

ABSTRACTS

GENECAR meeting

September 23-25 in Umeå

**Application of DNA based tools for genetic research, molecular breeding,
and management and monitoring of genetic resources**

WOOD FORMATION; APPROACHES AND INSIGHTS TOWARDS AN UNDERSTANDING OF ITS MOLECULAR REGULATION

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A bridging task in tree biotechnology research is to identify and functionally understand genes underlying traits of commercial interest. Development of microarray analysis and access to the genome sequence in poplar has greatly contributed to advance this research. At Umeå Plant Science Centre (UPSC), scientists have used cryomicrotome dissection/microarray analysis to reveal key genes in wood formation. This technique visualizes transcript profiles across all wood forming tissues, and also with high resolution across specific wood forming tissues. A recent contribution to the UPSC transcriptional wood formation database is the profiling of gene expression across S2 to gelatinous cell wall layers in tension wood formation. This approach has allowed us to identify and separate candidate genes involved in the biosynthesis of major cell polymers of secondary walls, because lignin and hemicellulose biosynthesis is silenced when biosynthesis of gelatinous layer is initiated. Information from UPSC wood transcriptomics is used by academics, and by SweeTreeTechnologies (STT) in their large-scale gene knock-down program in poplar. Within FuncFiber (The Swedish Centre of Excellence in Wood Science (www.funcfiber.se)), more than 30 transgenic lines with preliminary phenotypes in wood properties emerging from this effort are currently under investigation in collaboration between STT and academic research. FuncFiber is an interdisciplinary program that combines biology, chemistry and chemometrics. Within the network state of the art technology for FT-IR wood imaging and NMR analysis of whole ball milled cell wall samples have been developed for wood phenotyping. The databank of transgenic trees with altered wood properties will not only be used for detailed gene-by-gene research, but also in a systems biology approach to take advantage of the enormous information provided by spectroscopy/spectrometry techniques to provide more insight into our understanding of wood formation, from genes to wood properties.

A COMPREHENSIVE GENOMIC APPROACH TO IDENTIFY GENES THAT GOVERN NATURAL VARIATION OF COMPLEX TRAITS IN SPRUCE

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White spruce (*Picea glauca* [Moench] Voss) is a keystone species within Canada's boreal forest ecosystem and 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. In addition to a suite of wood chemical and physical property traits, we focus on the timing of terminal bud formation. This adaptive trait is a major determinant of the length of the growing season and thus influences productivity. To discover the genes that underlie these complex traits, we have developed a genome-wide mapping approach that combines high-throughput genotyping with large-scale gene expression profiling. Current efforts aim to identify thousands of orthologous SNPs located in expressed regions, then link these SNPs to adaptive variation through various genome scan strategies. Our approach includes QTL mapping and co-localization with mapped genes, candidate gene association studies in unstructured populations, and SNP-scans to identify outlier gene loci that may differentiate adaptive characters among natural populations. Due to the conserved nature of genes across *Picea*, exciting opportunities for the interspecific transfer of genomic information are now forthcoming.

DOES NATURAL VARIATION IN POPLAR WOOD QUALITY AND GENE EXPRESSION INFLUENCE SACCHARIFICATION FOR BIOETHANOL PRODUCTION?

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This is a new project which aims to explore how bioethanol yield can be optimised in the future from lignocellulosic second generation energy crops such as *Populus*. It is already recognised that first generation food crops are often not sustainable for bioethanol production, with a poor energy balance and that step-change is required to improve this process if green plants are ever to be harnessed as part of the biorefinery concept. Here we wished to investigate natural genetic variation in an F₂ mapping population of *Populus* in wood chemistry, wood physical properties and wood yield and to relate this to saccharification potential and also to underlying differences in patterns of gene expression. Wood density varies significantly in this population and in choosing a set of high and low density wood extremes we have found a number of genes that are differentially expressed from analysis of cambial cells using the *Populus* affymetrix array. We have recently taken these extreme genotypes and have estimated glucose yield in a saccharification assay, with differences are also apparent. Our current research will be focussed on the links between this potential, areas of the *Populus* genome determining saccharification (QTL for saccharification) and in identifying underlying genes using a genetical genomics approach. Through this research we hope to capture natural genetic variation in saccharification and ethanol yield, developing genes and markers for future breeding and improvement.

GENOMICS OF ADAPTATION TO BIOTIC AND ABIOTIC STRESSES IN CONIFERS

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The genetic basis of adaptation to biotic and abiotic stresses in forest trees is quite well understood in many cases, largely by using the common garden experimental approach. Such information is useful for guiding reforestation programs through well-defined seed zones or breeding zones. The individual genes underlying these complex traits, such as drought or cold tolerance, are, however, largely unknown. Genomic approaches such as quantitative trait locus (QTL) and association mapping are being used in trees to begin to dissect complex adaptive traits to their individual gene components. In recent years, we have used the association approach to identify genes controlling drought tolerance, cold tolerance and resistance to fungal pathogens. For example, candidate genes such as *dehydrin*, *Cu/Zn superoxide dismutase* and a *wrky-like* transcription factor are associated with carbon isotope discrimination (a measure of water-use efficiency) in loblolly pine. Genes from the lignin biosynthetic pathway and a couple of transcription factors were associated with resistance to fungal pathogens in loblolly pine. Now that it is experimentally possible to identify individual genes controlling complex adaptive traits, and furthermore the effects of alleles at these genes, it will become possible to develop forest health diagnostic tools to help manage forest tree populations.

A MODEST PROPOSAL TO FIND DNA VARIATION ASSOCIATED TO PHENOLOGY IN NORWAY SPRUCE: COMBINING DNA SEQUENCE ANALYSIS AND GENE EXPRESSION STUDIES

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Conifers have huge genomes on which we know so far precious little. Similarly, our understanding of the genetic control of traits important for adaptation, such as bud set, remains limited. On the other hand, a great deal is known about genome structure and the genetic basis of traits such as flowering time in the model species, *A. thaliana* and genomic resources are being developed in conifers. Conifers and *A. thaliana* diverged some 300 Mya and budset is not flowering time. Yet, we will argue that a “candidate gene” approach, starting from what is known in *A. thaliana* and that combines DNA sequence analysis, QTL mapping and gene expression studies is possibly the best course of action to start to understand the genetic control of phenology and identify markers for marker assisted selection. In this talk and in the accompanying talk by Ulf Lagercrantz we will present some results obtained in Norway spruce, using this approach.

THE GENETIC BASIS OF NATURAL VARIATION IN BUD PHENOLOGY ACROSS A LATITUDINAL GRADIENT IN EUROPEAN ASPEN (*POPULUS TREMULA*)

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The initiation of growth and dormancy represents critical ecological and evolutionary trade-offs between survival and growth in most perennial plants and latitudinal clines in important phenological traits related to the annual development cycle are commonly observed in many plants. In aspens (*Populus* spp.) the most important environmental cue regulating the initiation of dormancy is a shortening of the photoperiod and QTL mapping experiments have implicated genes in the photoperiodic pathway in the control of growth cessation. Here we present data from a study on the genetic basis of variation in phenology in European aspen (*Populus tremula*) across a latitudinal gradient. Genetic differentiation at neutral markers is low despite strong differentiation in several phenology traits, demonstrating that populations are adapted to the local photoperiodic regime. Sequence variants found in a few of the genes in the photoperiodic pathway show associations with variation in phenology and explain a sizable fraction of the natural variation in phenology. There are, however, no indications that these genes have genetic differentiation that exceeds genetic differentiation at neutral markers.

ADAPTIVE CLINE TO LIGHT QUALITY IN SCOTS PINE: A PROTEOMIC APPROACH

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Traits like timing of growth, dormancy and frost hardiness show strong local adaptation in pines. Temperature, photoperiod and light spectral quality (wavelength) have been proposed as the environmental cues governing them. Light spectral quality varies significantly from northern to southern latitudes in Scandinavia where Scots pine populations are a good example of well-studied adaptive differentiation. Scandinavian Scots pine populations provide an excellent scenario to prove the role of light quality in local adaptation, and to search for the underlying genes. We performed a pilot study to characterize the response to different light treatments (blue, red, far-red, white) of Scots pine hypocotyl elongation along a latitudinal cline in Sweden. The pilot study has shown a significant differential response to red light and far red light and GxE interaction. The next step is to perform the experiment in a larger association mapping population in Scots pine. Seeds are being collected from a total of 900 mother trees from northern, middle and southern Sweden. Seeds will be germinated and grown under four light wavelengths (white, red, far-red, blue) and darkness. Hypocotyls will be measured and collected at -80 °C for proteomic analysis. A first bulked exploratory total proteome profile will help to identify some candidate proteins showing differential expression patten. Latitudinal changes in protein expression level are also expected to be reflected in the anatomical architecture of the hypocotyl tissue. Therefore, hypocotyl transverse sections will be collected and fixed in paraffin for anatomical characterization. Correlation between expression level and anatomy in the hypocotyl will help to understand the genetics behind local adaptation to light quality.

MOLECULAR REGULATION OF FLOWERING AND GROWTH CESSATION IN ASPEN

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Day length controls flowering time in many plants. The day-length signal is perceived in the leaf, and this signal is transduced to the shoot apex where floral initiation occurs. In *Arabidopsis*, the day-length response depends on the induction of the *FLOWERING LOCUS T (FT)* gene by the gene *CONSTANS (CO)*.

In contrast to annual plants like *Arabidopsis*, forest trees display a perennial growth behaviour characterized by a very extended juvenile phase before flowering, and, in temperate regions of the world, an annual cycling between growth and dormancy. We have shown that the CO/FT regulatory module is functionally conserved in the aspen tree where it controls the timing of flowering. However, unexpectedly, it also controls the short-day induced growth cessation and bud set normally occurring in the fall. We show that the differences in critical daylength for growth cessation can be explained by a difference in the phase of expression of the aspen ortholog of the gene *CO* leading to induction of *FT* at the different critical daylengths. The difference in critical daylength between tree provenances is a highly adaptive trait, and this is one of very few examples where the ecogenetic mechanism to such a variation has been determined. Taken together, these data suggest that FT is not a specific regulator of flowering, but might have a more general role in regulating biological processes controlled by variations in day length. We are now investigating the ecogenetic basis for the difference in phase of *CO* expression and *FT* activation displayed by various aspen tree provenances and we will discuss these findings.

THE CIRCADIAN CLOCK AND TIMING OF SEASONAL GROWTH IN HYBRID ASPEN

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The circadian clock is an endogenous biological timer, which is set to about 24 hours and keeps its rhythm by resetting itself to local time each day by the regular changes in light and temperature. In hybrid aspen (*Populus tremula* x *P. tremuloides*, Ptt) the clock is important for synchronization of daily events but also, as shown, crucial for seasonal events such as growth cessation and bud set. The putative aspen oscillator genes LATE ELONGATED HYPOCOTYL 1 and 2 (PttLHY1 and PttLHY2), as well as TIMING OF CAB 1 (PttTOC1) have been knocked down in hybrid aspen using RNA interference (RNAi) and their resulting phenotypes was investigated by microarray and real-time PCR. Also, plants carrying the heterologous *Arabidopsis* promoter COLD AND CIRCADIAN RHYTHM RNA BINDING 2 (CCR2) fused to firefly luciferase (LUC), enabled us to follow the expression of CCR2 by measuring the amount of emitted light. Strong down regulation of PttLHY, or PttTOC1 by RNAi results in trees with shorter internal periods. The shortening in period affects the phase of clock associated gene expression and leads to a shorter critical day length for growth in these trees. Thus, RNAi lines continue to grow when wild type trees stop under 15 hours light/ 9 hours dark. Our studies show that the circadian clock is participating in deciding the critical day length for growth in hybrid aspen.

A NORWAY SPRUCE FLOWERING LOCUS T HOMOLOG IS IMPLICATED IN CONTROL OF GROWTH RHYTHM IN CONIFERS

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Norway spruce as many other trees show a strong latitudinal cline in growth cessation and bud set. This variation is to a large extent determined by a genetically controlled response to photoperiod. To identify genes controlling this variation we have adopted a combined approach comprising candidate gene mapping and analysis of DNA variation (see talk by Martin Lascoux) combined with functional studies to identify and verify those candidate genes. In an effort to identify molecular components of the photoperiodic pathway in Norway spruce, we isolated homologues to photoperiod genes in *Arabidopsis* to study their function. In these studies, a tight correlation between growth rhythm and expression pattern of one Norway spruce FT-like gene (*PaFT4*) was observed over a range of experimental conditions. This suggests that one Norway spruce homologue to the *FT* gene, which controls flowering in angiosperms, is also a key integrator of photoperiodic and thermal signals in the control of growth rhythms in gymnosperms. The data also indicate that the divergent adaptive bud set responses of northern and southern Norway spruce populations, both to photoperiod and light quality, are mediated through *PaFT4*.

DIFFERENTIAL EXPRESSION OF DEHYDRINS IN NORWAY SPRUCE DURING BUD BURST INITIATION

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Initiation of flushing and cold deacclimation is likely quite water demanding for rehydration of meristems and recovering metabolic pathways needed for temperature and light sensing and preparation to active cell division. Therefore water stress related genes could play an important role in providing of bud burst timing processes. One of the most extensively studied classes of dehydration protective proteins are the dehydrins (DHN).

Here we report the sequence analysis of several differentially expressed Norway spruce dehydrins. Norway spruce ESTs were identified in our in-house Norway spruce EST database (more than 13000 ESTs) including an early and late flushing SSH derived EST libraries (more than 2700 ESTs). On the basis of ESTs, 12 full length and/or full ORFs cDNAs and three 5'-partial cDNAs were obtained, representing eight genes from three distinct types of dehydrins. Using qRT-PCR, the differential expression of these genes during bud burst initiation and bud flushing has been studied. For most of the dehydrin transcripts, the levels in late-flushing spruces were significantly higher than in the early-flushing families. Based on the transcript data, we propose that in Norway spruce, dehydrins are clearly related to the timing of bud burst and can play an important protective role during winter dehardening and early stages of bud burst initiation. Their abundance is neutrally or negatively correlated with the starting of bud burst. The reduction of transcription of some of the dehydrins (*PaDhn1*, *PaDhn4.6*, *PaDhn5*, *PaDhn6*, *PaDhn2* and *PaDhn3*) thus coincides with the reduction in frost hardness in shoot tips. Obtained results had been confirmed in the bud burst initiation experiment fulfilled in controlled conditions in Finland.

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A MEMORY FROM EMBRYO DEVELOPMENT

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The anticipated change in global climate will most likely alter the geographic distribution of plant species, and will challenge their evolutionary capabilities. Several conifers species reach sexual maturity late, and the long generation interval could make them less able to respond to the rapid changes in temperature by evolutionary means. However, in Norway spruce (*Picea abies*) we have discovered a mechanism that contributes to a fast response to climate changes. The timing of dehardening and bud burst in spring, leader shoot growth cessation in summer, bud set and cold acclimation in the autumn, are processes that are advanced or delayed according to the temperature during female sexual reproduction. Conditions colder than normal advance the timing whilst temperatures above normal delay the onset of these processes. The altered performance lasts for many years. The seedlings actually remember the temperatures and photoperiod prevailing during zygotic embryogenesis and seed maturation. We show for the first time that regenerated plants, cloned through somatic embryogenesis, express a memory of the temperatures applied during embryo development whilst growing in a common greenhouse environment. The warmer the *in vitro* temperature applied, the later the regenerated plants formed terminal buds in the common environment the second growth season. They could also remember the temperature during *in vivo* zygotic embryo development in the seed cones, despite being subjected to the *in vitro* propagation. The differences were very large, and similar in size to a provenance separation of 4 – 6 degrees of latitude.

DEFENSE MECHANISMS AGAINST FUNGAL INFECTIONS IN FOREST TREES; TOWARDS AN UNDERSTANDING OF THEIR MOLECULAR COMPONENTS AND POSSIBLE APPLICATIONS IN FUTURE FOREST TREE BREEDING

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Trees have a multitude of constitutive and induced defense mechanisms against fungi. Structural and chemical antifungal components are important but also the nutritional and phenological properties of trees. In addition, trees have an endophytic and symbiotic microflora that contributes to their protection. In order to minimize the costs over evolutionary time, plants have evolved recognition systems of potential invaders that trigger induced responses. The recognition is mediated through jasmonate, ethylene and salicylate pathways and includes tissue-like reactions e.g. the hypersensitive response, traumatic resin ducts, polyphenol cells, necrophylactic periderm etc. Chemically these responses include de novo production of terpenes and resins, phenolic substances, reactive oxygen species etc. Different fungal pathogens react differently to the tree as an environment and host. Biotrophic pathogens have the requirement for living host cells for their nutrition while necrotrophic pathogens typically kill host cells via toxins before degrading them. The defense mechanisms are differentially effective towards different pathogens, and for induced reactions rapid responses are pivotal. It is therefore not likely that there exists a superresistant tree. In order to elucidate the mechanisms involved a wide range of techniques have been employed including microarray techniques, proteomic and metabolomic approaches, differential expression studies, transformation of trees and pathogens, and comparisons with the model plant *Arabidopsis*. For resistance studies it is desirable to work on defined host and pathogen genotypes, e.g. clonal lineages or mapping populations. For future breeding programs it would be possible to include resistance testing against a suite of severe pathogens or alternatively use genetic markers to guide the selection. In cases when the molecular mechanisms involved are known, the information should be included in the programs.

IDENTIFICATION AND ANALYSIS OF DEFENSE RELATED GENES IN NORWAY SPRUCE

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Our goal is to study what gene products (mRNA and proteins) are important for trees in order to fend off pathogens. We are interested in the genetic basis of resistance focusing on the molecular basis of both the local and systemic defence responses of trees. So far we have focused on the host defense against necrotrophic fungi and studied the expression and role of defensins, peroxidases, chitinases, CHS, PAL, CAD and a number of other host gene products.

Earlier work using clonal trials (in both Sweden and Norway) indicate that there is a genetic basis for resistance to the root-rot causing fungus *Heterobasidion annosum* s.l.. We have studied the timing and spatial signaling of the defense response in mature Norway spruce trees as well as seedlings and found that the molecular responses differ. Our studies suggest that the time from wounding and infection to induction of defense-related expression is shorter in resistant spruce clones indicating a more efficient host defense response than in susceptible trees.

PATHOGENOMIC APPROACHES IN HETEROBASIDION-CONIFER INTERACTIONS: ORGAN AND PATHOGEN SPECIFIC INDUCED RESPONSES

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Host–pathogen interactions have been studied mostly in agricultural crops, with very little work done in forest trees, particularly gymnosperms. Due to the paucity of our knowledge about interactions in forest tree pathosystems, much of the information have been drawn with reference to agricultural systems. Although gymnosperms and angiosperms diverged during evolution several million years ago, a lot can be learnt from studies conducted in agricultural crop pathosystems. In conifer tree pathosystems, much of the basic molecular research have mostly been conducted using the root and butt rot fungus *Heterobasidion annosum* as a model. However, although the biology and genetics of this most economically important conifer pathogen *Heterobasidion annosum* sensu lato has been studied, knowledge about mechanisms of defence responses, resistance and pathogenicity remains largely unexplored. A major set back for the conifer pathosystem is the lack of a suitable model system. Additionally, the long life cycle, size of the mature trees and long timescale of many of their diseases make working with these plants inherently difficult. Equally, absence of any host genotype in the Pinaceae with total resistance against *H. annosum* further complicates detailed molecular analysis of the interaction.

In this presentation, I will outline our efforts using a transcriptomic approach to analyse specificity in responses of pine seedling roots to *H. annosum* infection. As there are no avirulent strains of *H. annosum*, we used *Laccaria bicolor* (an ectomycorrhizal symbiont) and *Trichoderma aureoviride* (a saprotroph) as non-pathogen models to determine whether the observed pine responses to *H. annosum* attack were indeed specific. The results indicated that pine was able to recognize all three fungi and specifically distinguish whether they were pathogenic, neutral or beneficial microorganisms. An additional transcript profiling study investigated whether the documented responses to *H. annosum* infection were organ specific. Comparison of transcript profiles of pine needles and roots challenged with root (*H. annosum*) and shoot specific (*Gremmeniella abietina*) pathogens, indicated that the responses were more organ-specific than pathogen-specific. A particular gene encoding an antimicrobial peptide (Sp-AMP) that was consistently induced during the pathogenic interactions was over-expressed in *Pichia* sp and further characterised. The recombinant peptide was shown to have inhibitory effect against mycelia and germinating spores of *H. annosum*. Finally, a novel combination of molecular and genomics approach will advance our knowledge of this pathosystem that will have direct relevance to conifer trees. This will obviously form the basis for increasing conifer tree resistance through selection or genetic engineering.

GENOMIC-BASED BREEDING IN FOREST TREES

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Genetic improvement in forest trees has traditionally been based on phenotypic mass selection. Significant genetic gains have been achieved in a number of species throughout the world, however the breeding cycle time is quite long and the cost of phenotypic selection can be significant. A complementary approach is to employ indirect selection on genetic markers linked to traits of interest. The quantitative trait locus (QTL) mapping approach was first tried to dissect complex traits to individual genes, but because linkages could only be resolved to 10-20 cM intervals, marker alleles linked to favorable QTL alleles might easily recombine in subsequent generations, thus limiting or negating the marker-based breeding approach. In recent years, it has been shown that the association genetics approach can identify markers (usually single nucleotide polymorphisms (SNPs)) very tightly linked to quantitative trait nucleotides (QTNs), if not being the functional polymorphism itself. This is due to the rapid decay of linkage disequilibrium in tree populations. First-generation association studies were limited by the number of candidate genes (10-100) that could be used in association studies, but now it is possible to screen tens of thousands of genes and SNPs within these genes. Association genetics in trees now approaches the full genome scan approach used in human studies to dissect complex disease. It is now realistic to expect that marker-based breeding can be brought to full application in a number of breeding programs within the next five years.

BREEDING WITHOUT BREEDING: PARADIGM SHIFT OR A PIPE DREAM

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Traditional tree improvement programs start with deliberate phenotypic selection of candidate trees followed by rounds of recurrent selection characterized by cumulative incremental gain advancements. While effective, the process is elaborate, quantitatively oriented, and follows sophisticated breeding theories. Breeding and testing start with the selection of pre-determined mating designs, requiring years to complete and often is not error free, followed by experimentally elaborate, large scale, multiple sites field testing. This process is costly, takes long time to complete, and most of all, requires sustained organizational commitments for its continuation. Here I present an innovative approach that allows the capture of the genetic gain attained through conventional tree improvement programs without conducting any crosses or the establishment of progeny test trials. The method is called “Breeding Without Breeding” and is based on combining the use of phenotypic pre-selection of better individuals, informative DNA markers for offspring fingerprinting, pedigree reconstruction to assemble full- and half-sib families after wind pollination, and simple quantitative genetics analyses to identify elite genotypes for seed orchards establishment. BWB’s potential and applicability is illustrated through a retrospective study of a “conventional” Douglas-fir breeding program. Results demonstrated the method’s effectiveness in capturing substantial amount of the genetic gain with relatively minimal efforts and allowed the development of a framework for its utility as an alternative to conventional breeding programs.

COMPARATIVE MAPPING OF SPRUCE AND LOBLOLLY PINE

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Conifers have large genomes (approximately $3-4 \times 10^{10}$ bp), and as the genome sequence of a conifer will not be available in the near future, the use of comparative mapping enables the transfer of information about genome structure and organization between conifer species. Recently, large EST databases have been developed for various conifer species, and these can be utilised to efficiently identify potential orthologous and other markers. We have developed a genetic linkage map of white spruce (*Picea glauca*) using bioinformatically identified COS, SSR and orthologous markers, as well as AFLP markers. This map was aligned with the reference loblolly pine map, which provides a framework for further targeted development of orthologous markers to elucidate the fine scale syntenic relationship between spruce and pine. We developed a system of mapping SNPs within these COS markers using a DNA mismatch digestion enzyme. Digestion with CJE (celery juice extract) identifies all SNPs within a PCR product, and enables this PCR product to be mapped. This is useful for transferring these markers to other species or crosses, as a particular SNP mapped in one cross need not be mapped in another, rather a different SNP on the same orthologous PCR product can be mapped in a different cross. All that is required is the PCR amplification of orthologous sequences from the species to be compared (and a SNP segregating within the mapping populations for each species). This property goes some way in overcoming the reduced information content inherent in (mainly) bi-allelic SNP markers.

INTEGRATING MOLECULAR GENETIC METHODS IN SEED SOURCE MANAGEMENT AND BREEDING ACTIVITIES OF NORDMANN FIR AND OTHER ABIES SPECIES

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Denmark has had a breeding programme for Nordmann fir (*Abies nordmanniana*) and Noble fir (*Abies procera*) since 1992. The former is used for production of Christmas trees, of which Denmark produces around 10 million each year; the latter is mostly used for greenery production. Besides that there is research in other *Abies* species such as Subalpine fir (*Abies lasiocarpa*) and

The Pacific Silver fir (*Abies amabilis*) – this research is mainly aimed at finding complementary Christmas tree species.

In 2002 a PhD project was started in order to integrate the use of DNA markers in the breeding and seed source management of Nordmann fir. This work is still going on, and efforts are now also made to expand this research to Noble fir and other *Abies* species. The presentation will give various examples of studies where the DNA markers (microsatellites) have or are being used to explore relevant issues and research hypotheses. This include studies of cryptic dysfunctions in clonal seed orchards, barriers to hybridization between European silver fir and Nordmann fir, seed source certification of imported provenances and male-female complementarity in relation to seed orchards with low number of clones. Also new methods of breeding will be discussed, including conversion of half-sib trials to full-sib trials and conversion of production stands to genetic field trials.

GENETIC VARIATION OF NORWAY SPRUCE IN SPACE AND TIME: COMBINED ANALYSIS OF A MITOCHONDRIAL MINISATELLITE AND FOSSIL POLLEN DATA

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Survival in glacial refugia and postglacial colonization are thought to be important determinants of the genetic structure we see in present populations at higher altitudes. However, few studies have tested genetic patterns against independent data from fossils and paleoclimate. In this study, we combined data of a minisatellite located in the mitochondrial *nad1* gene from 4876 trees (369 populations) with recently compiled fossil pollen data. We detected 28 haplotypes which formed a northern and a central European clade. In northern Europe, a single major gene pool was detected by SAMOVA, while in central Europe several distinct gene pools were identified. Three of the SAMOVA groups in central Europe were large and highly divergent, probably reflecting diversification during several glacial periods. When combined with pollen data, we identified five main sources for expansions in Europe during the Holocene: the Russian plains, the eastern Alps, the Bohemian massif, the West Carpathians, and the southern part of the East Carpathians. Genetic diversity was relatively high in most source areas both in northern and central Europe. Differentiation among the oldest populations on the Russian plains was very low, indicating a large continuous population at the time of expansion. In contrast, the oldest populations in the eastern Alps and the western Carpathians were more differentiated, suggesting longer-term isolation in separate refugial pockets. Colonization across vast areas in northern Europe did not lead to a large loss in genetic diversity but to an increase in population differentiation, a pattern concordant with pollen data which suggest that colonization was rapidly taking place supplemented with long-distance dispersal. In central Europe, diversity was kept over much shorter distances, possibly as a result of population bottlenecks. Along some migration routes in central Europe, population admixture from different refugia probably took place, increasing genetic diversity.

HYBRIDIZATION AND GENETIC VARIATION IN DANISH POPULATIONS OF EUROPEAN CRAB APPLE (*MALUS SYLVESTRIS*): IMPLICATIONS FOR CONSERVATION

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The European crab apple (*Malus sylvestris* (L.) Mill.) is an insect pollinated species native to most of continental Europe and the British Isles. The species has a scattered distribution within Denmark, where it is mainly found in small populations although some larger populations (>300) exist. *Malus sylvestris* has been planted in the Danish landscape for decades in shelterbelts and amenity plantings. Previously, such plantings have to a large extent been of non-local origin, but the interest in using native seed sources have increased in recent years.

Malus sylvestris was included in the Danish program for conservation of genetic resources of native woody species since 1994, but efficient conservation activities have been complicated by

- Risk of hybridization with the omnipresent domesticated apple *M. ×domestica* Borkh.
- Lack of knowledge on pollination distances
- Risk of genetic erosion due to historic or present low population sizes
- Risk of substantial gene flow from the imported origins into remaining native populations

In order to reduce this uncertainty, we initiated research that could shed light on a number of key aspects including selfing rates and gene flow, levels of genetic diversity and genetic differentiation, crossability between *Malus sylvestris* and *M. ×domestica*, synchrony in flowering time between *Malus sylvestris* and *M. ×domestica*, and levels of hybridization/introgression levels in wild populations candidate for in situ conservation.

The studies lead to a number of interesting results:

- Very low levels of selfings in natural populations (1%)
- Gene flow mainly confined to small distances, but a few large distance pollinations were observed
- No sign of significant inbreeding through either non random mating or genetic drift in the wild populations.
- *Malus sylvestris* appears to hybridize easily with *M. ×domestica* in controlled crosses, and no hybridization depression could be observed up to the stage of full germination
- The frequency of putative hybrids was substantially lower than previously expected in the native Danish stands when DNA markers were applied to supplement morphological indices of hybridization.

The results have assisted identification and management of Danish *in situ* conservations stands, but have also provided new tools for seed source establishment and management.

USING MARKERS IN BREEDING, TESTING AND SELECTION IN LOBLOLLY PINE – CURRENT AND FUTURE RESEARCH

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Molecular genetic markers have many potential applications in tree breeding, including verification of the identity of genotypes, validation of the parentage of seeds from controlled crosses, and identifying genomic regions associated with phenotypic variation in traits of interest. The costs of molecular genetic marker analysis are still relatively high, and the most efficient and cost-effective ways to apply genetic markers in breeding programs are still uncertain. This presentation will summarize important major genes (rust R genes, Cad-nl gene, and QTLs) discovered in loblolly pine during the last decade and applications of genetic markers in loblolly pine (*Pinus taeda* L.) breeding, testing and selection of North Carolina State University Cooperative Tree Improvement Program. Current and upcoming research projects to use markers for genetic analysis in pine breeding populations will be described.