F.G. TIBBITS¹,
Desmond J. STACKPOLE², René E. VAILLANCOURT² and Gerd BOSSINGER¹

¹The University of Melbourne, School of Forest and Ecosystem Science and Cooperative Research Centre for Forestry, Creswick, Victoria 3363, Australia, ²University of Tasmania, School of Plant Science and Cooperative Research Centre for Forestry, Private Bag 55, Hobart, Tasmania 7001, Australia
lmcmamus@unimelb.edu.au

Eucalyptus globulus is the major pulpwood species grown in temperate regions of the world. Despite this, seed sources are largely unimproved. Improving eucalypt seed sources by conventional breeding is difficult due to long generation times, difficulties in measuring phenotypic traits particularly when looking at mature trait development, and inherent within tree variability in important traits during growth and maturation. An alternative approach involves the detection of associations between allelic variation (at the sequence level) and phenotypic variation which would allow for marker assisted selection. In such an approach, we have collected DNA from cambial scrapings of 810 *Eucalyptus globulus* trees covering 10 populations, 18 subpopulations and 370 families planted in a provenance-progeny trial, growing near Latrobe, Tasmania, Australia (Gunns Ltd). This trial comprises 570 families, planted in two-tree plots, with five replicates and 21-28 incomplete blocks. Phenotypic studies showed heritabilities (h^2) of sufficient magnitude to apply to the present study (density 0.44, cellulose 0.27, and pulp yield 0.36) with standard errors below 20%. Korrigan (endo-1, 4-beta-glucanase), a gene implicated in plant cell wall modification and/or cellulose biosynthesis, has been identified as a functional candidate and we have designed primers using publicly available korrigan sequences of *Eucalyptus globulus*, *Populus tremuloides* and *Arabidopsis thaliana*. Here we present progress towards single nucleotide polymorphism (SNP) discovery and LD mapping to test for associations between korrigan variation and measured phenotypes.

FINE-MAPPING OF SEX-RELATED MARKER, AND IDENTIFICATION AND CHARACTERIZATION SEX-DETERMINING GENES IN ASPEN (*POPULUS TREMULA* L.)

Birte PAKULL, Sandra PAASCH, Torsten MARKUSSEN, Matthias FLADUNG*

BFH, Institute for Forest Genetics and Forest Tree Breeding, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany
mfladung@uni-hamburg.de

A preliminary consensus map of *Populus tremula x tremuloides* has been constructed from an interspecific hybrid population of 66 seedlings of the cross Brauna 11 (*P. tremula*) x Turesson 141 (*P. tremuloides*). The map was constructed based on 205 AFLP- and 29 SSR-markers covering 1875cM on 19 linkage groups. In a search for sex-correlated markers a single locus correlating to a male factor ("G-locus") in *Populus* was mapped close to two flanking AFLP-markers.

The aim of the proceeding work is a ratifying and fine mapping of the respective genome region. The constructed genetic map of aspen will be aligned with other genetic maps of the genus *Populus* by means of an expansion of the F1-progeny mapping population and a subsequent screening for more sex-correlated AFLP and SSR-markers. Some already mapped AFLP-markers and new rendered molecular markers close to the preliminary G-locus will be converted into sequence specified SCAR-markers, which can be used as probes for the screening. Reverse complement SSR-primers may be usable as starting point for sequencing reactions with subsequent designing of probes out the resulting sequences.

Furthermore a constructed BAC-library will be screened with the aim of constructing and characterizing a BAC-Contig containing the region of the preliminary G-locus. Final aim is the identification of potential sex-determining genes and the development of sex-specific molecular markers.

ELIMINATION OF MARKER GENES AND TARGETED INTEGRATION OF TRANSGENES VIA THE FLP/FRT-SYSTEM

Tobias SCHENK², Dirk BECKER², Horst LÖRZ², Matthias FLADUNG^{1*}

¹BFH, Institute for Forest Genetics and Forest Tree Breeding, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany, ²University Hamburg, Ohnhorststr. 18, D-22609 Hamburg, Germany
mfladung@uni-hamburg.de

Compared to annual plants, the problem of expression variability is more crucial for trees having long vegetative cycles. To address the issue of biological safety of transgenic plants both the problem of expression variability as well as the removal of antibiotic genes have to be considered. Antibiotic resistance genes are needed for recognition of transgenic plants during the process of transformation, however, are without any use at later developmental stages. EU-law demands field-grown transgenic plants without antibiotic selection genes starting with 2008 or 2009.

Expression variability is more crucial for trees having long vegetative cycles, thus, single-copy transgenes need to be placed precisely into the pre-defined genomic loci characterized for stable expression. Site-specific recombination (SSR) can be used to remove the marker genes or place a recognition target (reporter gene) within the genome so that the “transgene-of-interest” (TOI) can be precisely placed into the target.

Similarly to the Cre/lox-system the Flp/FRT recombination system will be tested. The advantage is that is not patent-protected. Only little information is available on the functions of these systems in wheat (*Triticum aestivum*) and Populus. The goals of this study are to isolate and characterize inducible promoters suitable for both plant systems, to verify the promoter induction in wheat embryos using transient expression assays, to clone vectors for excision of the marker gene npt-II as well as for targeted integration of the bar-gene in place of npt-II. To reach that goal transgenic wheat (biolistic) and poplar (*Agrobacterium*) plants will be generated carrying appropriate gene constructs. Transgenic plants will be screened for single copy integration, and subsequently, the Flp/FRT recombination system will be activated. To verify the recombination events removal of marker genes and the targeted gene integration will be investigated. For both species transgenic plants carrying the gene constructs are available. At present the number of integrated copies are studied.

CONSTRUCTION OF GENETIC LINKAGE MAPS IN ASPEN (*POPULUS TREMULA* L. AND *P. TREMULOIDES* MICHX.) AND THEIR USE FOR QTL MAPPING

Matthias MEYER^{1*}, Torsten MARKUSSEN³, Gerhard HELLE², Doris KRABEL¹, Matthias FLADUNG³

¹ Technische Universität Dresden, Institute for Forest Botany and Forest Zoology, Piennner Str. 7, D-01737 Tharandt, Germany, ² Forschungszentrum Jülich GmbH, ICG-V, Leo-Brandt-Str., D-52425 Jülich, Germany, ³ BFH, Institute for Forest Genetics and Forest Tree Breeding, Sieker Landstr. 2, D-22927 Grosshansdorf, Germany
matthias.meyer@mailbox.tu-dresden.de

European and quaking aspen, two of the most wide-spread tree species in the northern hemisphere, are important in pioneer forest ecosystems as well as in Central and North European short rotation coppices (SRC). The optimization of aspen cultivars for farm cropping on set-aside agricultural land is an important objective of forest tree breeding in the European Community. Particularly in a changing global climate, poplar (*Populus* spp.) cultivars well adapted to drought and heat are badly needed. The present studies were aimed at providing linkage maps for aspen and at the detection of markers linked to relevant traits concerning sex, drought tolerance and wood anatomy.

Using SSR and AFLP marker data, three moderately dense molecular genetic linkage maps were constructed for European aspen (*P. tremula* L.) and one for quaking aspen (*P. tremuloides* Michx.). We applied the two-way pseudo-testcross strategy and investigated one hybrid and one non hybrid F1-segregation population. Additionally, consensus maps harbouring markers of all segregation types were constructed for the mapping pedigrees with the software package JoinMap®. The mapping efficiency is discussed with regard to heterozygosity levels and genetic similarity of the parental trees. In both independent mapping populations the average maternal recombination distance was reduced resulting in approximately 19 % longer maps for female trees.

The maps derived from the non hybrid population (*P. tremula* x *P. tremula*) were used for the mapping of QTL's concerning drought tolerance and wood anatomy of younger trees in SRC. To determine drought tolerance, contents of stable isotopes ($\delta^{13}\text{C}$ / $\delta^{18}\text{O}$), vessel traits, wood density and parameters of hydraulic architecture were measured in the growth rings of the seven-years-old offspring. The proportion of the values of the respective trait in 2003 and in 2002 was used to describe the reaction to drought. 2003 was an extremely dry year in Central Europe with a long-lasting and strong drought. Loci linked to the sexual differentiation of poplar were mapped in the hybrid aspen population (*P. tremula* x *P. tremuloides*). The sex of the trees was determined in the fifty-years-old progeny.

We plan to establish PCR-marker tests for the early selection of desired phenotypes concerning all investigated traits. At present, the maps obtained for the hybrid population are being enriched with additional SSR markers available from the International *Populus* Genome Consortium.

QTL FOR MYCOSPHAERELLA LEAF DISEASE RESISTANCE AND WOOD PROPERTIES IN *EUCALYPTUS GLOBULUS*

René E VAILLANCOURT*, Jules S FREEMAN, Simon P WHITTOCK and Brad M POTTS

School of Plant Science and Cooperative Research Centre for Forestry, University of Tasmania, Private Bag 55, Hobart, Tasmania 7001, Australia

R.Vaillancourt@utas.edu.au

QTL analysis of a clonally replicated (ortet and one ramet), outbred (T7/KI7//T144/KI5 = family one) F2 *Eucalyptus globulus* family (112 genotypes) allowed dissection of the genetic control of resistance to *Mycosphaerella* leaf disease. Maps of the maternal and paternal parents were merged into a consensus containing 169 markers (34 SSR and 135 AFLP loci) in 11 linkage groups. We assessed damage caused by natural *Mycosphaerella* leaf disease infection that occurred shortly after planting and before vegetative phase change. Growth and phase change were monitored annually for 7 years. Wood density was measured from cores taken on seven year old trees. Cellulose and lignin content were measured from cores scanned using NIRA. The F2 was genetically variable, with significant differences detected amongst genotypes for all traits. Two unlinked QTL explained up to 62% of the phenotypic variance in *Mycosphaerella* severity (myco1, interval mapping LOD = 6.2*, myco8, LOD = 11.4***). Validation of these QTL used two other clonally replicated F2 families of similar size planted in the same trial (Woolnorth). Both QTL were significantly associated with resistance in family two (T7/KI157//KI5/KI157), while only myco1 was significantly associated with resistance in a third family (KI2/KI161//KI5/KI157). Resistance in family three appeared to be controlled by a single Mendelian factor, with resistance being dominant (71 resistant: 20 susceptible, $c^2 = 0.44ns$). We also studied the environmental stability of resistance with genetic stock planted at three different and widely separated locations and all naturally infected with the disease. There was good correlation between the level of *Mycosphaerella* damage (r varied from 0.63 to 0.82) across sites. Strong QTL were also found for wood density and cellulose content in family one. However, these have not been validated as yet. Validation and new QTL discovery will be undertaken through mapping of several populations using the Diversity Array Technology™ (DArT) which will be developed for *E. globulus*, *E. grandis* and five other eucalypt species in 2007. DArT is a high throughput genotyping technology that uses DNA array technology. The relatively low cost of DArT markers will allow researchers working on different crosses and species to use a common set of markers, which will ultimately allow the eucalypt genomic community to share positional information on QTL and genes affecting important traits.