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WFGA
Western Forest Genetics Association

IUFRO
Breeding and Genetic Resources of Pacific Northwest Conifers

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Forest Genetics 2013

A Joint Meeting of the

Canadian Forest Genetics Association (CFGA)

Western Forest Genetics Association (WFGA)

International Union of Forest Research Organizations (IUFRO) Population, Ecological and Conservation Genetics (Working Group 2.04.01)

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ABSTRACTS FOR TALKS AND POSTERS

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Presentations

Plenary Session 1

Tuesday July 23, 8:30 – Mt. Currie South

Ecological genomics of bud burst in temperate trees

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Monitoring of the timing of bud burst (TBB) during the past decades in temperate trees has shown that TBB has shifted substantially due to global warming. Climatic predictions suggest that this trend will continue in the future. These observable variations *in situ* demonstrate the important plastic phenological response of trees to climate change, but raise the issue of the consequences they will have on the fitness of the trees. I explore the various potential adaptive responses by considering the different evolutionary drivers contributing to TBB variation (divergent selection, assortative mating and gene flow) and by using theoretical and experimental approaches. Computer simulations suggest that the phenotypic shifts will enlarge the genetic differentiation along the same direction than the phenotypic cline due to the combined effect of assortative mating and gene flow. Such consequences may reduce fitness for species exhibiting counter-gradient variation, and increase fitness for species that follow co-gradient variation. I will further compare these predictions to observed variations at the phenotypic and molecular level in populations sampled along altitudinal/latitudinal gradients reproducing temperature gradients. Contrasting slopes of genetic clines are indeed observed between species (oaks vs beech) exhibiting co- and counter- gradient variation along the altitudinal/latitudinal gradients. Clines at the phenotypic level translated in a limited number of cases to clinal variation of allelic frequencies at candidate gene loci.

Tuesday July 23, 9:10 - Mt. Currie South

Forecasting future ranges: current limitations and next steps

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One of the most ubiquitous and well-documented types of biological responses to climate change is the shifting of species' geographic ranges to higher latitudes and altitudes. Because species are shifting at individualistic rates, changes in geographic ranges are rippling up to create important yet poorly understood changes in community structure and ecosystem functioning. Thus, a pressing challenge for ecologists, biogeographers, and conservation biologists is to more accurately predict the extent and rate of future range changes by different species. Tools that are currently available for forecasting future ranges often make simplistic assumptions about current range-limiting factors and fail to incorporate evolutionary processes. I will synthesize case studies of the perennial wildflower *Mimulus cardinalis* across its elevation and latitude ranges in western North America. Using field studies, lab experiments, and demographic models, these studies aim to determine the relative importance of different range-limiting factors and assess how local adaptation and eco-evolutionary dynamics influence the process of range shifts.

Tuesday July 23, 9:50 - Mt. Currie South

Genomic selection in *Eucalyptus*: current advances and the way forward

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The prospects of accelerating breeding for complex traits in forest trees by Marker Assisted Selection (MAS), has been one of the driving rationales of large forest tree genome projects. We were assured that we would be able to look at the tree's alleles at quantitative trait loci (QTLs) or even directly at genes and determine its breeding or genotypic value. This included the implicit assumption that by the genetic dissection approach we would have already established the effects of all important alleles during the life of the tree, in every population and environment. However, despite important advances in QTL mapping and association genetics (AG) in forest trees, it seems that ascertaining these effects is proving much more elusive than originally thought. QTLs numbers and QTL effects are under and overestimated respectively, while candidate-gene based AG captures negligible fractions of trait heritability to be useful to breeding. Notwithstanding the limitations of early genomic technologies, an overoptimistic outlook of the architecture of complex traits and an underestimation of the phenotypic plasticity of forest trees most likely explain why MAS has not made it yet in the 'real tree breeding world'.

The inability to ascertain the effects of QTLs has now caused a paradigm shift in molecular breeding. We are now moving from trying to discover genes or genomic regions and their individual effects, back to dealing with the aggregate of the genome, just like quantitative genetics, although with a much higher resolution driven by high density genotyping. We can now predict the Genomic Estimated Breeding Value (GEBV) of an individual using Genomic Selection (GS) [1]. Differently from MAS, where a small subset of marker-trait associations are declared using stringent statistical thresholds and then used for selection, GS estimates all marker effects simultaneously, retaining all of them as predictors of performance. The rise of GS was based on technical breakthroughs and declining costs of high-throughput genotyping, coupled to improved statistical methods to deal with large datasets. GS works on the principle that linkage disequilibrium (LD), provided by dense whole-genome genotyping, is sufficient to track all relevant QTL effects for the target trait(s) which are expected to be in LD with the queried markers. The extent of marker–QTL LD, dependent on the effective population size and marker density, has therefore the largest impact on the prospects of GS [2]. This impact will intensify as generations of selection advance because higher marker densities enable GS accuracy to persist over time due to a slower decay of LD [3].

Experimental studies in two *Eucalyptus* breeding populations [4] and in loblolly pine [5] head started GS in forest trees. Experimental accuracies averaging 0.5 to 0.8 were estimated for traits such as volume growth, wood specific gravity, lignin content and composition, pulp yield and fungal rust resistance, converging well to predictions from deterministic [2] simulations and indicating potential gains of 50-200% in efficiency of selection. We are now at the brink of operational implementation of GS in eucalypt intensive forestry in Brazil. To this end we developed a fully public 60,000 SNP Illumina *Infinium*™ genotyping chip for *Eucalyptus* species (EucHIP60K.br), based on whole genome resequencing and SNP discovery in 240 unrelated trees of 12 eucalypts species. This powerful chip has now been successfully validated across all major species of the genus by genotyping almost 17,000 samples from 10 different breeding populations, yielding high quality polymorphic data for >50,000 SNPs. Designed with one SNP every 10kbp on average, the chip supplied an inter-marker spacing of polymorphic markers (MAF>0.01) between 15.4kbp to 32.3kbp. Substantial increases in prediction accuracies of GS models were obtained with SNP data generated with the EucHIP60k.br.

The prospects of GS in tree breeding are very promising [6]. GS can increase the rates of genetic gain per unit time through increased accuracy of estimated breeding (for parent selection) or genotypic (clone selection) values, reduction of generation time and optimization of genome-directed mate allocation. How to incorporate GS into a *Eucalyptus* improvement program will vary on a case by case basis, following a cost/benefit analysis coupled to the adoption of innovative breeding tactics. Several issues are currently the focus of GS research, including: (1) modeling of non-additive effects; (2) accuracy of predictions, inbreeding and reduction of selection response as generations of GS advance; (3) GS modeling across different populations and environments; and (4) logistic issues such as special nursery infrastructure, sample collection and tracking. Being it a publicly accessible genotyping tool the EucHIP60k.br will allow assembling increasingly larger datasets, opening unprecedented opportunities of large scale meta-analyses of genotypic and corresponding phenotypic data. Not only this will empower genomic selection, but should also enhance genome-wide association studies, gene discovery and population genomics experiments in species of *Eucalyptus*.

Acknowledgments
PRONEX FAP-DF/CNPq grant NEXTREE (193.000.570/2009) and a CNPq Research fellowship.

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Adaptation and Climate Change – Session 2

Tuesday July 23, 10:50 - Mt. Currie South

Estimated reduction of suitable climate habitat for *Pinus pseudostrobus* and experimental altitudinal upward assisted migration for accommodating climatic change

Cuauhtémoc Sáenz-Romero[1], Gerald E. Rehfeldt[2], Dante Castellanos-Acuña[1], Roberto A. Lindig-Cisneros[3], Juan Manuel Ortega-Rodríguez[4], María Consuelo Marín-Togo[4], Xavier Madrigal-Sánchez[4]
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A bioclimate model predicting the presence of *Pinus pseudostrobus*, the economically and ecologically most important conifer species in the Trans-Mexican Volcanic Belt and Guatemala highlands, was developed using the Random Forests classification tree, for contemporary (average 1961-1990) and future climates (decades centered in the years 2030, 2060 and 2090) using three General Circulation Models (Canadian, Hadley and Geophisical Fluids Dynamic) and two emission scenarios (A2, elevated emissions and B1 or B2, low emissions). Results suggest that for México and Guatemala, the area occupied by the *Pinus psedostrobus* climatic niche should diminish 51.3 % by 2030, 68.3 % for 2060, and 89.6 % for 2090. Areas remaining suitable in the future were always projected for higher and higher altitudes. Because altitudinal clines are well-documented for this species, the results indicate the need to realign contemporary populations to the climate for which they are adapted, which will occur at different sites than today. We tested this hypothesis at the forest of the Native Indian Community of Nuevo San Juan Parangaricutiro, Michoacán, western México, by experimentally shifting populations of *P. pseudostrobus* and *Pinus devoniana* (=*P. michoacana*) upwards in altitude. The latter species is naturally distributed at altitudes below the former and thus would be the intuitive candidate to replace the former. At 2 years, we found very high survival (> 98 %) and a gradual decrease of growth as the altitudinal shift increases. When *P. pseudostrobus* populations were moved +300 m (the shift needed to accommodate the climate change projected for year 2030), relative growth in seedling height decreased 15 % (5 % per each 100 m of elevation shift) in comparison to local populations. *P. devoniana* grew as much as the local population when moved +300 m, but decreased 30 % when moved +600 m (again, 5 % per each 100 m of elevation shift). This suggests that assisted migration might be a viable strategy to realign genotypes to projected climates for year 2030, although achieving less growth than natural populations under contemporary climate in the short term.

Tuesday July 23, 11:05 - Mt. Currie South

Landscape genomics of adaptive genetic variation in long-lived populations of a California oak, *Quercus lobata*

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Quercus lobata (valley oak) is an endemic widespread oak in California that occurs in grasslands, woodlands, and riparian forests, but is now in serious jeopardy due to habitat loss and threat of global warming. These oak populations have occupied unglaciated regions for at least 120,000 years without significant shift in the climate niche, although there were some local contractions and expansions. This stability provides ample opportunity for local adaptation in response to climate, though temporal fluctuations may reduce those effects. Using population samples from throughout the species range, we will examine three types of evidence for local adaptation. First, we examine the association of gradients in allele frequencies of SNPs at selected candidate genes with climate variables and identify several genes associated with budburst and cold tolerance that are significantly correlated with climate gradients. Then, we report the results of an environmental association analysis based on whole bud/leaf transcriptome data sampled from widely distributed localities. Once we control for the background genetic structure, we find outlier SNPs associated with environmental gradients in growing season precipitation that suggest additional candidate genes. Finally, we will present our genome-wide association study of SNPs associated with climate gradients, based on SNPs discovered through reduced-representation genome sequencing approach. Overall, these three approaches produce evidence that local adaptation is shaping the distribution of climate-associated adaptive genetic variation in natural populations.

Tuesday July 23, 11:20 - Mt. Currie South

Assisted migration of tree populations to address climate change in western Canada

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Commercial forestry programs commonly use locally collected seed for reforestation under the assumption that tree populations are optimally adapted to the local environment. This assumption however, may no longer be valid for many forest ecosystems in North America given the warming trends and changes in precipitation patterns observed over the last 25 years. Here we use a bioclimate envelope modeling approach to project suitable species habitat and estimate the adaptational lag for populations of 15 major commercial forestry species in western North America under observed climate trends (1970s to 2000s) and multi-model projections for the 2020s, 2050s, and 2080s.

We find that on average, populations already lag behind their optimal climate niche by approximately 130km in latitude, or 60m in elevation (Gray and Hamann, 2013. Climatic Change, 117: 289-303). Our models further show an average lag of approximately 310km in latitude or 140m in elevation by the 2020s, with the most pronounce geographic lags identified in the Rocky Mountains and the boreal forest. For application, we are able to derive a general formula where 100km northward shift is equivalent to an approximately 44m upward shift in elevation, which in principle could be applied to guide assisted migration of planting stock in reforestation programs together with the projected species distribution maps provided.

Additionally, we generated detailed seed transfer matrices based on an ecosystem classification system, specifying seed source and planting target locations in Alberta to help guide reforestation decisions in the province (Gray and Hamann, 2012. PLoS One, 6(8): e22977). We find that genotypes of species that are adapted to drier climatic conditions will be the preferred planting stock over much of the boreal forest that is commercially managed. Interestingly, no alternative species that are currently not present in Alberta can be recommended with any confidence. Finally, we observe large uncertainties in projections of suitable habitat that make reforestation planning beyond the 2050s difficult for most species.

Tuesday July 23, 11:35 - Mt. Currie South

F_{ST} Outlier tests in genome scans for local adaptation: when do they tell the truth and what are we missing?

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Next-generation technology has made it possible to obtain large amounts of genomic data. But how do we find locally-adapted genes from this mountain of data? One method is to look for outliers in the distribution of F_{ST} (F_{ST} outlier tests, FDIST (Beaumont and Nichols 1996) and BAYESCAN (Foll and Gaggiotti 2008)). Using a large-scale landscape genetics simulator, we compared these two programs for three demographic histories that are common to tree populations: isolation-by-distance, expansion from one refuge, and expansion from two refugia with secondary contact. The latter two demographies were non-equilibrium scenarios. We found a large number of false positive F_{ST} outliers with these demographic histories, especially with the refugia scenarios. We show that the default parameters in Bayescan produce more false positives than FDIST, but that the number of false positives can be decreased with the prior-odds parameter without affecting power.

We also propose that the FDIST method can be improved by simulating the actual number of populations in the data, rather than by the number of samples collected.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Tuesday July 23, 11:50 - Mt. Currie South

Perceptions of adaptation in forest management

Molly M. Moshofsky, Reem Hajjar, Robert A. Kozak
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Forest management is presently undergoing major changes to adapt to a changing climate. The objective of this research is to examine the variation in perceived acceptability of potential forest management interventions that can mitigate the risks of climate change among stakeholders in British Columbia (BC) and Alberta (AB). A mixed method research design was developed that included surveys, focus groups, and Q-sort analysis. Online surveys were administered to the general public and leaders of forest-dependent communities in BC and AB to determine their levels of acceptability of various intervention strategies for forest adaptation (ranging from a "do nothing" approach, a status quo option, assisted migration, and genetically engineering optimal growth in future climates), as well as to assess their knowledge and perception of risk of climate change and reforestation technologies. Online surveys were also administered to registered professional foresters and biologists in BC, assessing their levels of knowledge and their perceptions of efficacy of assisted migration strategies. In addition to the surveys, focus groups were conducted in four case-study communities to provide an in-depth analysis of stakeholder perceptions. Three qualitative focus groups were conducted in each community and an exit Q-sort was administered to measure perceived acceptability to a set of nine forest adaptation management scenarios. With analysis underway, preliminary results indicate that professional foresters perceive the status quo management option as the most acceptable, indicating in Q-sorts that the natural regeneration scenario is perceived better than scenarios featuring assisted migration in some cases. Business owners were observed to perceive the status quo scenario as most acceptable, in some cases remarking on the importance of adapting human behaviour to the changing environment. Environmentalists' responses varied widely by region with some regarding assisted migration as the most acceptable and some preferring the status quo. Ultimately, it appears that a hesitance to accept human capability to project climatic and ecosystem changes on the landscape informs the perceived acceptability of all management scenarios.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Tuesday July 23, 12:05 - Mt. Currie South

Early survival and climatic damages of white, Engelmann and interior spruce populations in Alberta

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As part of the adaptation to climate change, British Columbia in collaboration with Alberta initiated a new series of provenance trials for white, Engelmann and interior spruces with populations sampled from across western USA and Canada. This series is replicated on eighteen sites (15 in BC, 2 in Alberta and 1 in the Yukon Territory). The series builds on the knowledge gained from previous provenance trials of white and interior spruce in Alberta and British Columbia in the past 30 years. The new trials were designed to measure the climatic effects of transferring tree populations from as far south as latitude 36° in Arizona to as far north as latitude 65° in the Yukon Territory. The trials located in Alberta at Whitecourt Mountain (54°N, 817m) and Hay River (59°N, 370m) are of great interest in that they

provide an environment that is colder and more continental than trials in British Columbia that are more maritime and located in the south.

When tree mortality is assessed in older field trials, it is impossible to identify its causes, which impede out ability to link tree mortality to adaptation to climate and the potential effects of climate change on forestry. Limitations encountered when using data from older provenance and progeny trials have led us to modify trial assessment procedures so that the causes of tree mortality can be identified during the first five years of field establishment. During the first five years of the BC-Alberta spruce provenance trials we have found that,

Much of the tree mortality occurs during the first few years of field establishment. Four years in the field at Hay River, survival was 30 – 67% for provenances from warmer environments in the USA and southern BC; 82 – 94% for provenances from cooler environments in northern BC, Alberta and Northwest Territories; 72 – 79% for provenances from Montana and Idaho. The correlation between survival and negative degree days (NDD) was 0.53 ($P < 0.0001$) showing that trees from regions with cooler winters had better survival at Hay River than provenances from regions with warmer winters.

At Hay River, assessment at age 3 years showed that on average, less than 7% (range 1 – 10%) of trees for provenances in northern BC, Alberta and Northwest Territories suffered diebacks. Provenances from the USA and southern BC had as high as 31% dieback. The correlation between percentage dieback incidences and NDD was -0.72 and -0.77 during the second and third field season, respectively. The corresponding correlations at the Whitecourt Mountain were -0.55 and -0.53. This shows that provenances from cooler environments suffered less dieback than provenances from warmer environments. The correlation for dieback incidences between Hay River and Whitecourt were 0.59 and 0.63 during the second and third field season, respectively.

Generally, preliminary assessment of these two trials suggests that transferring populations for long climatic distances could potentially harm forest health and productivity through repeated diebacks at least during the stand establishment stage.

Tuesday July 23, 1:15 - Mt. Currie South

Broad- and fine-scale genetic structure of a spruce (*Picea sitchensis* x *P. glauca*) hybrid zone spanning a climatic gradient

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Historic colonization and contemporary evolutionary processes contribute to broad- and fine-scale patterns of genetic variation and differentiation among populations. However, teasing apart the respective influences of these processes remains a challenge. This is particularly true of natural hybrid zones, where standing genetic variation may result from evolutionary processes both preceding and following contact, influencing the evolutionary trajectory of hybrid populations. Where adaptation to novel environments may be facilitated by interspecific hybridization, teasing apart these processes will have practical implications for forest management in changing environments.

In this study, we evaluated the genetic architecture of the economically and ecologically important *Picea sitchensis* (Sitka spruce) *x P. glauca* (white spruce) hybrid zone along the Nass and Skeena river valleys in northwestern British Columbia using chloroplast, mitochondrial and microsatellite loci, alongside a complementary study using single nucleotide polymorphisms (SNPs). Sitka spruce mitotype 'capture' throughout the introgression zone point towards asymmetric gene flow, congruent with patterns observed for microsatellites and SNPs, indicating extensive long-term introgression and widespread recombination with more Sitka spruce than white spruce ancestry in hybrid populations. Significant and complementary clinal variation for marker-based hybrid indices based on both neutral microsatellites and candidate gene SNPs suggest ancestry is strongly influenced by climate and geography. These results indicate extrinsic selection plays a strong role in the distribution and structure of this hybrid zone. Indeed, highly parallel results for replicate transects along climatic gradients provide support for the importance of extrinsic selection at a broad scale, fitting expectations of the environmentally-determined bounded hybrid superiority model of hybrid zone maintenance. Finally, broad-scale patterns of variation, combined with fine-scale analysis of candidate SNP-specific patterns of introgression revealed a suite of candidate loci that may be targets of extrinsic or intrinsic selection, particularly along precipitation gradients. This broad- and fine-scale analysis of genetic structure indicates the importance of historic and contemporary gene flow, environmental selection, and their interaction in shaping genetic variation within this hybrid zone. These results have important implications for selection of seed sources for current reforestation within this ecologically transitional area, and appropriate scale and direction of seed transfer relating current genotype-climate associations to future climate predictions for this region.

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Hamilton, J.A., and Aitken S.N. Genetics and morphology of a spruce (*Picea sitchensis x P. glauca*) hybrid zone along a precipitation gradient. Accepted at American Journal of Botany.

Tuesday July 23, 1:30 - Mt. Currie South

Chasing drought tolerance in *Populus*

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Introduction:
The cultivation of poplars (*Populus* spp.) is favored for forestry and reclamation purposes throughout the northern hemisphere where they represent a commercially important resource. More recently, hybrid poplars are planted and grown to provide feedstock for pulp and paper production and a carbon-neutral alternative to fossil fuels (Balatincz and Kretschmann, 2001; Yuan et al. 2008). Poplars may also help Canada to meet its international commitments to reduce greenhouse gases by carbon sequestration. Poplars, however, are generally regarded as drought sensitive, which is a problem especially in the light of increasing summer droughts in the prairies. Several studies including one from the Intergovernmental Panel on Climate Change also predict increased drought frequencies on the Canadian prairies (IPCC, 2001).

Here, we sought to address four main objectives related to drought tolerance in poplar:

1. To evaluate drought responses in hybrid poplar clones commonly planted in the Canadian prairies and subsequently generate a drought-tolerance ranking.
2. To use the contrasting responses of the least and the most tolerant clones to identify gene expression responses that correlate with drought tolerance.
3. To rapidly screen the role of identified poplar genes in drought tolerance by overexpressing them in transgenic *Arabidopsis* plants and assess the plants for altered drought responses.
4. To alter the expression of poplar genes identified in objective 3 in transgenic poplar in a manner that may improve drought tolerance.

Results:
We first compared nine hybrid poplar clones commonly grown on the Canadian prairies with respect to height growth, leaf formation, water potential and relative water content during controlled drought in a greenhouse setting. We found significant differences in responses between clones for all four criteria. In particular, one clone stood out as being tolerant to drought. Likewise, one clone was particularly sensitive to drought. The ranking generated from this analysis can inform the choice of clones for deployment at drought-prone sites.

Secondly, a set of genes that are induced by drought was identified from the literature (Bogeat-Triboulot et al., 2007; Plomion et al., 2006; Street et al., 2006; Wilkins et al., 2009) and that based on their sequence similarity, they may be involved in different aspects of drought responses such as stomatal movement, osmotic adjustment, ABA signaling and cellular water conservation. We thereafter compared their expression in the least and the most drought tolerant poplar clones during drought stress. This comparison yielded a subset of genes whose expression differed significantly in the two clones, including three genes with high similarity to genes in *Arabidopsis* implicated in ABA signaling and drought tolerance.

To further test the function of these three differentially expressed poplar genes, we initially overexpressed them in transgenic *Arabidopsis*. We measured seed germination, root growth, fresh weight accumulation and water loss in wild type and transgenic *Arabidopsis* plants. Based on these parameters, transgenic *Arabidopsis* plants overexpressing a poplar ortholog exhibited drought hypersensitive and ABA-insensitive phenotype. In addition, we also observed a molecular phenotype in the form of reduced expression of genes with known function in drought tolerance providing evidence that the tested poplar gene is a negative regulator of drought tolerance also on the molecular level. Similarly, transgenic *Arabidopsis* overexpressing a second poplar gene showed a reduced water loss during drought stress indicating that this gene is a positive regulator of drought tolerance.

In light of these promising results, we have introduced the same DNA constructs into poplar plants. We intend to present our first analysis of drought responses of these plants as well as a perspective on how our results can be used for drought tolerance breeding in poplar based on both transgenic technology and marker-assisted selection based on natural variation.

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Geographic variation in phenology behaviour and response to drought of cork oak populations is crucial to cope with climate change

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Cork oak is widely distributed in the Western Mediterranean region, spanning a range of different environmental conditions, and frequently dominating open woodlands of high conservation and socio-economic value. Cork oak woodlands spread-out for over 715000 ha in Portugal, covering 21% of the national forested area and 30% of the world's cork producing area. Cork oak is well adapted to the seasonal drought of Mediterranean climate, following several decades of warming- up and frequent drought years. However, since the 70's, maximum and minimum temperatures have risen in Portugal about 0.5 ºC each decade, corresponding to twice the average world temperature increment. In addition, since longer, more frequent, and more intense drought periods are expected, stress caused by the expansion of arid and semi-arid climate throughout the country will affect the species distribution. Consequently, not only established stands may be prone to tree mortality, but also the current reforestation effort may be jeopardized by low survival rates attributed to the use of unsuitable genetic material.

It is expected that, through genetic adaptation and/or phenotypic plasticity, cork oak populations may have developed significant differences in fitness and the traits related to it. In this context, provenance trials are the best resource of material to assess the variability between and within populations from seed sources sampled in a wide range of locations (stands) covering the geographical distribution of the species. This will enable to assess the levels and patterns of genetic variation for growth and traits determining adaptation to a specific environment, hence providing crucial information to select appropriate seed sources for planting, as well as to develop sustainable breeding and gene conservation programs. In 1998, multi-site provenance trials were established at three locations in Portugal, as a part of the “European Network for the Evaluation of Genetic Resources of Cork Oak for Appropriate Use in Breeding and Gene Conservation Strategies”. Results from these field trials at 7 years from planting pointed out that seed origin must be considered in reforestation programs, since survival, growth and adaptive traits (phenology, water use efficiency - wue) showed significant differences among populations. Responses to drought (a major limitation to cork oak regeneration) of four contrasting populations, selected according to their field performance for growth, phenology and wue, were further studied under controlled-environment conditions where drought stress was induced. In addition, morphological (total height, root/shoot biomass), physiological (wue, water potential, relative water content and gas exchange) and biochemical (maximum quantum yield of PSII) traits were assessed in 6-month seedlings produced from acorns collected in the stands representing the four contrasting provenances. Results from the controlled experiment provided an indication that drought adaptation was mainly related to early stomatal closure and root investment, with these traits showing significant differences between the studied populations. We have also observed significant differences in growth rhythm, as well as dissimilar temporal drought responses, in the populations under study. In conclusion, both field trials and controlled environment showed consistent results and indicated an important influence of geographic origin on growth performance and wue.

Tuesday July 23, 2:15 - Mt. Currie South

Species specific versus parallel evolution of local adaptation in two white pine species: *Pinus monticola* and *Pinus strobus*

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Under rapid global warming, it is critical for us to better understand the capacity of forest trees to adapt to a changing climate, especially for species that are more at risk such as five-needle pines. Western white pine (*Pinus monticola*) and eastern white pine (*Pinus strobus*) have fragmented ranges and have suffered declines due to harvesting, fire suppression and the white pine blister rust. We identified and compared patterns of genetic diversity and adaptation to climate in these two species by using a set of 267 orthologous genes, including candidate genes for growth, bud phenology and resistance to biotic and abiotic stresses. From those genes, assays for ~200 single nucleotide polymorphism (SNP) markers for each species were developed. Each set of SNPs was genotyped on range-wide samples of 362 *P. monticola* individuals (61 populations) and 840 *P. strobus* individuals (133 populations). Genotyping resulted in 158 and 153 successful SNPs for *P. monticola* and *P. strobus* respectively. Depending on the species lineage and statistical models chosen, F_{st} outliers analyses revealed that from 4% to 25% of SNPs were candidates for natural selection, with the number of outliers being higher in *P. strobus* than in *P. monticola*. Similarly, ~25% of *P. monticola* SNPs and ~30% of *P. strobus* SNPs were significantly associated with one or more climate variables. Furthermore, out of the 79 genes containing SNPs in both species, ~25% showed signature of selection simultaneously in both species. These results indicated that a considerable amount of SNPs, genes and gene families are under strong selection in both *P. monticola* and *P. strobus*. However, a larger number of SNPs and genes that have contributed to local adaptation are specific to each species. This indicates that parallel and species specific evolution can have occurred to shape adaptation to

climate in these two conifer species, which have diverged ~20 million years ago.

Tuesday July 23, 2:30 - Mt. Currie South

Representing cold tolerance in models of tree growth

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Predicting effects of climate change on productivity of boreal and temperate forests requires a quantitative description of how cold adaptation mediates tree growth, yet this comes with the challenge of disentangling genetic differentiation from effects of ontogeny and climate. In this study, we calibrated a model of thermal and hydraulic limitations against 4.9 million periodic forest inventory measurements of relative growth rate (RGR), distributed across 34 North American species/subspecies sample groups, to develop a general framework for representing cold tolerance. Values of leaf-specific hydraulic conductance (K_L), derived from optimal fit against each sample, were correlated with the long-term average annual number of days with freezing (DWF) and the product of climate moisture index (CMI) and DWF (R^2=0.90, p<0.001). We interpret this as the selection of increased immunity against freezing-induced embolism at the expense of reduced hydraulic efficiency. Increased drought sensitivity in cold climates is partially balanced by increased cold hardiness, represented by setting reference growth at 10 °C in an exponential function of temperature (G_{10}) as an inverse function of DWF and setting direct sensitivity to freezing (δ_F) as a function of K_L. The framework was equally applicable within the distributions of two tested species (Douglas-fir and white spruce). Hence from a modeling perspective, the overall cold tolerance of trees predetermines how heat and water budgets limit immediate RGR by defining states of hydraulic efficiency and cold hardiness, while low hydraulic efficiency in cold-adapted populations controls the biomass sink by lowering the stem height and leaf area ratio that narrowly avoid drought-induced hydraulic failure over the long term. Estimating these traits using a climatological approach, by species or population, provides a foundation for representing maladaptation in spatially-explicit model predictions of tree growth under future climate change scenarios. We discuss the additional potential to map a cold tolerance index (CTI) based on geographically-weighted regression of satellite observations and ongoing efforts to test levels of hydraulic compensation and plasticity in G_{10} by comparing the model with tree-ring measurements.

Tuesday July 23, 2:45 - Mt. Currie South

Glacial refugia and modern genetic diversity of western North American tree species

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Interglacial warm periods, such as the current Holocene, are the exception to the Quaternary norm. The evolution of North American tree species, subspecies, and genetic varieties has therefore primarily taken place in landscape with extensive continental ice and restricted temperate climate environments. Here, we reconstruct historical biogeographies and glacial refugia of western North American trees to evaluate the merit of biogeographic hypotheses regarding the existence of glacial refugia in Beringia and along the Pacific coast, the evolution of subspecies in widespread conifers, and the origin of Pacific Northwest inland rainforests. We use a species distribution modelling approach, validated against 3,571 fossil pollen and packrat midden records from 835 study sites. Genetic diversity was measured as allelic richness and expected heterozygosity, compiled from published studies for 473 populations of 22 tree species. We find that species with strong genetic differentiation into subspecies and varieties had widespread and large glacial refugia. In contrast, species with restricted refugia show no differentiation and little genetic diversity, despite being common over a wide range of environments today. In a regression tree analysis, 66% of allelic richness could be explained by the total size of glacial refugia. Expected heterozygosity was best explained by the number of glacial refugia as expected from genetic theory (27% variance explained).

Habitat reconstructions further provide strong support for the existence of glacial refugia in Beringia and Haida Gwaii for many species, and suggest only a recent emergence of coastal Pacific Northwest tree species in inland rainforest.

Tuesday July 23, 3:15 - Mt. Currie South

Matching current forest genetic resources to future climates

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One of the major concerns about the impact of climate change on ecosystems and forest trees is the mismatch between the climate that a forest ecosystem or a tree species is adapted to and the climate that the ecosystem will experience in the future. If such a mismatch occurs, the health and productivity of the ecosystem are likely to be compromised. Therefore, matching current forest trees with future climates is critical to ensure our future forests are adaptable to future climates. Niche-based bioclimate envelope models have been widely used to project future geographic distributions of ecosystem climate niches, which can be used as the basis for climate matching. However, challenges arising from model accuracy and the uncertainty of future climates make it difficult to apply the model projections with confidence in developing adaptive strategies in natural resource management.

We addressed these challenges through 1) using high-resolution and high-quality climate data generated by ClimateWNA; 2) optimization of Random Forest modeling approach (a machine-learning classification approach); and 3) consensus projections based on multiple climate change scenarios. We found that our bioclimatic envelope model accurately predicted the distribution of current ecosystems in British Columbia (BC). Based on our predictions, over 20% of the BC landscape has already shifted to climate envelopes of different ecosystems since 1970s. Projected shifts in BC ecosystem climates are substantial under climate change, which may seriously compromise the health of the ecosystems and impose challenges to land managers and policymakers. However, projected expansions of climate niches for several productive ecosystems may provide an opportunity to increase forest productivity and carbon sequestration capacity in BC if appropriate species and populations are matched for future climates. The consensus projections have been used in species selection and seed transfer and other projects. A web-based platform has also been developed to allow users to visualize and access to high-resolution climate data and the projections. To address the among-population variation within a species in the climate matching, we have developed population genecology functions, response functions and a universal response function for Lodgepole pine. A climate-based seed transfer system is being developed using both consensus projections and the universal response function.

Tuesday July 23, 3:30 - Mt. Currie South

Photoperiodic competency for bud dormancy induction in *Populus balsamifera*

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Bud dormancy is an important overwintering mechanism for woody perennials and is induced in most species during late summer by an increase in night length and/or low temperature. Adaptively, however, it would be important for newly flushed growth to avoid responding prematurely to a similar day-length that occurs in early spring. To investigate this phenomenon, two growth chamber experiments were conducted on four genotypes of balsam poplar (*Populus balsamifera* L.), originating from two latitudes. Rooted cuttings were moved into inductive, short-photoperiod conditions at weekly intervals after flushing at either 15°C or 20°C. Plant heights were measured every other day. Plants were harvested weekly and RNA were extracted for RT-qPCR on genes known to be involved in dormancy induction, and also for transcriptome sequencing followed by RT-qPCR to identify additional genes marking photoperiodic competency which were validated by qRT-PCR. Analysis of height growth cessation data showed that before a certain age, no matter how soon plants were transferred to short day conditions, they continued to grow until they became competent to respond to photoperiod. The different genotypes became competent at different times (18-40 days since flush), indicating possible genetic variation in this trait. Once competency was attained, it took plants 7-20 days to cease height growth under short photoperiod depending on genotype, experiment and time since competency acquisition. Leaf number data revealed that competency acquisition and the transition from preformed leaf emergence to neoformed leaf production are two independent processes. Temperature did not appear to influence the development of competency, though it slightly increased the rate of height growth cessation. RT-qPCR results indicated three promising gene markers for competency: Potri.017G051100 (G6), Potri.001G222000 (G7) and CONSTANS 2 (CO2). Increased expression for G6 and G7 was observed post-competency relative to pre-competency, and changes in expression varied between leaves and stem tissue. In contrast, CO2 peaked in abundance at the age of 32d from bud flush, coinciding with competency acquisition. This research offers new insight into molecular mechanisms that may underlie the acquisition of photoperiodic competency, which, in a warming climate, may cause phenological mismatch in deciduous boreal tree species. By advancing dates of bud burst, earlier springs may result in plants becoming competent too early, causing premature height growth cessation and loss of productivity.

Tuesday July 23, 3:45 - Mt. Currie South

Geographic patterns of adaptive variation in interior spruce and lodgepole pine in Western Canada

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In western Canada, both interior spruce (*Picea glauca*, *Picea engelmannii*, and their hybrids) and lodgepole pine (*Pinus contorta*) are important commercial forest tree species. However, a changing climate may lead to mal-adaptation and thereby decrease forest productivity and threaten forest health. Assessing the adaptive characteristics of populations within these species is important for the development of forest management strategies to match reforestation stocks with predicted future climates. This research, conducted as part of the AdapTree project, investigates phenotypic variation of lodgepole pine and interior spruce at the landscape level. Seeds were collected in British Columbia and Alberta from over 250 locations, germinated and grown in environmental chambers with four different temperature and moisture regimes. Phenotypic traits (i.e. height, diameter, budbreak, budset and cold hardiness) were measured to investigate variation among sampled populations. Central objectives in the analysis are to: 1) assess phenotype-climate correlations and 2) examine geographic patterns of phenotypic variation to then 3) determine groups of populations having similar phenotypes as the basis for seedzone delineations. Phenotypic traits will also be used in future genome-wide association studies to determine the genomic basis of local adaptation to climate.

Individual climate variables could explain between 5 and 25% of the variance in measured growth and adaptive traits. Variables such as mean warmest month temperature and growing degree-days best explained variance in growth, whereas variables related to winter temperatures correlated best with cold hardiness. Geographic distribution of phenotypic variation in interior spruce indicated a separation of cold hardy populations east of the Rocky Mountains from susceptible populations in the west. The most rapid growth was observed for seedlings originating from the interior valleys of British Columbia. High elevation interior spruce populations were distinguished by late budbreak and early budset. Lodgepole pine showed similar patterns with the exception of a few frost hardy and simultaneously well growing populations from Alberta. Provenances from *Pinus contorta* ssp. *contorta* the BC coast stood out from interior ssp. *latifolia* with late budbreak and high susceptibility to frost. Multivariate partitioning of genetic variation using a regression tree approach suggests 11 seed zones for interior spruce and 9 seedzones for lodgepole pine that account for 18% and 14% of the total observed phenotypic variation, respectively.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Tuesday July 23, 4:00 - Mt. Currie South

Identifying putative adaptive variation among and within hybridizing lodgepole pine and jack pine

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The most recent outbreak of the mountain pine beetle is unprecedented. Millions of hectares of pine forests in western Canada and the United States have been decimated, causing economic loss and affecting ecosystem health. The main host affected by this outbreak has been lodgepole pine (*Pinus contorta* var. *latifolia*), which shares a co-evolutionary history with mountain pine beetle (*Dendroctonus ponderosae*). The outbreak range for mountain pine beetle underwent major range expansion into northern Alberta in 2006; in 2011, we used molecular markers to identify successfully attacked lodgepole × jack pine hybrids, and jack pine (*Pinus banksiana*), a new host species. With the range expansion into a new environment and host we are faced with many unknowns regarding spread-risk potential and tree response. For example, how will genetic differences between lodgepole, jack pine, and their hybrids affect mountain pine beetle population dynamics, including reproduction? Will the beetle become endemic in this expanded range? Will expansion continue east and north, beyond the borders of Alberta? How will changing climate affect these dynamics? With heightened uncertainty regarding spread-risk potential, our aim is to identify host genetic factors that may contribute to mountain pine beetle spread risk. To do this, we developed genomic resources using two-year old lodgepole and jack pine seedlings exposed to biotic and abiotic stresses to increase the expression of potentially informative genes. From these we generated cDNA libraries from root, needle and stem tissues that were sequenced on a 454 next-generation platform for each species. The resulting transcriptome assemblies were mined for SNPs; following extensive filtering, 1 536 SNPs were selected for population typing using Illumina's Golden Gate assay. A total of 549 individuals were typed, comprising lodgepole pine, lodgepole x jack pine hybrids, and jack pine selected from both within and beyond the region currently affected by mountain pine beetle. From this analysis, 472 SNP loci were identified that were both variable and exhibited good amplification. Using these data, we have identified SNP loci that are putatively under selection by conducting three complementary approaches. First, we used outlier detection (BayeSCAN, Arlequin) to identify SNP loci that exhibit greater or lesser genetic differentiation than expectations based on all loci. Second, we used environmental correlation analysis (MatSAM and bayENV) to examine the relationship of alleles with a number of environmental variables. Finally, we used multinomial regression (INTROGRESS) to identify SNP loci that exhibited either more or less frequent degrees of introgression across the lodgepole x jack pine hybrid zone than expected. From consensus of these methods, we identified potential candidate loci across all samples (12), and within lodgepole pine (10) and jack pine (7). Comparison across the different methods highlights the potential for false positives and emphasizes the application of multiple methods as a best practice to ensure relevant results. Candidate loci identified by these methods are associated with genes that encode products putatively involved in important processes such as water transport, transcriptional regulation,

metabolism and stress response. Based on these findings we will carry out additional genotyping to better characterize the spatial distribution of these alleles which will allow us to test for associations with mountain pine beetle spread patterns. Our application of genomic resources to identify spread-risk factors in a non-model, complex system is a novel approach that could be extended to other forest-insect interactions.

Tuesday July 23, 4:15 - Mt. Currie South

Natural adaptation in *Populus trichocarpa* and implications for climate change

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Black cottonwood poplar (*Populus trichocarpa*) is a widespread, deciduous tree occurring west of the Rocky Mountains from Alaska to northern California and spanning a wide range in local growing season and climate. *Populus trichocarpa* shows strong relationships in many traits relating to its natural distribution along this latitudinal cline. We investigated inherent trait variation within *P. trichocarpa* using over 2000 trees grown in a common garden at Vancouver (representing replicate plantings of 461 collected genotypes within 136 provenance localities). We focused on seasonal (phenological) events, biomass accumulation and growth rates, canopy traits, isotopes, and gas exchange-based traits. We also estimated broad-sense trait heritability and overall population patterns with ~29K single nucleotide polymorphisms (SNP) in 3518 candidate genes using Illumina sequencing. Our findings showed that common garden trees demonstrated substantial variation in traits, many of which were repeatable year to year, and most traits correlated with latitude, length of day (photoperiod), and annual temperature but not with precipitation or moisture indices. Trait heritability estimates were highest in phenology traits and moderate to low in biomass and other ecophysiology traits. In general, traits with higher heritability also had higher correlation to biogeographic variables with few exceptions. Overall genetic population structure was clinal, and related directly to the latitudinal cline from our accession collection. Many SNPs contributing to this structure are described genes, including clock genes and other photoperiodic related genes. The strong relationship between most traits in *P. trichocarpa* with latitude, length of day, and temperature suggests that selection is driven by a complex environment. This indicates that natural migration and/or inherent biological response to climate change in *P. trichocarpa* may be diminished by a strong photoperiodic requirement.

Breeding Pacific Northwest Conifers – Session 3

Tuesday July 23, 10:50 - Boardroom

Cooperative advanced-generation breeding and testing of western hemlock in Oregon and Washington

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First-Generation Testing

The IFA-PNW “Progressive Tree Improvement System” was launched in 1966 due to the failure of grafted Douglas-fir clonal orchards at that time (Silen and Wheat 1979). The emphasis was on forming local cooperatives to share costs, and on progeny testing large numbers of trees using wind-pollinated seed on multiple sites in small testing zones.

Western hemlock is a prolific and dominant species in the coastal forests of the US Pacific Northwest, and often outcompetes coastal Douglas-fir in the highest-rainfall areas close to the coast. It is the second most important timber conifer in the US PNW. Interest in this species increased markedly in the 1990s after growing incidence of Swiss Needle Cast disease on Douglas-fir on the Oregon Coast, and the white pine weevil on Sitka spruce from Oregon to Alaska.

The first-generation testing phase ran for western hemlock from 1972 till 1995, during which over 1,300 first-generation parents were tested in five breeding units. Height was measured at five and 10 years from seed, while diameter was measured at age 10 and sometimes at age 14 or 15. Unlike Douglas-fir, branching traits (e.g., forking and ramicorns) and stem sinuosity were not scored. Age-5 results from one of these programs are presented in Foster and Lester (1983).

Second-Cycle Testing

Design and Implementation

A single second-cycle breeding and testing program (HEMTIC) was developed in 1992, from the middle of the Oregon coast (44°30’N) to northern Vancouver Island (51°00’N). Genetic materials were selected from six first-generation programs based on age-10 height growth. HEMTIC differed from the Douglas-fir second-cycle cooperatives in several ways:

- In addition to Oregon and Washington, there was active involvement and leadership from the province of British Columbia.
- No forward selections from open-pollinated tests were used, due to the lower heritabilities compared to Douglas-fir.
- Resources were divided into a main and an elite population. The main population had 150 parents (chosen from over 1,500 parents) crossed in six-parent disconnected diallels forming 342 crosses. The elite population of 30 parents had 166 crosses (Jayawickrama 2003) and was tested in both single-tree plots and family blocks establishment.
- The number of crosses per parent was much higher, 5 in the local diallels and up to 18 in total.
- Full-sib family blocks were established in addition to single-tree plots.
- Test sites were not fenced.
- Neither intensive mechanical site preparation nor frequent herbicide application were used, due to western hemlock’s sensitivity to these treatments.

Data collection, analysis and selections

Age-5 heights and incidence of basal forking were measured and analyzed from 86,375 progeny trees and checklots by the end of 2004, and age-10 data was analyzed by early 2010. Heritabilities for these traits are shown in Table 1 and are generally considerably lower than for coastal Douglas-fir at the same ages. As a result of the lower heritabilities predicted gains were also lower than for coastal Douglas-fir, but there was still solid evidence for an overall gain for the second-cycle population over unimproved controls (4.1% gain for HT10, 11.3% gain for VOL10).

Third-Cycle Breeding and Testing Cooperators in OR and WA have supported moving forward to a third-cycle program. The strategy is largely similar to the second-cycle strategy. Differences between the 3rd- and 2nd cycle strategies include: (1) The “elite” population will be much smaller; (2) A maximum of four crosses per parent; (3) Fewer progeny planted per cross per site; (4) Use of a standard group of Oregon and Washington woodsrun controls on every site; (5) Test sites would be fenced; (6) A slightly smaller number of selections (about 120); (7) The 3rd-cycle breeding population is

Figure 1. Location of second-cycle western hemlock sites in Oregon and Washington. Over 130,000 test trees were planted and tested on 19 sites between 1997 and 2001.

Table 1. Heritabilities of second-cycle western hemlock (HEMTIC) sites.

Heritabilities	Trait								
	Age-5		Age-10						
	HT	Basal Forking	HT	HT510	DBH	VOL	Stem Forking	Ramicorn Branching	Stem break
Range of within-site	.04-.17	.03-.15	.07-.26	.04-.25	.03-.17	.05-.19	.01-.02	.02-.08	.02-.04
Across-site narrow-sense	0.058	0.062	0.077	0.067	0.048	0.057	0.011	0.023	0.016
Across-site family-mean	0.655	0.639	0.743	0.758	0.665	0.695	0.249	0.461	0.191

Ages calculated from seed. HT= height, DBH= diameter at breast height, VOL= DBH2 * HT, Stem forking = incidences of stem forking, ramicorn branching = incidences of ramicorn branching. HT510 = height increment from age 5 to 10.

comprised mostly of untested 2nd-cycle forward selections from full-sib tests (the largest component), with perhaps 30 high-gain 1st-gen parents unused in second-cycle HEMTIC; (8) Some germplasm from higher elevations in the Coast Range and Cascades will be included; (9) A small cloned breeding population may be included; and (10) Additional traits such as wood stiffness may be used as selection criteria.

A total of 152 forward selection candidates were identified for the third-cycle breeding population and grafted (in 2006, 2010 and 2013). This will be reduced to 90-100 selections based on predicted gains, response to stimulation and to restrict the number of related entries. Stimulation and pollen collection began in 2012.

Acknowledgements

Charlie Cartwright and John King (BC Ministry of Forests), Dan Cress and Jess Daniels (NWTIC), Jim Hargrove (Quinault Indian Nation), Jessica Josephs (Rayonier Timberlands), Greg Johnson and Ron Haverlandt (Willamette Industries) were key players in getting HEMTIC underway. The Oregon Department of Forestry, Rayonier Forest Resources, The Campbell Group, Hancock Forest Management, Stimson Lumber, Quinault Indian Nation, Hampton Lumber, Green Crow Forest Management, Bureau of Land management and Plum Creek Timber Company are current members of HEMTIC in Oregon and Washington.

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Sixty years of genetic improvement for coastal Douglas-fir in Oregon and Washington

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First-generation testing of Douglas-fir

Forest tree improvement got underway in the US Pacific Northwest in 1954, with several organizations starting tree improvement programs by selecting trees and grafting them into clonal orchards. Graft incompatibility soon became evident and dampened enthusiasm for grafted clonal orchards (Silen and Copes 1972). The IFA-PNW “Progressive Tree Improvement System” was launched in 1966 (Silen 1966, Silen and Wheat 1979), emphasizing forming local cooperatives to share costs, and on progeny testing large numbers of trees using wind-pollinated seed in small testing zones (believing that was not appropriate to move Douglas-fir seed far from the source). Improved seed was to be delivered from seed orchards established using full-sib crosses made on the parent trees.

First-generation test establishment continued till 1993, during which over 25,500 first-generation Douglas-fir parents were tested in 115 breeding units, ranked based on 2.4 million surviving progeny trees. Most trials were established in either “reps-in-sets” or “sets-in-reps” design. The typical measurement schedule was five, 10 and 15 years from seed. Height was usually measured each time, and diameter at age-15 and sometimes age-10 as well. The incidence of ramicorn branches, forks and stem sinuosity was assessed in some testing programs, once the inheritance of these traits was established (e.g. Temel and Adams 2000).

Second-cycle cooperative breeding and testing

The second-cycle breeding and testing strategy was developed around 1997 based on global analyses of first-generation data, computer simulation and inferences from other breeding programs (Johnson 1997, 1998a, 1998b; Johnson et al. 1997). It was concluded that full-sib breeding had several advantages over open-pollinated breeding, that there would be little marginal gain per test beyond six successful progeny tests (in terms of ranking families), that two or three crosses would give a reasonable estimate of a parent’s GCA, and that final selection around age 12 years would be efficient. Eighty-six first-generation testing programs were merged, mostly in the north-south direction, into 11 larger testing zones operated by eight second-cycle cooperatives.

Breeding populations for second-generation cooperatives ranged from 112 to 429 crosses. Within a breeding population breeding groups were constructed, with each breeding group originating from a single first-generation program. This resulted in sublines to manage inbreeding, and multiple populations to maintain locally adapted gene complexes. About 1 in 10 first-generation parents or families were selected to move forward into the second cycle. Most selections were made on age-15 height; information on DBH, stem form and wood specific gravity were also considered. The breeding population for each testing zone included parents or progeny from the "local" breeding groups within the testing zone, and only the highest-ranked selections from breeding groups originating further away from the zone. Each selection was generally used in two crosses, with some elite selections used up to four times. Some elite crosses were made across first-generation zones.

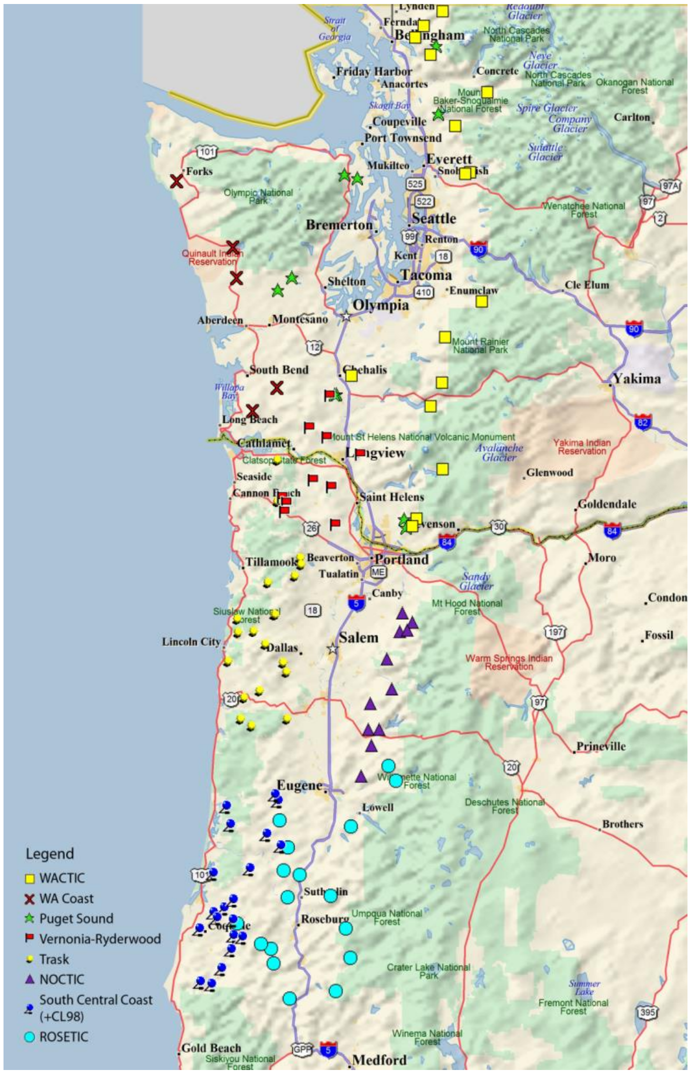

Figure 1. Distribution of cooperative second-cycle Douglas–fir test sites in Oregon and Washington

A total of 2,640 pair-crosses were planted in 115 tests between 1999 and 2012 (see Figure 1), and about 455,000 test trees planted of which about 364,000 were measure trees. Five to eight tests were established per test series using “alpha” design (a few in “sets-in-reps design”), with 20 trees per cross per site in single-tree plots. Test sites contained 50 - 283 full-sib crosses. Only six of the 115 sites were established over 900 meters elevation. Multiple phases within a testing program were linked by at least 10 common linker crosses and additional linker parents. Unimproved checklots were included in all tests planted after 2001. No polymix tests were established.

All sites were established with one-year old containerized seedlings and fenced to prevent browse. The goal was to control herbaceous competition for three years, and more harmful competitors (such as Scotchbroom and aggressive hardwoods) until crown closure. Tests are being measured twice, at seven and 10-12 years. Recent research has established little benefit to waiting beyond age-10 for final selections for growth rate (Ye and Jayawickrama 2012). Height, diameter, forking, ramicorns, sinuosity and budburst rating are scored by age-7, fall cold hardiness has been scored in four programs and acoustic velocity is currently being scored in one program. Genetic gains are predicted using BLUP, after adjusting for spatial trends (Ye and Jayawickrama 2008)

Third-cycle cooperative breeding and testing

The strategy is largely the same as in the second cycle. Further consolidation of breeding zones will take place, the merits of adding polymix tests is being debated, and test sites will be kept small to maximize heritability. Over 960 forward selections from second-cycle tests have already been grafted in seven breeding orchards. Elite first-generation selections omitted from second-cycle programs or only involved in suboptimal pairings are also being included. Selections are organized in sublines of 10-15 trees with all relatedness confined to within those sublines. Over 270 crosses have already been attempted. Test establishment may start as early as 2016.

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Tuesday July 23, 11:20 - Boardroom

The lodgepole pine breeding program in British Columbia: Breeding for multiple traits

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Lodgepole pine (*Pinus contorta* var. *latifolia*) is a very important commercial tree species in British Columbia east of the Coastal Mountains. In 2012, 107 MM seedlings were planted which represented 53% of the total provincial planting program. Population and quantitative genetics research has been conducted for over 45 years and has focused on growth as a target trait for breeding activities and an indicator of adaptive differences among populations. Currently there are five seed zones that have progressed to the second generation of progeny testing. The second generation tests are composed of F1 crosses derived from parents which were selected for growth and wood density based on data from open pollinated progeny tests. These tests are approaching 10-years of field testing and are being assessed for growth, infection by pathogens and attack by insects. While stem volume continues to be the primary trait of interest for breeding activities, we are investigating the feasibility of integrating other traits into the breeding program.

Three pathogens have been identified as high priority for breeding activities based on consultation with foresters and pathologists: comandra blister rust (*Cronartium comandrae*), dothistroma needle blight (*Dothistroma pini*), and western gall rust (*Endocronartium harknessii*). We are actively monitoring progeny tests for the occurrence of these pathogens and continue to survey mature progeny tests that have been attacked by mountain pine beetle (*Dendroctonous ponderosae*). Resistance and tolerance to western gall rust and dothistroma needle blight are the most promising with heritability estimates of similar magnitude to other traits such as growth and wood density. Resistance and tolerance to comandra blister rust infection has very low heritability. Symptoms

caused by comandra blister rust are similar to those of white pine blister rust (*Cronartium ribicola*) on western white pine and the life-cycles of both rusts are very similar with multiple spore stages and alternating hosts. There is no significant genetic correlation between growth traits and resistance to these three pathogens. We are currently investigating the possibility of screening progeny for resistance using field tests established in high hazard areas and controlled inoculation experiments. We are also conducting controlled crosses among parent trees selected for tolerance and resistance. The heritability of resistance and tolerance to mountain pine beetle and its associated fungi is similar in magnitude to other traits of interest but the relationship with growth is more complex. There was strong selection by beetles for large trees in southern progeny tests but less in northern progeny tests. The infrequent nature of epidemic outbreaks of mountain pine beetle makes it a low priority for selection and breeding activities.
A very significant challenge for the lodgepole pine breeding program in BC is poor seed production from established seed orchards in the north Okanagan. Seed production in the north Okanagan has historically been much lower than from seed orchards located in other regions of the province with lower seed-per-cone yields and higher numbers of cones-per-ramet. Seed loss occurs at the end of July and beginning of August of the second year of cone development but the mechanism is not yet clear. Ongoing research is now focusing on the short window in which seed loss occurs to help determine the possible causes and actions necessary to help boost seed production in established north Okanagan orchards.

The integration of innovative techniques to help set priorities and improve efficiencies in the breeding program is key to the advancement of the lodgepole pine genetics program in BC. We are using climate envelope modelling to help prioritize future breeding activities by ranking breeding populations for importance based on changes in the size of seed zones. A similar approach is being used to model the relationship between pathogen infection and climate to help predict the impact of diseases at the landscape level and prioritize disease screening research. We are adapting controlled inoculation procedures from other conifer-pathogen systems to improve efficiency in screening for resistance and tolerance and help to identify mechanisms of resistance. We are also investigating the use of genomic selection to improve efficiencies in the breeding program by reducing testing time and costs for phenotyping complex traits. The future of the lodgepole pine breeding program will continue to build on the legacy left to us by the hard work and dedication of the many researchers and technicians that preceded us.

Tuesday July 23, 11:35 - Boardroom

The past, the present and future of the coastal Douglas-fir tree improvement program in British Columbia

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Coastal Douglas-fir was the first conifer subjected to tree improvement efforts in BC. Intensive phenotypic selections were made by Alan Orr-Ewing starting in 1957. It was expected that these intense selections would translate into immediate gains in seed orchards and as a consequence, progeny testing was not considered an integral part of the program. By 1966, a total of 455 selected trees had been grafted and were growing in breeding arboreta and seed orchards. In 1972/73, it was decided to produce a pedigreed population for the testing and selection of superior genotypes as well as for the estimation of genetic parameters and the establishment of a long-term breeding population. This gave rise to EP708, which formed the basis for all future improvement of coastal Douglas-fir. EP708 was a 6-tree partial, disconnected diallel breeding design extending over 8 years (11 sites/year), crossing a total 372 parents in 62 diallels. By the early 1990, a total of 991 trees were forward selected from many of the 88 test sites planted. Breeding for the next generation was structured into sublines to manage future inbreeding. A total of 32 sublines were established with up to 12 parents per subline. Crossings were done in four series. Series 1 was planted in 1999 and Series 4 was planted in 2010. Series 1 to 3 were complimentary tests (GCA testing of parents and full-sib family blocks), while Series 4 is a full-sib test using single tree plots on four sites. GCA tests were planted on 4 sites/Series, while the full-sib blocks were established as 25 tree plots per full-sib family on two sites for each Series. Forward selections (age 12 years from seed) were based on mid-parent BVs obtained from the GCA test for Series 1 and based on combined blup analysis for Series 2. Wood density was either estimated using the resistograph (Series 1) or increment cores (Series 2) taken from half of the GCA test seedlings. Breeding values of selected trees average around 25% gain, expressed as volume gain at age 60. Trees with BVs for wood density below -5% were not selected. Breeding for the next generation will be done using 4 parent diallels for each of the 32 sublines. It is anticipated that the N_e for this breeding population will be around 70 to 80.

Tuesday July 23, 11:50 - Boardroom

Genetic and environmental control of Douglas-fir stem form in the Pacific Northwest

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The value of wood products is determined by tree volume and stem quality. Stem form defects, such as forks and ramicorn branches, reduce stem quality and, therefore, tree value. Foresters in the Pacific Northwest have observed that the frequency of stem form defects seems to be associated with rapid growth and proximity to the coast. In addition, past research studies have indicated a positive genetic correlation between growth and stem defects. Nonetheless, the relative roles of genotype and environment on the frequency of stem form defects are still unclear. The objectives of this study were to *i)* identify environmental characteristics that explain variation in the frequency of forks and ramicorn branches; *ii)* examine whether rapid plantation growth is associated with an increase in stem defects; *iii)* determine how much variation in stem defects can be explained by differences in growth; *iv)* examine whether there is a relationship between stem form and proximity to the coast; *v)* estimate genetic and environmental correlations between stem defects and growth traits; and *vi)* estimate heritabilities and genetic gains for stem defects and growth traits.

To achieve these objectives, data from 22 first generation operational breeding programs within the Northwest Tree Improvement Cooperative (NWTIC) were analyzed. We examined 40 environmental characteristics (climate, soils, and topography), and did not find any evidence that they explain variation in stem form defects. We found that the frequency of stem form defects increased with increased growth and increased proximity to the coast. We also found that forks and ramicorn branches were heritable and were generally positively genetically correlated with growth. However, genetic correlations were variable among programs. Direct backward selection on stem form traits could result in a decrease in defect frequency between 3 and 28%. Selection solely on growth traits (i.e., indirect backward selection) had a small potential to increase the frequency of stem form defects (1-4%). Because of the variability in genetic correlations between growth traits and stem form defects, it is also possible to select simultaneously for growth and fewer defects.

We now have a better understanding of the genetic and environmental control of Douglas-fir stem form. There is ample evidence to suggest that stem form should be included as a selection criterion in operational breeding programs, particularly in breeding programs that plan to deploying material close the coast or on “high” sites. The genetic correlations between stem form and growth traits should be examined in later generations, as the potential to increase the frequency of stem form defects may change, particularly in programs that had large positive genetic correlations between growth and the frequency of stem defects.

Tuesday July 23, 12:05 - Boardroom

Results from Douglas-fir provenances tests: The base for a breeding program in Germany

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In the first part of the presentations selected results from two provenance trails will be reported. Seed of 54 provenances of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) from 8 states of the USA and 2 provenances from Canada collected within the natural range of the species was sown in 1962 for the first trial. In spring 1965, a field trial was established in the forest district of Nordhorn, north-western Germany, to test the suitability of Douglas-fir provenances on former agricultural land. In the field trial several traits were measured or assessed regarding growth performance, and susceptibility to biotic agents. Regarding these characters the provenances show a great variation. Regarding growth considerable changes in ranking were still observed between age 11 and age 33. At the age of 33 the provenances from coastal Oregon and Washington hat the largest diameters. These provenances were as well resistant to *Rhabdocline* needle cast disease. Provenances from the southern interior were partly heavily attacked by the fungus. Some of them failed totally at age 33. To assess the quality of provenances, especially for exotic tree species, older aged provenance trials are essential.

In the second trial was established with provenances from Washington and British Columbia and progenies from stands in Germany. Most of the offspring from the German stands had a better height growth than those from North America. Survival rate of the German progenies was at average

Based on the results of the previous trial and further tests a breeding program was projected for the next 15 years. This will be presented in the second part of the presentation. The program consists of 4 packages: (1) analysis of existing provenance trials, (2) evaluation of gene flow, (3) establishment of progeny trails with off-springs from seed-orchards, (4) establishing of breeding populations and tests on draught stress and frost tolerance under controlled conditions. The estimated costs of the program are 7 Mio € (9.2 Mio US$). Grants of about 2/3 of the costs are necessary. To initiate the program a proposal is submitted for a 3.5 years period (2 Mio €).

Tuesday July 23, 1:15 - Boardroom

Douglas-fir: A history of breeding in New Zealand and some challenges for the future with climate change

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Douglas-fir (*Pseudotsuga menziesii*) has been grown in New Zealand since 1859 and is now the second most important planted exotic forest tree. With around 109,000 ha planted, the species represents 6% of New Zealand's planted forest estate. Douglas-fir is grown mainly in the South Island of New Zealand, in colder, wetter environments.

Douglas-fir breeding was initiated between 1957 and 1959 when provenance trials were established across a large number of sites in both the North and South Islands of New Zealand. Origin of the material was from Washington and Oregon (1957), and from coastal California and Oregon (1959). Following an analysis that demonstrated the superior growth rate of Oregon- and Californian-origin material, further collections were made from these areas. Additional breeding trials ranging in location the central North Island to the south of the South Island in were established in 1996.

There are three environmental challenges to increasing the profitability from Douglas-fir in New Zealand. Firstly, resource quality varies with temperature and water availability. Cooler temperatures lower the density and stiffness of the wood. Second, Swiss needle cast (SNC), caused by the pathogen *Phaeocryptopus gaeumannii*, results in a 32% reduction in tree growth (New Zealand-wide average). This productivity loss is greatest in the warmer, wetter regions of the North Island. Third, New Zealand temperatures are projected to increase with climate change. Temperatures are predicted to rise, particularly on the east coasts of both the North and South Islands by approximately 2°C.

The challenge to tree breeders is how to deal with the loss of productivity in warmer regions due to SNC, and with the lower wood stiffness in the cooler areas all in a changing climate. In particular, we are interested whether the gain achievable in wood stiffness from breeding will be sufficient to compensate from lower wood stiffness in cooler sites now and into the future.

Breeding for wood stiffness is important to achieve wood quality on some sites. Wood stiffness has not been measured routinely in the breeding programme. A sub-set of 60 families at three sites has been assessed in the 1996 trials. Wood stiffness was found to be highly heritable (narrow-sense heritability of 0.49, age 11-13 years). Importantly, little change in rankings was found across three sites. This result needed confirmation. We also needed to know if wood stiffness would still be highly heritable across all the 238 open-pollinated families tested so that selections made would definitely deliver heritable variation.

New wood stiffness assessments are currently underway. We will use the new data to confirm the high heritability across a wider genetic sample. We will also examine the effect that sub-sampling of families has on genetic parameters and genetic gain for wood stiffness in NZ Douglas-fir. We will estimate genetic gain and determine if the gain achievable will be sufficient to compensate for lower wood stiffness on cooler sites.

Understanding the relationship between gains achievable in wood stiffness (even with climate change) and needle loss due to SNC will be vital for the future profitability of Douglas-fir production. Currently, SNC damage is restricted to the North Island and the northern areas of the South Island. Modelling under different climate scenarios indicates that SNC will move down further into the South Island, into the core growing region for this species. We will examine the genetic relationship between these growth, wood quality and disease resistance traits and determine trade-offs necessary (if any) to provide robust trees for planting.

Changes to breeding and deployment plans will be discussed.

Tuesday July 23, 1:30 - Boardroom

Breeding of radiata pine in Australia

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The objective of the national tree improvement program for *Pinus radiata* in Australia is to breed, select and deploy genetic material with improved biological characteristics for traits of commercial importance. Breeding began in the 1950s with plus tree selection and the establishment of seed orchards by various companies and state and federal agencies. The STBA breeding cooperative, established in 1983 in South Australia, continues to expand, consolidating genetic resources on a national basis, providing cost efficiencies and enhancing the rate of genetic gain for this major plantation species.

Economic objectives for plantation growing for structural timber markets are fundamental to the breeding and deployment programs. Changes in the environment due to changing climate and markets are evaluated within this framework.

There is effectively a single breeding population with a single national objective. We are still deciding on how best operationally to manage the population for regional performance, different products and risk traits associated with some pests and diseases. New software tools (SELECT and MATE) have helped breeders manage relatedness and limit the change in group co-ancestry to ensure inbreeding does not build up too quickly in the advanced generation population. Some effort is also directed towards conserving native provenance material for risk management purposes as well as infusion of genes from tested selections to broaden the genetic base.

The breeding program moved to a rolling front strategy in 2000, where breeding, testing and selection activities are done on an annual basis. This has reduced costs and generation interval by avoiding resource and biological bottlenecks, and maintained the skills of field technicians and breeders (particularly in reproductive biology). It has enhanced the rate of genetic gain per unit time, as well as delivering new information and genetic material (tangibles) to cooperative members on a regular basis. Infusion of 30-50 new selections each year into a dedicated multi-age breeding arboretum allows about 250 crosses pa - many more than was previously achieved per generation.

Progeny tests and deployment trials are established on an annual basis, and across years to sample all of the plantation estate. Families are also being exchanged with the RPBC in New Zealand for collaborative testing in series of trials across Australasia. Families with parents in common provide genetic linkage among trials across sites and years. Treatments are stratified into groups of families with similar breeding values for growth to reduce competition among single tree plots and provide large plots to monitor long term stand growth. Traits measured at various ages include growth, tree form, wood properties (density and stiffness using acoustic tools), and damage due to pests (pine aphid) and diseases (dothistroma, pine pitch canker, phytophthora and spring needle cast).

All data and information is stored in the national DATAPLAN database. The web based system has been operating since 2001 and currently stores ten million measurements on 650,000 *Pinus radiata* genotypes in over 300 trials, with a total of 60 million measurements on four million genotypes in 11 species. We are currently incorporating new trial data (more than 80 trials) for radiata pine from a previously independent state based breeding program in WA, following Forest Products Commission joining the national cooperative in 2013. This will further broaden the genetic base and increase selection intensity for all members.

The TREEPLAN genetic evaluation system has been used routinely since 2001 for breeding value prediction using multi-generation multivariate (-site, -trait and -age) BLUP. BLUP allows us to account for previous selection and monitor trends in genetic improvement over time by having objective comparisons across time and space. Variance components are estimated within trials (and across subsets of trials) using ASREML and other tools. TREEPLAN genetic values (EBVs, EGVs and SCAs) are currently predicted for 32 selection criteria (measured traits) using data from more than 160 trials in the multivariate analysis. Genetic values are generated for the clearfall harvest age breeding objective traits of growth (MAI on a regional basis, m^3/ha/yr), stem straightness (SWEEP, mm/m), BRANCH size (cm), and timber STIFFNESS (GPa). Economic indices using appropriate economic weights (based on various production systems and end use processing) are produced for each genotype, family, aggregate and genetic group.

Standard errors and accuracies are now routinely reported as measures of reliability for all types of genetic values for all pedigree entities (individuals, families, aggregates and genetic groups), and for all trait types (selection criteria, objective traits and profit indices). Genetic values for all the population are updated regularly (at least annually) as new data is collected. Realised gain trials are used to audit the predictions and ensure scaling factors are appropriate, particularly for MAI. Implementation of changes in economic models and other assumptions derived from research projects is straightforward within this framework.

The STBA does not itself produce seed and plants for deployment. This is done by members and a licensee. STBA provides selections for use in propagation systems, as well as objective information to assist seed producers, nurserymen, and forest growers make informed decisions about what material is best for their situation. This is not a simple task, as the breeding program generates complex and vast amounts of information for consideration in deployment.

The SEEDPLAN decision support system has been developed within the DATAPLAN and TREEPLAN framework for industry use after pilot testing. Its purpose is to improve the use of improved seed and clones in deployment under different situations. Tools assist with selecting genotypes to best balance gain and diversity (SELECT), placement of ramets in seed orchards (DESIGN), estimating the composition of seed harvested (COMPOSE), and customising the economic indices for the different growing environments and production systems (INDEX). A tool for allocating seed and plant lots to stand types in an optimal manner to maximise enterprise profitability (MATCH) has been prototyped, but is still under development.

Other studies have focused on juvenile wood and timber stiffness. The potential impact of climate change is given some consideration, but mainly in terms of characterising the genetic resource for a greater range of environments. A better understanding of GxE patterns and associated environmental factors is helping classify site types across Australasia for breeding and deployment. Current research suggests rainfall, temperature and altitude are largely responsible for observed patterns of genotype by environment interactions. It is anticipated some refinement of site type classifications for screening and deployment of genetic material will improve overall gain.

Molecular markers have been used in the past for fingerprinting to check pedigree error rates in the breeding and deployment populations. Genomic selection is not being used operationally in the tree improvement program for radiata pine at this time. Research projects have generated some marker data, but it has been too little to warrant incorporation in routine evaluation. It is anticipated next generation genetic values will increasingly rely on additional information from molecular genetic studies.

Breeding values incorporating non-neutral marker information can currently be done in TREEPLAN by using a synthetic pseudo-continuous trait. Multi-locus marker-trait responses are combined into a prediction of genetic merit for a trait based on genotypes which have phenotypic and/or marker information. This approach has been used successfully in *Eucalyptus nitens*. We anticipate advances in genotyping technology will make it feasible to test individuals for hundreds of thousands of single nucleotide polymorphisms simultaneously at an affordable price. A genomic relationship matrix based on neutral markers can be merged with the average co-ancestry matrix currently used. This should increase the accuracy of breeding value prediction, by better accounting for the Mendelian sampling term.

Alleles of large effect can be incorporated directly into the model, utilising all phenotypic data to better identify their effects. The opportunity for radiata pine is largely with harvest age traits that are only expressed later in the life of the tree, or are expressed rarely, such as with pests and diseases, or are very expensive or impractical to measure. It will be necessary to continue collecting phenotypic data to ensure precision of estimates.

In summary, the radiata pine program has undergone substantial change in the past decade with the development and adoption of economically defined breeding objectives, screening for new pest (essigella pine aphid) and disease traits (pine pitch canker), increased measurement of stiffness, development of DATAPLAN software and data consolidation in national database(s), development of TREEPLAN software for comprehensive industry wide genetic evaluation with regionalised genetic values, a rolling front operational program, development of selection, breeding and deployment (SEEDPLAN) tools, gene conservation coordinated nationally, improved security of the national genetic resource (breeding population), and a focused research portfolio with rapid adoption of results and findings.

Tuesday July 23, 2:00 - Boardroom

Gains in weevil resistance for Sitka spruce: Comparisons between 1st generation and F1 trials and implications for Sitka spruce in the Pacific Northwest

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White pine weevil (*Pissodes strobi* Peck (Coleoptera: Curculionidae) is one of the most devastating pests of young spruce (*Picea* spp.) and pines (*Pinus* spp.) in North America. The weevil is a native insect that occurs across Canada and the northern United States. In eastern North America it is a major pest of Eastern white pine (*Pinus strobus* L.) and introduced Norway spruce (*Picea abies* (L.) Karst.), but in the west it mainly attacks spruce species - Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is particularly susceptible. Finding and incorporating resistance to this pest has been the main focus of a breeding and research program for Sitka spruce, carried on for over two decades by the BC provincial, and the Canadian federal, forest services and universities. First generation screening of Sitka spruce parents occurred in open-pollinated or clonal trials. Thirty of these 1st generation trial sites were monitored annually for up to 10 years for weevil attack. Over 1/3rd of these had been artificially infested by augmenting the trial trees with weevils. This was quite effective in getting early screening results and parents were chosen from these trial screenings to produce an F1 population. At this stage results are being monitored from the F1 trials from both augmented and non-augmented trials. A marked reduction in weevil damage is noted in the F1 compared to the first generation trials - even in the F1 trials that were augmented. Work is ongoing at discovering the mechanisms behind this resistance and current knowledge and concerns are discussed. This improvement for weevil resistance, together with silvicultural changes means that Sitka spruce can once again be considered as a viable plantation species in British Columbia and the Pacific Northwest. This program is arguably one of the most successful pest resistance breeding programs for plantation forest species in North America.

Tuesday July 23, 2:15 - Boardroom

Breeding and seed production of conifers from the Pacific Northwest in Sweden

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Three conifers from the Pacific Northwest are currently of serious interest for use and genetic improvement in Sweden.

Lodgepole pine

Lodgepole pine (LP) is the most frequently used introduced tree species in Swedish forestry. It covers some 600 000 hectares and is the third largest species to be planted with 16 million seedlings sold in 2011, which is about 7 % of the total planting stock. Prolific early flowering and ease of clonal propagation make LP a good species for tree improvement, with shorter generations and higher rates of genetic improvement than for Norway spruce or Scots pine. In addition, lack of background pollination enhances realised gain in seed orchards. After a peak of planting LP in the late 1980s, the annual planting decreased – a trend that has only recently reversed. Today's interest in greater forest production has led to increased planting of LP. This has also initiated the production of new seed orchards, which are expected to give a total gain in growth rate of about 25% compared to unimproved trees.

The first plus-tree selection in natural forests in Canada for grafting trees into seed orchards in Sweden was initiated in the early 1970's. In the late 1970's, a cooperative of forest companies initiated and financed a seed orchard programme to make Sweden self-sufficient in improved LP seed. This also initiated the establishment of a long-term breeding programme, which included new plus-tree selection in natural forests in Canada, progeny testing, and establishment of seedling seed orchards. There are a total of 3 232 plus-trees available as candidates for the LP breeding population. These include plus-trees imported as grafts, selected progenies from plus-trees, and selections in commercial Swedish plantations. This material covers much of the native distribution range of LP north of latitude 50°N in Canada.

A new breeding plan for LP has evolved and put into practice. Based on research findings, operational experience and analysis of different breeding strategies, the new strategy will be by forward selection within clonally replicated full-sib families after field testing. This option best utilises resources already invested in the breeding, and provides options for long-term breeding and selection into seed orchards. A new generation of trees for forward selection is formed without delay. Breeding gains for LP under alternative long-term strategies have been quantified. Long-term gain for a programme initiated with 50 trees would give 5% gain per generation in the breeding population for individual forward selection and 11% gain for clone testing, corresponding to an annual gain of 0.26 and 0.65% per year, respectively.

Currently, the plan is to structure the LP breeding populations as 11 advanced-generation sub-populations which cover the climate variation in northern Sweden. Each closed sub-population has at least 50 unrelated trees, resulting in a meta population of some 550 trees. In general terms, the breeding of LP focuses on trees that are efficient in using limited resources, and grow stem wood more rapidly. A selection index is constructed from the measured traits, their heritabilities, and economic weights of the breeding objectives.

The interest for Douglas-fir and Sitka spruce is increasing but from a low level. The main reason for these species is a wish for spreading risks to more species than Norway spruce due to expected impact of global warming, especially in Southern Sweden.

Sitka spruce

For Sitka spruce 65 new plus trees were selected in the 1990's and tested together with materials from Danish, Norwegian and British breeding programs. Progenies from the new Swedish selections turned out comparatively well indicating a land race effect in those selections. Experiment means showed 25-50 % superiority over Norway spruce check-lots. British improved materials also look promising for south Sweden. The Norwegian material did not perform well due to the Alaskan origin being to northern. Two seed orchards will be established with backwards selected tested plus tree clones and some forward selections among progeny. Long term strategies for breeding and seed production are being developed.

Douglas-fir

Intermittent breeding activities have been carried out from time to time. In 1995, 65 plus trees were selected in South Swedish well performing stands. Progeny tests were established and forward selections were carried out after analysis.

A series of three combined provenance and progeny trials in South and Central Sweden were analyzed 18-19 years after planting. A general pattern was the presence of significant differences for growth traits between provenance groups of inland and coastal origin and even larger differences between provenances within groups. The most useful variation, however, was detected between open pollinated half sib families. No serious adverse correlation estimates were found between growth and stem quality traits. Growth traits and susceptibility for Rhabdocline needle cast seemed to be favourably correlated. Strong additive genetic correlation estimates between the two southern trials suggested that the same regeneration material can be used all over the southern part of Sweden.

Grafted seed orchards will be used as a first step of capturing the genetic gain by forward selection of plus trees within the best open pollinated families in good provenances. Seedling seed orchards, established after seed collection from selected individuals, is as an alternative low cost method for mass propagation, and would also serve as clone archives for the breeding population in a low budget breeding program.

Tuesday July 23, 2:30 - Boardroom

Western hemlock forest genetics program for British Columbia

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Western hemlock is the commonest tree on the British Columbia coast. It is roughly 30% of the annual volume harvested and estimated to be about 60% of timber inventory for the maritime region. As a consequence of its potential, tree improvement activities commenced early on with parent tree selections starting in the late 1950's. First progeny trials were established in the early 1970s and a provincial breeding program was launched in the second half of that decade under Mike Meagher. With the recession of the early 80s and consequential budget restrictions, activities were restricted to maintenance of existing sites and provenance seed collections placed in the hands of the Douglas-fir breeder, Jack Woods. The hemlock program revived with the economy, as forest industry put weight behind their investment in the species requesting, in the words of Western Forest Product's John Barker, that if they were to have seed orchards they should be tested. John King was engaged as breeder, and seeing that few parent trees were tested to that point, set out with others, to develop the Hemlock Tree Improvement Co-operative (HEMTIC) in the 1990s. Its course is well described by Jayawickrama and Ye in their description of endeavors in the US Pacific Northwest.

For hemlock in BC unstable economics of the new millennium combined with the fecundity of the species led to reliance on natural regeneration. Planting numbers have fallen from a high of about 9 million a year in the early 1990s to as little as 10% of that recently. As a consequence, it was decided by the guiding committee for tree improvement for the province, the Forest Genetics Council of BC, that the program should be put on hold. This has meant once again activities are limited to securing long term research installations, and finishing planned measurements of field trials.

The current program has several facets. Gene conservation status has been assessed and as expected for a prolific, invasive, shade tolerant species, risk of loss of genetic variation is relatively low. For genecology research there is a broad network of almost 40 field installations covering most of the environments in which hemlock occurs in BC. Measurements to age 10 years are fairly complete for the trials and reporting on features of the genetic architecture and adaptation is planned for the near future. Preliminary analysis suggests a latitudinal cline of decreasing growth capacity from South to North. With elevation, at least for coastal seed sources, there appears to be a step cline with a fairly abrupt differentiation of low and high altitude genotypes. Longitudinally trends within the coast, transition and interior regions are not detectable, but movement of seed from one of these regions to another does not have positive outcomes.

For investigation of growth and yield of improved genotypes versus wild type plants, 3 series (9 test sites) were established. These tests confirm gain estimates from single tree progeny trial plots by area based 0.1 ha plots. As well, 6 of the trials have varied planting densities in order to predict effects from competition. Results to date confirm expectations for gain. In addition, comparison of wild stand progeny to controlled-cross orchard mean lots suggests inbreeding depression results in a loss of about 5% in early height growth.

Breeding to identify genotypes superior in desired traits ceased almost a decade ago, but progeny tests established then are just now yielding reliable data. Poly-cross (Px) trials of high elevation parents age 12 years are due for measurement this fall, with new breeding values subsequently. Similarly, measurements of polycrosses of backwards selections from the HEMTIC program have been carried out recently, with final rankings due to be tabled this fall. Forward selections from HEMTIC second generation testing have been ranked and the best 120 genotypes grafted for inclusion in clonebanks, but planned Px trials to confirm the breeding values are not to be carried out. Also this fall, tests of best BC parent trees not ranked in time to be included in the HEMTIC second generation will have a final measurement. Unlike the earlier second generation trials, the parents were cloned to allow for more effective within family selection.

Prospects for the future of the hemlock forest genetics program are likely limited. Despite 2 year delays for natural regeneration versus planting, inbreeding depression, patchiness, clear evidence that local seed is surpassed by more southerly sources and increasing genetic gains in seed orchards, the method is inexpensive and often fairly successful. Perhaps declining rotation lengths, or rising genetic worth of seed, either from our orchards or those of the Pacific Northwest, will lead to resurgence of this program once again.

Tuesday July 23, 2:45 - Boardroom

Age-7 Results from a Douglas-fir Realized Genetic Gain / Stand Management Trial

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Introduction

Realized genetic gain is vitally important to those who fund tree improvement and use its results. Single-tree-plot progeny tests do not serve the purpose well following crown closure because of high competition among trees with different sizes (Magnussen, 1989; Ye *et al.*, 2010). Evaluating how genetically improved materials respond to different silvicultural treatments (e.g., stand density, weed control, etc.), can only be done in large-block field trials. Verifying realized genetic gain is expensive and takes a long time. In the mid-1990s, two realized gain trails of Douglas-fir were established in the north Oregon Cascades and British Columbia (St Clair *et al.*, 2004; Stoehr *et al.*, 2010; Ye *et al.*, 2010). Realized gains were evident for growth rate in these studies.

The Grays Harbor Realized Genetic Gain / Type IV trial was the first large block-plot genetic gain testing effort in the public domain for Douglas-fir in the Washington coastal area, and was established by the Stand Management Cooperative, the NW Tree Improvement

Cooperative and the USDA Forest Service PNW Research Station.

Trial Overview

Three genetic levels (*G*) of seedlots were tested from the Grays Harbor breeding zone: (1) *Unimproved* (a random sample of 50 wild trees distributed throughout the zone). (2) *Intermediate* (pair crosses among 20 parent trees with an intermediate level of genetic gain). This seedlot is only tested in the genetic gain trial portion (i.e., 10’ spacing with standard vegetation control). (3) *Elite* (pair crosses among 20 highly ranked parent trees). All parents in the *Elite* and *Intermediate* populations originated from the Grays Harbor vicinity, and were selected based on their growth performance in the progeny tests.

The trial has six sites, three each established in 2005 and 2006 using one-year old containerized seedlings. Each site contains 22 square plots (100 trees / plot). There are three planting densities (*S*): (1) low - 15 × 15’, 200 SPA, (2) intermediate - 10 × 10’, 440 SPA, and (3) high - 7 × 7’, 889 SPA. Two types of vegetation control (*V*) were applied. Nineteen of the 22 plots are common to all sites and received complete weed control to maintain at least 80% bare ground until crown closure. These 19 plots at each site form a response surface design with greatest replication of all gain levels at 10’ spacing. The remaining three plots received standard weed control (i.e., one complete weed kill during site preparation). These plots form a randomized incomplete block design. Thus, a total of 132 plots, comprising 14,800 measure trees were established within fenced plantations. Each gain level was represented by 10 trees from each of 10 families. A schematic of one site is shown below in Figure 1.

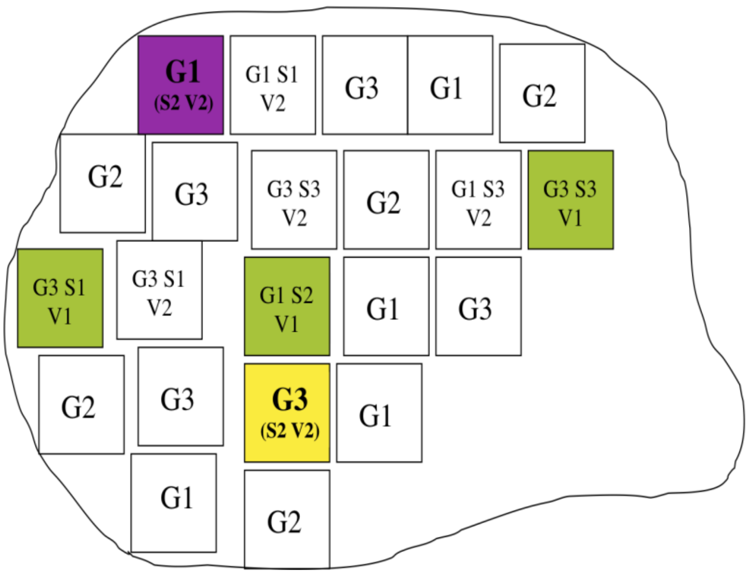

Figure 1. Plot layout of one of the Grays Harbor Genetic Gain / Type IV trials

G1, G2, G3 – unimproved, intermediate and elite seedlot, respectively

V1, V2 – standard and complete weed control, respectively

S1, S2, S3 – 15x15’, 10x10’, and 7x7’, respectively

Data were collected from all sites six seasons after planting, for height (ht7), diameter at breast height (dbh7), average crown width (acw7), crown base height (cbh7), number of incidents of forking (fk7), number of incidents of ramicorn branching (rc7), and stem sinuosity score (su7). The last thee traits (fk7, rc7, su7), were subject to square root transformation before mixed model analysis.

Results

By age-7 there were 12,289 surviving trees. Survival (surv7) varied from 72 to 90% within sites. Analyzed across sites, the genetically-improved seedlots had significantly greater height and diameter than the unimproved seedlot (*P*<0.001), with larger crown base height (*P*=0.01) and fewer incidences of ramicorn branching (*P*=0.001). While differences between gain levels for forking were non-significant, least-square means for forking were marginally lower in the intermediate and elite lots. Realized genetic gains were slightly higher for diameter (12.5% - intermediate, 12.2% - elite) than for height (10.0% - intermediate, 10.7% - elite), and were 27.6% (intermediate) and 29.5%(elite) for tree volume.

No significant difference was found between elite and intermediate seedlots for growth rate. We expect a difference to appear after the onset of strong inter-tree competition. The lack of significance in seedlot × spacing and spacing × survival may imply that inter-tree competition at age 7 is still weak.

Trees planted at the narrow spacings (7’ and 10’) were on average taller (~5%) and had greater diameter (~4%). Spacing had not, however, significantly impacted tree survival (*P*=0.25) yet. Differences between the two levels of vegetation control were insignificant except for dbh7 (*P*=0.05), cbh7 (*P*=0.02), and surv7 (*P*=0.05), with the complete control having slightly better diameter growth, smaller crown base height, and higher survival. We speculate that there would be larger gains from complete weed control in areas subject to more severe summer droughts.

Realized gains varied greatly among full-sib families within each improved seedlot for all traits, and within-seedlot variation was much larger than among-seedlot variation. The highest realized gains for age-7 height, diameter, and volume for a full-sib cross were 23.6%, 27.3% and 73.8% respectively, based on about 480 surviving trees for that cross.

The overall predicted gains were quite comparable to the realized gains. The average realized gains (elite + intermediate) were 10.4% for ht7, 12.3% for dbh7, and 28.3% for vol7. The corresponding average predicted gains from the progeny trials were 11.7% for ht10, 11.8% for dbh10, and 30.8% for vol10.

Conclusions

Results indicate that genetic gains are readily obtainable from Douglas-fir tree improvement programs and, averaged over the crosses, close to those predicted from progeny trials.

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Population Genetics and Genomics – Session 4

Tuesday July 23, 3:15 - Boardroom

The Tria Project: Using genetics and genomics to assess the vulnerability of the pine host landscape in the face of mountain pine beetle range expansion

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The current epidemic of the mountain pine beetle (MPB; *Dendroctonus ponderosae*) is unprecedented in recorded history, impacting more than 28 million hectares of pine forests in western North America. Lodgepole pine (Pinus contorta var. latifolia), with a range overlapping that of MPB, has been the main species of pine affected by the present outbreak. From its historic range in the interior of British Columbia, MPB has spread across the Rocky Mountains into more northerly, easterly and higher elevation forests than previously documented. Long distance dispersal events marked the arrival of MPB into northern Alberta in 2006, where lodgepole pine naturally hybridizes with jack pine (*Pinus banksiana*), a boreal species whose range spans eastward to the Atlantic coast. We used species-distinguishing markers to redefine this hybrid zone, and demonstrate that MPB has undergone host range expansion to pure jack pine. Lodgepole pine shares a co-evolutionary history with MPB, and is thought to have acquired constitutive and induced defences that render greater protection against MPB than evolutionarily naïve hosts such as jack pine. We are using biochemical, physiological and functional genomics to test the hypothesis that molecular aspects of host suitability differ between lodgepole and jack pine. There is evidence that host suitability is influenced by environmental factors, so we are also testing the hypothesis that water limitation affects host suitability features of both species. The development of lesions in both seedlings and mature trees following inoculation with the MPB fungal associate Grosmannia clavigera was slower in jack pine than lodgepole pine; lesion development in both species was delayed by water deficit. Terpenoid oleoresins are an important component of the pine defense arsenal that the tree invokes against mountain pine beetle and other pests and pathogens. The monoterpene profiles of lodgepole and jack pine differ, leading to the possibility that these dissimilarities contribute to differences in host quality between these two species. Towards identifying how these differences are manifested at the genetic, molecular and biochemical level, transcriptomic resources have been mined to identify and characterize genes whose products are implicated in biosynthesis of these monoterpenes. Transcriptome-scale analyses by microarray reveal that thousands of genes are invoked in the response of pines to G. clavigera infection, that there are substantial differences in responses of lodgepole and jack pine, and that water limitation alters this transcriptional programme. In tandem with this research, we are using population genomics to characterize the lodgepole pine, jack pine, and lodgepole – jack pine zone of introgression as a means to develop an “index of naiveté” that can be used to inform risk analysis frameworks. Information arising from this integrated research programme also has the potential to inform tree improvement and reforestation strategies in the wake of this destructive forest insect pest.

Tuesday July 23, 3:45 - Boardroom

Comparative genomics of spruce and pine

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Using two types of genomics resources, full-length cDNAs (ESTs) and BAC libraries, we studied several aspects of evolution along the white spruce-loblolly pine lineage. Previous studies have documented conservation of chromosome number and linkage groups in several species of this family. Our analyses of

ESTs indicate a quite dramatic slowdown of nucleotide substitution rates, perhaps up to ten fold relative to angiosperms, in line with reduced nucleotide diversity within each species. Conifers also have higher dN/dS ratios ("slow but not low"). A simple model is presented that illustrates that natural selection is more effective at a single locus than when two loci are selected at once, providing one possible explanation of higher dN/dS in species with lower diversity. We sequenced 96 random BACs in each species (ca. 10 mb in each species) and also an apparent homologous region containing the lignin biosynthesis gene, CCoAMT. Identification of transposons with long terminal repeats shows that transposition has been ongoing at comparable rates in both species since their divergence (over 120 MYA), but in both species, there seems to be a peak at the start of the Cenozoic era (65 MYA), corresponding to a period of climate change (Paleocene–Eocene Thermal Maximum). CCoAMT coding regions were almost 100% identical, but flanking regions showed low homology. Comparisons of random BACs with draft genome sequence assemblies in both species illustrate that BACs containing complex large repeat structures are left out of the spruce and pine genome assemblies, while other BACs aligned quite well with the draft genome sequences.

Acknowledgement: This work is part of the Treenomix and SMarTForests project funded by Genome Canada, Genome British Columbia and Genome Quebec, a Science Opportunity Fund grant by Genome British Columbia. We would also like to thank Agnes Yuen, Anh Nguyen, Sarah Chao and Stewart Murray for their contributions to the project.

Tuesday July 23, 4:00 - Boardroom

Genetic dissection of expression variation in white spruce (*Picea glauca*)

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In forest biology, two diciplines stand out as benefitting from high-throughput genomics analysis. One of them is ecological and evolutionary genomics, the goal of which is to find genes linked to evolutionary processes in ecological contexts (Feder & Mitchell-Olds, Nat Rev Genet 4:649-655). The other is genetical genomics, which aims at dissecting the genetic architectures underlying variation in complex molecular phenotypes such as global gene or protein expression (Jansen & Nap, Trends Genet 17:288-391). Bridging these two disciplines would give scientists greater resolution in studying the genetic basis of ecologically important evolutionary questions. This however requires that the studied species is amenable to the assessment of genetic effects on phenotypes. Although genomics analysis can now be applied to almost any organism, the list of species that are genetically tractable has remained relatively restricted. This problem is even more pronounced regarding species with dominant roles in their respective ecosystems. In conifer trees, for example, standard genetic experiments that address the phenotypic effects of genetic variants have remained largely unfeasible. Such studies would however be essential to our understanding of the eco-evolutionary dynamics that drive genetic variation.

We have explored an approach to track genetic effects on gene expression that is based on the analysis of global gene expression levels in the haploid, meiotic seed tissue of gymnosperms, the megagametophyte. Microarray analysis of megagametophyte sets from two white spruce trees allowed the identification of gene expression differences between maternal alleles that segregated in Mendelian ratios, and which were therefore most likely caused by single genetic variants (Verta *et al.*, Mol Ecol 22:2369-2379). A relatively large frequency of analyzed genes exhibited simple Mendelian segregation in expression levels in our white spruce dataset, compared to public datasets of plant, insect and mammal model species. While being consistent with high levels of heterozygosity in white spruce, the higher frequency of Mendelian over polygenic effects on gene expression points towards a lower level of connectedness in gene networks in the case of white spruce. On the other hand, as well as being overrepresented in genes involved in stress response, expression variation showed a bias towards duplicated genes. Partitioning of expression variation between duplicated genes was consistent with a transition from relaxed selection on young copies to unequal selection pressures on older paralogs. Furthermore, the evolutionary implications of expression variation are underlined by our observation that expression variation in most genes was affected by independent genetic variants. Because the effects of single genetic variants seem to be limited to only few expression traits, natural selection could act on the expression variants independently. Our results therefore support the idea that adaptation in some traits may be achieved through alternative molecular solutions in different subpopulations of wide-spread conifers.

We are currently conducting an RNA-seq experiment in megagametophytes and the associated, self-fertilized embryos in order to gain insight into the genetic loci that are associated with heritable expression variation, and to examine some of the above predictions in more detail. Both genotypes and gene expression levels can be estimated in a single RNA-seq experiment, enabling the identification of genetic variants that co-segregate with gene expression levels. Our approach takes full advantage of the sequence data because the haploid nature of the megagametophyte tissue allows unequivocal identification of haplotypes. In addition, gymnosperm embryos are produced by fertilized archegonia which derive from cells of the megagametophyte, and thus the maternal genome of the embryo is identical to that of the megagametophyte. Megagametophyte haplotype information can therefore be used as *a priori* information in calling genotypes in the associated embryos. Analysis of self-fertilized embryos will allow us to characterize the phenotypic effects of genetic variants in a diploid tissue. In addition, comparisons between homo- and heterozygote embryos allow the determination of dominance effects between allelic expression levels. This aspect is important when predicting the evolutionary dynamics of genetic variants affecting gene expression, because the balance between drift and natural selection as drivers of allele frequencies and phenotypic evolution depends on dominance relationships between alleles (Lemos *et al.*, PNAS 105:14471-14476).

In total, we have generated over 1.3 billion 100 bp paired-end Illumina reads, distributed to 66 sample pairs. To date, we have aligned the reads to the white spruce gene catalogue, called segregating variants, defined linkage groups and performed association tests of gene expression against local SNP variants in the megagametophyte samples. We identified over 10 800 genes that contained segregating SNP variants between the maternal alleles. Allele-specific (i.e. local) effects on transcript accumulation seem to be common, affecting nearly a third of the genes, with the *p*-value threshold of 0.001. We expect to be in a position to give accurate estimates on the frequencies of local and distant genetic effects on gene expression, their effect sizes and heritabilities in the two studied tissues, as well as their dominance relationships, within the next two months.

Taken together, our analyses indicate that RNA-seq coupled with megagametophyte sampling provides a very powerful and cost-efficient method to identify segregating genetic variants in coding regions in conjunction with defining gene expression levels. In addition to serving as a proof-of-concept for linking genetic variants to molecular phenotypes in spruce, our analyses will build knowledge on the network effects and dynamics of genetic variation with phenotypic impacts.

Tuesday July 23, 4:15 - Boardroom

Genetic variation in urban forests: case study of oak (*Quercus robur*) populations from Helsinki city in southern Finland

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Urban forests are well recognized and valued, among others, for the enhancement of biodiversity at habitat levels. However, urban forests and trees are classified as more vulnerable and subject to genetic fragmentation incidents as being isolated from natural populations in the natural habitats. The dominating hypothesis then may include that such kind of forests and trees are fragmented populations and therefore suffer from the consequences of reduced genetic diversity and increased genetic differentiation. When consider urban forests sustainability and related management issues, it is important to reflect the maintenance needs for trees at individual as well as at population levels. Population genetics of urban forests addresses related research questions that consider urban forests' sustainability in terms of its genetic richness and fitness, vulnerability as related to genetic adaptation as well as the role of gene flow and the surrounding urban environment. Pedunculate oak is common forest tree species in central Europe, while in Finland oaks grow naturally in relatively small isolated stands in the southernmost parts of the country. In Helsinki city, oak populations also exist as initially man-made plantations in popular recreational areas. Reports on pollen-mediated long-distance gene flow within natural stands of oak species, suggested possibilities of positive contribution of background pollination to the maintenance of genetic diversity in such semi-isolated forests.

The aim of this work was to assess the extent and pattern of genetic diversity in originally man-made oak populations in three islands in Helsinki city. Within this aim, the specific objectives were to: 1) estimate how much genetic variation is retained at inter-generational levels; 2) to highlight on the factors that naturally may influence maintenance of genetic variability of oaks under semi-isolated environments, such factors include possible gene flow and the role of background pollination from outside.

The results showed that, over the five tested microsatellite loci - there were no significant differences in hetrozygosities between trees and seedlings generations in each of the tested locations. The same trend was observed considering the three populations as one group, with two generations, trees and seedlings. The overall number of scored alleles among trees and seedlings was 20±6.0 and 19.6±4.2 respectively; expected heterozygosity was 0.839±0.099 and 0.836±0.081 respectively; observed heterozygosity was 0.713±0.099 and 0.739±0.114 respectively. The results also indicated that genetic variability is well maintained among trees and seedling populations with genetic variability exceeded 90% explained by within populations' variability as source of variation. Little, but significant genetic structuring exists among populations at geographical locational level rather than at intergenerational level. Background pollination, on the other hand, contributed by up to 26% of the genetic diversity in seedling generations within each population and 9% among the three populations. Comparably, results from the studied populations showed slightly higher heterozygosity than unpublished results from natural oak population in southwest Finland. These results indicated that, urban fragmentation as such may not constitute an absolute factor that obstructs beneficial gene flow in urban landscape, but a combination of different factors such as factors related to the biological nature of plant species as well as the surrounding environment in the specific urban landscape.

Plenary Session 5

Wednesday July 24, 8:15 – Mt. Currie South

Sitka spruce breeding in Britain: Dawn of a new era

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Although Sitka spruce (*Picea sitchensis* (Bong.) Carr) is native to the Pacific Northwest it is well suited to the moist warm climate of northern and western Britain. There are now 700 000 hectares of Sitka spruce planted in Britain producing around 6 million cubic metres of timber annually over a roughly 40-year rotation. The most suitable origin of seed for most of Britain is from Queen Charlotte Islands, BC although seed sourced from Washington or even Oregon State, USA survives and grows well on milder sites in the extreme south and west of the Britain.

The breeding programme commenced in the early 1960s along traditional lines selecting superior phenotypes in plantations, half-sibling progeny testing and re-selection of the best parents based on an index of growth rate, stem straightness and an indirect measure of wood density. Tested clonal seed orchards are now mature and productive.

The first full-sibling progeny trials combining some of the early re-selected parents are now over 20-years old. The best progenies are re-created through controlled pollination and the resulting seed is sold to nurseries who multiply this scarce resource by striking roots on cuttings taken from stock hedges raised from the seed. Rooted cuttings are deployed as full-sibling family blocks.

Currently all the Sitka spruce planted is based on improved material derived either from seed orchards (approx 75%) or from vegetatively propagated material (25%). Clonal forestry is not currently being practised due to perceived lack of demand and institutional resistance; technological problems are largely overcome but not yet proven to be economically viable.

Policy makers tend to hold the view that the achievement of further gains through breeding is not justified due to the cost that this would entail as well as the time it would take. This is particularly the case when research funding is generally being reduced, and being diverted from Sitka spruce towards other species. There is however, an appreciation that we need to protect the Sitka resource we have created in terms of resilience to climate change and out-breaks of disease. At the same time there is a demand to make faster and cheaper progress in certain areas of breeding particularly wood quality and its component parts.

A Sitka spruce Marker Aided Selection project started in 2004 with the planting of a large scale clonal trial replicated on 3 contrasting site-types. At each site there are 4,500 clones made up of 1,500 clones in each of 3 full-sibling families. There are 4 replicates of the same 4,500 clones on all 3 sites. Initially, work concentrated on looking for microsatellite markers and later moved onto SNPs. In 2007 we became an external partner of 'Genome BC'. In 2008 we became partners in 'NovelTree' a 4-year EU funded project which enabled us to collaborate with Roslin Institute. Within this project we used the RAD approach to genotype large numbers of SNP markers and used these to predict the performance of trees for 6-year height and 5-year bud-burst. These results provide the first DNA-markers that have been linked to a phenotypic trait of Sitka spruce growing in Britain, albeit for a large number of individuals within one full-sibling family. This demonstrates an important step forward for conifer tree breeding in Britain.

DNA-marker work continues under a new 4-year EU contract called 'ProCoGen' during which time Forest Research and Roslin Institute aim to find markers for a wood-quality traits such as density or micro-fibril angle in all three full-sib families in our trial.

Breeding for multiple pest resistance in western redcedar (*Thuja plicata*) under a changing climate

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Western redcedar and yellow cypress breeding programs, despite being in place for approximately 20 years, are the newest species under improvement in BC. Western redcedar has a number of unique biological features including ease of vegetative propagation, ability to self with minimal inbreeding depression and early reproduction, all of which have influenced the program strategy. Currently, the focus has been on volume, cedar leaf blight and deer resistance, as well as the most important economic trait, heartwood durability. A number of populations have been developed and current focus is on breeding for broad spectrum resistance in one future population. Yellow cypress also propagates vegetatively, but unlike western redcedar, seed orchards have not been successful due to environmental challenges. A clonal forestry approach has been taken and currently selected clones for growth and form are being managed in donor hedges and rooted cuttings deployed operationally. Research has recently focussed on establishing orchards in environments conducive to seed production. Since the breeding program currently is not moving on to advanced generation breeding, it is important to have an alternative to clones for future deployment of selected populations.

Modelling and exploring genotype by environment interaction using factor analysis, reduced tree models and hierarchical clustering in multi-environment tree breeding trials

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Accurate prediction of the genetic value of individuals is crucial for all breeding programs, but none more-so than tree breeding programs which typically have extremely long generation intervals of up to 35 years. Prediction and selection for many traits is based on phenotypic data acquired from so-called multi-environment trials (METs). METs usually include (genetic) trials grown over many years and locations, the latter chosen to represent the environments for which deployment will occur.

One of the main reasons for use of a MET is to determine whether individuals (that is, genotypes) will perform similarly across the range of environments relevant to the objectives of the breeding program. The varying performance of genotypes in different environments is known as genotype by environment (GxE) interaction. It is well known that GxE interaction can be partitioned into heterogeneity of scale and cross-over interaction (Muir et al. 1992), and it is the latter that can ultimately affect rates of genetic gain unless selection and deployment strategies are developed to capture the associated specific adaptation.

In general, GxE interaction appears to be relatively important for tree growth rates (see for example Johnson and Burdon, 1990; Carson, 1991; Matheson and Wu, 2005) but there is little evidence to suggest that this is being adequately described and exploited in tree breeding programs. Progress towards a solution has been hampered by practical and methodological issues. A key issue is that a comprehensive and genetically connected MET data-set is required to reliably estimate genetic correlations between environments (Apiolaza, 2012). However, it is not uncommon in many historical tree breeding MET data-sets for there to be few parents in common between environments. There are various reasons for this, including limited resources, propagation issues or simply poor planning.

Due to the typically poor connectivity in tree breeding MET data-sets, historically only simplistic statistical models, such as compound symmetric models, or piecemeal approaches based on pair-wise bivariate analyses have been used for modelling GxE interaction.

In this talk we present a one-stage approach to the analysis of tree breeding MET data that incorporates both genetic effects (that is, additive and where appropriate non-additive effects) and non-genetic effects associated with aspects of the experimental designs (for example, blocking, sets and plots). GxE interaction is modelled using a factor analytic model (Smith et al. 2001) which simultaneously provides an estimate of the genetic correlation matrix for all environments. The genetic correlation between two environments is inversely proportional to the amount of cross-over interaction for these environments. We exploit this by using a model-based clustering of environments based on the estimated genetic correlations in order to produce meaningful “mega-environments”. By definition all pair-wise genetic correlations within a mega-environment are similar so that “generalised” main effects may be obtained separately for each. The approach is illustrated using a large MET data-set from the Radiata Pine Breeding Company program, which spans 37 years with over 320,000 observational units.

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Adaptation and Climate Change – Session 6

Wednesday July 24, 10:35 - Mt. Currie South

Black spruce seed source transfers for future Ontario climates

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Focal point seed zones are species-specific GIS algorithms developed to provide best-adapted, site-specific choices for seed transfer. They are based on regressions of growth/survival observed in common garden trials across a range of test climates. Thus, biological variation (e.g. height or survival) at the population level is expressed in terms of local climate patterns. Previous work with jack pine had demonstrated that temperature variables were most important for its future seed zone delineation. For black spruce, however, precipitation variables were equally important to temperature variables for future seed source delineation. Based on these observations, three black spruce seed source portfolio trials 'preadapted to climate change' were established in northern and central Ontario based on four future climate models: CGCM, CSIRO, MIROC and NCAR. Predicted best planting sites were 1) quite variable depending upon which model they were based and 2) were increasingly distant from present day distributions over first and second 30 year periods.

Wednesday July 24, 10:50 - Mt. Currie South

Early results from a Douglas-fir reciprocal transplant study

Brad J. St. Clair[1], Connie A. Harrington[1], Peter J. Gould[2]
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Genecological studies of Douglas-fir have shown genetic variation in adaptive traits that track geographic gradients in temperature and aridity of seed sources, suggesting natural selection for phenology, cold hardiness, growth, and biomass partitioning. Although genecological studies are useful for delineating seed zones and population movement guidelines, they necessarily make assumptions about local adaptation and the degree to which population differences are important for productivity and adaptation. We set out to test hypotheses of adaptation of coastal Douglas-fir using a reciprocal transplant design in which 120 families from 60 populations from 12 diverse regions from northern California to western Washington were planted at test sites in 9 of those regions in western Washington and Oregon. Results from previous genecology studies were used to ensure sampling of a diversity of populations, the presumed climates in which they evolved, and the climates in which they are now being tested. A diversity of populations and climates is critical to modeling population responses to climate in order to understand the effects of climate change on adaptation and productivity of native populations, and to explore options for assisted migration. Preliminary results are presented for survival and height at age 6. Overall survival was high at 97 percent, although trees from the California Coast had poorer survival, particularly at the most northern and higher elevation sites. Analyses of variance for height indicated statistically significant differences among test sites, regions, and populations within regions, as well as significant test site x region and population interactions. As expected, greater differences were found among test sites than among populations within test sites, with warmer sites having the greatest growth. Response functions at individual sites indicated local adaptation with better growth from populations that came from climates most similar to the test sites. Other adaptive traits being evaluated include budburst, cambial phenology, drought hardiness and cold hardiness.

Geographic variation and evidence for local growth superiority for coastal Douglas-fir: Rotation-age growth performance in a series of Douglas-fir provenance trials

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Introduction

Long-term provenance tests are commonly used to understand the geographic variation of the species, to select the best provenances for a specific region, and to explore provenance by environment interaction. Since two cycles of breeding have already occurred for Douglas-fir and genetically improved materials are widely used in the Pacific Northwest, selecting the best provenance is no longer the main purpose. Instead, quantifying provenance by environment interaction has become an important issue, as it will provide valuable information on long-term adaptation and breeding zone delineation.

Despite the fact that Douglas-fir is one of the world's most valuable timber species, it's provenance by environment interaction is not well understood. The only multiple-provenance, multiple-site, long-term provenance test in the Pacific Northwest was planted in 1959 in Oregon, Washington, British Columbia, and northern California (Ching 1965). Several publications based on this study were available at various ages, with inconsistent results (Ching and Bever 1960, Ching 1965, Rowe and Ching 1973, Ching and Hinz 1978, White and Ching 1985, and Krakowski and Stoehr 2009). Provenance by environment interaction appeared to be evident only after age 30 or later (Krakowski and Stoehr 2009). While White and Ching (1985) observed that the local provenances have greatly increased their growth rankings from age 9 to 25 at several locations, Krakowski and Stoehr (2009) concluded that provenances from relatively distant origins often grew as well or better than the local ones at age 45. No study focused on quantifying the level of local growth superiority except for simple comparisons of local and non-local sources at each location.

The main objectives of this study were (1) to investigate provenance x environment interaction and quantify the level of local growth superiority, and (2) to verify if geographically or climatically closely located provenances have similar volume growth.

Materials and methods

Reciprocal design - 16 provenances were planted at each seed collection site.

Complete block design, with 4 blocks/ site. Individual provenances were planted in 7 x 7-tree square plots surrounded by two rows of buffer trees of the same source at 1.8 m spacing.
Six of the 16 sites, located in OR and BC, were measured at age 46~52 (from seed) and used in this study. Sites were thinned and 24~25 undamaged, well formed, and well-spaced trees were measured in each plot.

Total height (ht) and diameter at breast height (dbh) at the final measurement (age 46~52) were collected. Stem volume (vol) calculated using Bruce and DeMars (1974) volume equations. Total volume per plot (vol_sum) calculated as the total vol in a plot for all living trees.

A set of 216 climate variables (including annual, seasonal, and monthly variables) generated by ClimateWNA (v4.70).

Analysis of variance (ANOVA) pooled across sites was conducted for each trait using SAS Proc GLM. The linear model included the following random effects: site, block within site, provenance, provenance x site, provenance x block (for ht, dbh, and vol only), and random error. In addition, single-degree-of-freedom contrasts were conducted for testing the relative performance between local and non-local provenances.

Least-squares means (LSMs) were estimated for each provenance across sites as well as within site. Rank correlations were calculated to depict the pattern of provenance x site interaction.

Local superiority is defined as the difference in volume between the local provenance and each non-local provenance. At each site, only the provenance originating from this site is considered as local.

Mantel tests were conducted to examine the existence of geographical (or climatic) spatial autocorrelation. The procedure was done using R package ade4 and based on 9,999 random permutations. Euclidean distances were based on weighted geographic coordinates (i.e., latitude, longitude, and elevation) or climate variables.

Regression analyses were conducted to examine the relationship between local growth superiority and Euclidean distance between provenance's original location and the test site.

Results and discussions

While there were significant differences between provenances, provenance ranking also changed from site to site. The best provenances varied widely in growth ranking while the worst provenances performed relatively consistently across sites. The rank correlations between sites were generally low (average $r = 0.31$ for vol and 0.16 for vol_sum).

While local provenances were not always the best performers in growth, positive correlations were found between local growth superiority in vol_sum and geographic distance (between provenance's origin and the test site) at each site ($r = 0.15$~0.64) as well as across sites ($r = 0.47$). This revealed a general pattern that the closer the provenance is to the test site geographically, the better rotation-age volume growth the provenance has at this site. This pattern was statistically significant considering all sites, and at two (i.e., Haney and Nimpkish) of the six sites.

At low-elevation sites, low-elevation provenances performed significantly better than high-elevation provenances. By contrast, high- and low-elevation provenances performed similarly at high-elevation sites. Across all sites, there was no difference between southern and northern provenances. For vol_sum, southern provenances generally performed better than northern provenances at the OR sites, while northern provenances performed better than southern provenances at the BC sites.

Across all test sites, results indicated that there was a significant spatial autocorrelation pattern ($r = 0.3$; $P < 0.05$), showing an overall trend of having similar rotation-age volume growth for geographically closely located provenances. Similar results were obtained when Euclidean distance was calculated using the climate variables instead of geographic variables (i.e., latitude, longitude, and elevation).

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Wednesday July 24, 11:20 - Mt. Currie South

Genetic strategies for adaptive resource management of US southern national forests in a changing world

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National strategic goals have been established to guide adaptive resource management of the US's national forests in changing times. Those goals are: Restore, Protect, and Respond. Within the national genetics program the "*National Genetics Strategic Plan*", "*Genetic Options for Adapting National Forests to Climate Change*" and the "*National Guideline on Conservation Genetics*" have been written to provide genetic guidance to resource management activities.

Genetic principles have always supported and guided reforestation efforts on the fourteen national forests in the Southern Region (R8). These national forests are the most diverse forested ecosystems in the US, existing in very fragmented landscapes. Numerous challenges, e.g. increasing populaces, predicted climate changes and forest health issues, have been impacting and changing the way these national forests are managed. These challenges precipitated a new paradigm in R8's Genetic Resource Management Program (GRMP), whose role is to contribute and support healthy, productive and sustainable tree populations on the national forests. As part of the adaptive process, R8's GRMP has evolved from traditional tree improvement to activities that include restoration, conservation and partnerships.

Providing seed for operational reforestation and restoration has long been the priority goal of the GRMP. The guiding principle, thus far, has been through the use of local seed sources. However the current practice of relying on seed sources best suited to past and current climate will need to shift to allow consideration of the sources of seed that will be best suited to future climates. In many cases, research or management attention is lacking, as is basic genetic information that is needed to guide decisions. To address this new seed zone modeling is being developed. A second priority goal for R8's GRMP is tree conservation. Over 140 tree species inhabit the southern landscapes. Using the ForGRAS model (Potter and Crane, 2010) approximately twelve of those tree species are considered threatened. More aggressive gene conservation activities are needed for species and populations most vulnerable to the current and future challenges. A third priority goal is partnerships. The GRMP coordinates with partners in the development and implementation of projects, most notably in tree conservation.

Wednesday July 24, 11:35 - Mt. Currie South

Sub-optimal populations: Postglacial refugia-induced melting-pot of *Populus tremuloides*

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Species under suboptimal conditions presented strong evolutionary significance, and are expected to be more vulnerable to climate change. The primary causes of sub-optimal populations are post-glacial refugia-induced population colonization and migration, adaptational-lag, margin-central population structure, and density-dependent selection. To test these hypotheses of sub-optimality, we investigated historical projections of species habitat with bioclimatic envelope modeling, and intra-specific quantitative genetic variances of *Populus tremuloides* based on series of reciprocal common garden experiments in western Canada and Minnesota State. We found two discernible refugia on the north and south margins of *P. tremuloides* historical habitat range. An increasing trend appeared in the additive genetic variances of adaptive traits such as bud break in Alberta populations that suggests a possible admixture and outcrossing of north and south populations. The "melting-pot" genetic structure is promising among the sub-optimal Alberta populations. The most likely reason for current sub-optimal conditions was complex postglacial meta-population dynamics, but other potential explanations are margin-central population structure, or density-dependent selection, where two divergent populations of lineages colonized and mixed from Alaska and south of Canada- US area the Although clear boundary between different mechanisms rarely exist. Our findings can guide management strategies to ameliorate aspen productivity.

Wednesday July 24, 11:50 - Mt. Currie South

Patterns of admixture and local adaptation in the interior spruce hybrid zone: Implications for forest management under climate change

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The patterns of admixture and the nature of selection responsible for the maintenance of the economically and ecologically important *Picea glauca x P. engelmannii* hybrid zone in western North America are investigated.

Genome-wide estimates of admixture based on a panel of 311 candidate gene single nucleotide polymorphisms (SNP) corresponding to 290 genes, were used to assess current levels of admixture and introgression and identify loci involved in reproductive barriers and adaptive differences between species. This data was combined with long-term quantitative data (growth, survival, bud phenology, and cold hardiness) and climatic data, to test assumptions of hybrid zone maintenance and to model future scenarios under climate change.

Our results suggest that the *P. glauca* x *P. engelmannii* hybrid zone is maintained by local adaptation to growing season length and snowpack. Hybrids appeared to be fitter than pure species in intermediate environments, which fits expectations of the bounded hybrid superiority model of hybrid zone maintenance. Adaptive introgression from parental species has likely contributed to increased hybrid fitness in intermediate habitats.

A total of 12 outlier loci corresponding to genes responsible for carbohydrate metabolism, signal transduction, transcription factors and others were found. Levels of admixture are very high and introgression is asymmetric towards Engelmann spruce, supporting recent studies using neutral markers.

While *P. engelmannii* ancestry is higher than *P. glauca* ancestry in hybrid populations, on average, selective breeding in managed hybrid populations is shifting genomic composition towards white spruce, potentially pre-adapting managed populations to warmer climates.

Wednesday July 24, 12:05 - Mt. Currie South

Guiding Douglas-fir seed selection in Europe under changing climates: Bioclimatic envelope model predictions versus growth observed in provenance trials

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Rationale & objective: Douglas-fir is an important tree species in its native range in North America, and in Europe, where it is was introduced more than 180 years ago due to its high productivity and wood quality. Many of these early plantations are experimental, with known origins of seed sources, and today they also serve as a unique experimental test bed to investigate how trees may react to climatic change. Here, we test if a model developed to guide assisted migration of Douglas-fir in North America under climate change can accurately predict the success of provenance transfers to Europe.

Approach: We first evaluated the performance of more than 700 Douglas-fir provenances, originating from coastal British Columbia to the interior mountains of New Mexico, that were tested in 120 European sites, from boreal conditions in Finland to dry and hot climates of Spain and Turkey. Provenance performance was compared at each test site and regionally ranked. Second, a regression tree-based bioclimatic envelope model for Douglas-fir populations was developed using North American data, then applied to current and future climates in Europe to predict where populations may find a suitable climate match.

Main findings: Model predictions of optimal climate matches for western and central Europe conformed well with observed provenance trial growth, but diverged under eastern Europe's continental climates. There was an expected north-south trend, with northern and southern coastal sources performing best at their corresponding western European latitudes. In Eastern Europe, however, interior Douglas-fir sources rarely outperformed coastal sources, even though they were predicted as best climate matches by the model.

Conclusions& applications: Climate trends observed over the last three decades may warrant changes in seed selection, and the bioclimate envelope predictions developed here appear accurate enough to guide this selection in Europe under contemporary and changing climates. Where model predictions and observed growth data diverge, test plantations may not have experienced rare climate extreme events to which the best performers may in fact be maladapted. In such cases, a safe compromise would be to use slightly less productive provenances with a better match among source and planting environments.

Wednesday July 24, 12:20 - Mt. Currie South

Genetic differences in Douglas-fir provenances control water use strategies and stem hydrology

Bela J. Bender[1], David Montwé[1], Laura V. Junker[2], Ingo Ensminger[2], Heinrich Spiecker[1]
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Douglas-fir is of outstanding economic importance in forestry in its natural distribution area in the Pacific Northwest as well as in western and central Europe. Since in those areas sites are almost expected to shift to significantly dryer conditions, selecting the most adapted planting material might be of increasing importance. In the present study we aim to elucidate the basic physiological mechanisms leading to different growth performance and drought resistance between Douglas-fir provenances. Understanding those mechanisms of adaptation to drought is important for identifying populations better adapted to future climate and for improving forest management planning.

We used 50 year old Douglas-fir trees in a common garden experiment in south-western Germany. Five provenances originating from British Columbia, Washington and Oregon, representing a natural north-south transect, were selected for this study. We surveyed three individuals per provenance by electronic point dendrometers during the 2011 vegetation season to detect variation in stem hydrology. Gas exchange of these trees was measured in May and July 2011 under dry and moist conditions to assess stomatal conductance. An additional 5 individuals per provenance were felled for retrospective growth analysis. This was repeated on three sites of the same common garden experiment with different climatic conditions.

In some provenances the diurnal swelling and shrinking processes of the stems were correlated to variations in air humidity. This relation decreased gradually from the selected provenances of winter-cold to those of winter-warm habitats. No correlations were found with soil water content. However, stomatal conductance showed the same gradient from high values in the northernmost provenance to low values in the southernmost. Therefore we assume that diurnal swelling and shrinking processes are influenced by genetically fixed differences in stomatal regulation which are an adaptive trait to optimize photosynthesis and minimize water loss by transpiration under dry conditions. In contrast to previous work, our results indicate that minimum wood density is higher for populations originating from warmer habitats, even though the differences were not significant. This indicates a higher mechanical resistance of the hydraulic system to drought stress, and could carry important implications for forest regeneration strategies.

Wednesday July 24, 12:35 - Mt. Currie South

Distribution and evolution of *Pistacia* in arid land

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The genus *Pistacia* L. (Anacardiaceae) consists of 11 species. Seven species are distributed from the Mediterranean basin to Central Asia (*P. atlantica*, *P. khinjuk*, *P. lentiscus*, *P. palaestina*, *P. terebinthus*, and *P. vera*), two species in Eastern Asia (*P. chinensis* and *P. weinmannifolia*), and two species from the Southwestern United States to Central America (*P. mexicana* and *P. texana*). *Pistacia aethiopica* was defined as a new species in 1980 by J. O. Kokwaro, however, its status has not been evaluated.

Pistacia is characterized by its dioecious reproductive system and homeochlamydic perianth (or naked flowers). The synapomorphies of a single apotropous ovule per locule place *Pistacia* within the Anacardiaceae.

The global distribution of species of the genus in relation to arid and semiarid zones and long-term climate change is the focus of our research. It is planned to be done by available GIS data sets (climate, landscape), remote sensing variables (reflectance, the first derivative of the reflectance, RS vegetation indices) and high spatial resolution satellite imagery for single-tree crown extraction and mapping. We hypothesize that long-term global climate episodes affect differentially the different species, limiting the distribution of those that are more vulnerable to drought and heat and *vice versa* in relation to the tolerant species. A better understanding of the species distribution related to drought, temperature change tolerance, and nutritional requirements are studied.

The availability of an extensive germplasm collection of *Pistacia* from various geographical regions around the globe has made it possible to evaluate their genetic relationships and the evolution of the genus.

Preliminary GIS data interpolation showed limited global distribution of the genus by climate and elevation. Additionally, biochemical content in the plants appeared to be an appropriate variable for species classification in separate clusters. High correlations are found between the specific portions of the first derivative of spectral reflectance and biochemicals' contents in the plants, cluster analysis was done and dendrograms elaborated. They showed not only distinctive classes of the different species but also similar patterns to phylogenetic analyses.

Dehydration in higher plants, either as a normal developmental process such as occurs during embryogenesis or induced by environmental stress, is accompanied by changes in gene expression.

We have identified and characterized a dehydrin protein in *Pistacia*, which appears to be associated with cold tolerance and drought. Dehydrins are abundant in all photosynthetic organisms from cyanobacteria to higher plants. Much dehydrin research has been directed at herbaceous species, but reports concerning dehydrins in woody plants are less numerous. Dehydrin accumulation in *P. vera* was found to be associated with cold tolerance, dormancy and/or seasonal changes.

The adaptation of *Pistacia* trees to harsh desert conditions and their longevity make them ideal candidates for reforestation in arid zones. A better understanding of the genetic relationships among the species would be an advantage for breeding programs in this genus.

Quantitative Genetics and Tree Breeding – Session 7

Wednesday July 24, 10:35 - Boardroom

Matching genotypes to current and future production environments to maximise radiata pine productivity and profitability

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Radiata pine (*Pinus radiata* D. Don) is the most important commercial conifer species in Australia and New Zealand. Significant progress has been made in the improvement of growth, form and wood quality traits through the understanding of their genetic control. However, the benefits of long-term investments in genetic tree improvement have not been fully realised, as improved genotypes should be matched to their specific growing environments and production systems to fully realize their genetic potential.

Current radiata pine breeding and deployment in Australia and New Zealand is based largely on state and regional boundaries or forest inventory zones rather than on rigorously mapped climatic and physiographic parameters. Current zones may not be delivering optimal genetic gains across the whole estate. To further improve radiata pine plantation productivity and to maximize realised genetic gain from breeding and deployment populations under current and future climates, it is necessary to: (*i*) delineate breeding and deployment zones based on site and climatic factors, and (*ii*) match genotypes with current and future production environments considering multiple objectives, such as maximizing growth, improving form, branching, resistance and wood quality.

Optimal breeding and deployment zones must consider genotypic responses together with physiographic and climatic information. Genetic information on breeding stock was combined with soil and climatic data of more than 300 test sites to pinpoint environmental factors affecting the response of radiata pine to environmental variation, and to provide a better understanding of the factors contributing to the observed genotype x environment interaction (GxE). An analysis of GxE was performed using multivariate analytical techniques and mapped using geographic information system (GIS) tools.

We explored different methodologies to account for GxE. In practice, the Radiata Pine Breeding Company (RPBC) in New Zealand prefers clustering of sites based on a factor analytic model involving a G×E term. To explain the clustering of sites in New Zealand based on geo-climatic variables we used several analytical techniques including visualisation based on Random Forest Classification and Multi Dimensional Scaling, Multiple Regression on Distance Matrices and Multiple Regression Trees.

The Southern Tree Breeding Association (STBA) in Australia prefers site classification based on modelling of genetic correlations. For trials in Australia, we created a database of genetic parameters as a basis for the modelling of genetic correlations. Site classification involved grouping trials into site types based on certain goodness of fit criteria to reduce the so called residual G×E, or minimise G×E within site types. Genetic evaluations with the TREEPLAN system, incorporating the new site classification will demonstrate the genetic gain resulting from the improved classification.

The environmental variables determining the G×E patterns or, so called, "drivers of GxE" were identified, and at the broadest, trans-continental scale, climatic variables such as temperature and rainfall were the most significant. However, at a local regional scale soils and topographical factors were of more significance. New site classification will be defined across Australia and New Zealand, and genetic gains that can potentially be obtained by accounting for genotype by environment interaction (G×E) evaluated. Our ultimate goal is to recommend best strategies to maximise genetic gain by matching genotypes with production environments and ensure immediate adoption of results into selection and deployment programs of the radiata pine industry.

Predictions of plantation yield at different plantation locations based on future climate forecasts typically do not consider potential for adaptation by using genetic selection. Climate change scenarios will be the superimposed on the "response functions" or "norms of reaction" models developed in this study, and responses of different genotypes will be extrapolated beyond current climatic conditions. Trials at the extremes of the radiata pine climate envelope will be particularly valuable to assess the effects of future climate change on growth and form traits. Climate change impacts on deployment zones will then be evaluated using (GIS) tools that we are currently developing. This study will determine the extent to which we can use genetic selection to mitigate some of the predicted negative effects of climate change on timber supply from the radiata pine plantation estate in Australia and New Zealand.

Wednesday July 24, 10:50 - Boardroom

Variation in reproductive capacity of lodgepole pine (*Pinus contorta* var. *latifolia*) in British Columbia

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Lodgepole pine is the most wide-ranging pine in North America. Populations in British Columbia vary widely in phenotypic and genotypic characteristics and differences between populations can be linked to local climate, or to geographic predictors. The effect of climate on variation in reproductive characteristics has never been examined, yet is vital to the production of seed necessary for reforestation. This study aims to determine the relationship between climate and variation in female cone and seed characteristics. The study makes use of the Illingworth provenance trial, sixty common garden plots that are distributed throughout British Columbia. Female cones from six source populations were collected at 22 sites during the summer of 2012. Variation in the average number scales and seed, and in average cone length were analyzed in relation to the climates of the test sites. Results indicate that wide variation exists between both sites and provenances in these reproductive traits.

Wednesday July 24, 11:05 - Boardroom

Pedigree analysis and fourth cycle breeding strategy in the North Carolina state university cooperative tree improvement program

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The build-up of inbreeding is expected to occur over time in any recurrently-selected breeding population. Breeding strategies aim to control coancestry and inbreeding while increasing genetic gain. As more cycles of selection are completed, the pedigree structure of a population becomes more complex. Designing crosses to optimize genetic gain and keeping the inbreeding at a desired level is challenging but necessary. We evaluated a number of software packages for their usefulness for analyzing pedigrees in the NC State University Cooperative Tree Improvement Program. Pedigree analyses suggest that there is minimal inbreeding in our loblolly pine (*Pinus taeda* L.) breeding populations after three cycles of selection. Out of 258 individuals tested in third cycle polymix trials with one or more known parents, only one individual (a self with F=0.5) has an inbreeding value greater than zero.

We used a Differential Evolution algorithm developed for animal breeding (Kinghorn 2011) to design the mating for the Cooperative fourth cycle. Simulations were carried out, testing various combinations of three different 'balancing' strategies between gain and coancestry, two different candidate mating pools, and two different maximum levels of parental use. Crosses between full- and half-sibs were discouraged by weighting against solutions that contain any recommended crosses with F>0.124. As expected, scenarios resulting in lower population diversity also achieved higher mean index values (mean breeding value). The mean F and the predicted progeny index of the fourth cycle population changed depending on the level of constraint placed on coancestry. For all the scenarios tested, mean progeny F for the recommended crosses ranged from 0 to 0.0018 and is not a problem. This is expected given the large number of parents in the breeding population and an initial mean F=0. Using the Differential Evolution algorithm to design crosses in sublines over several generations resulted in rapid increase in mean F and rapid leveling out of the expected mean index. In conclusion, pedigree analysis and the Differential Evolution algorithm appear to be effective tools for designing the fourth cycle breeding population for the Cooperative.

Wednesday July 24, 11:20 - Boardroom

How to add more seed cones on lodgepole pine

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Low seed cone number can limit seed production. By studying hormone profiles of parts of lodgepole pine long-shoot buds, we were able to devise a method to enhance seed cone production. In a long-shoot bud, developing seed cones and male cones are separated. High performance liquid chromatography-electrospray ionization tandem mass spectrometry in multiple-reaction monitoring mode was used to investigate phytohormone profiles in the proximal and distal portions of long-shoot buds. Higher concentrations of *trans*-zeatin riboside (*t*-ZR) and dihydrozeatin (dhZR) were found in the distal parts of long-shoot buds, whereas concentrations of isopentenyl adenosine (iPA), indole-3-acetic acid, abscisic acid (ABA), ABA glucose ester (ABA-GE) and phaseic acid (PA) were higher in the proximal parts. In long-shoot buds of genotypes with a history of high seed-cone yield, concentrations of *t*-ZR and the ratio of zeatin-type to isopentenyl-type cytokinins (CKs) were higher in the entire buds. In low seed-cone yielding genotypes, concentrations of *c*-ZR, iPA, ABA-GE and PA were higher throughout the long-shoot bud. ABA was higher in the distal portion. Exogenous gibberellins (GAs) applied by stem-injection or branch paste, reduced endogenous concentrations of ABA and/or some of its metabolites, such as ABA-GE. Treatments that combined GAs with CKs, such as thidiazuron or 6-benzylaminopurine, altered endogenous CK concentrations more than treatment by CK or GA alone. Application of bud paste with a mixture of GA_{4+7} and CK resulted in many female cones. Many of these were found in locations where previously only male cones are found. These ectopic PGR-induced seed cones develop normally.

Wednesday July 24, 11:35 - Boardroom

Inheritance of partial resistance to white pine blister rust in sugar pine

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Sugar pine, one of the white pines' most susceptible species to the blister rust fungus (*Cronartium ribicola*), has a major gene (*Cr1*) at low frequency in populations, that confers immunity to the disease. Sugar pines bearing at least one copy of *Cr1*, however, are vulnerable to specific virulence from a complementary gene (*vcr1*) in the pathogen. In the years since the appearance of the virulence gene, a form of resistance, partial (PR; aka, slow-rusting), was discovered, which is a suite of traits that reduces susceptibility to blister rust in pines challenged by either the avirulent (*Avcr1*) or virulent (*vcr1*) forms of the pathogen.

Sixteen sugar pine parents with known, suspected, or low PR were control-pollinated, resulting in 75 families in a mixed factorial-diallel design. In 2006, a plantation was established at Happy Camp in northwestern California to assess the degree and inheritance of PR. Progenies consisted of open-pollinated seedlings from survivors of previous epidemic waves on the same site (all presumed to carry some degree of PR), along with the control-pollinated families. Since 2006, all progenies have been naturally exposed to blister rust at the test-site, in which alternate host *Ribes* spp. have been interplanted among the rows. Progenies were evaluated in 2010-2012 after successive wave years of *C. ribicola* caused overall infection to reach 82%. In 2010, numbers of separate infections were counted on each seedling and characterized as normal susceptible or reactive PR responses; in subsequent years, only the presence of reaction type was noted. Mean infections per family ranged from 0.39 to 12 per tree, with numbers of infections in families with low PR increasing exponentially, suggesting epistatic effects. In 2012, families varied from 36 to 100 in the percentage of progeny with normal, susceptible infections; mortality is now ~60%. Families with the least infection tended to come from F1 PR parent survivors from prior epidemics. As expected, the presence of the *Cr1* gene in a parental genotype had no effect on PR responses of the progeny. We analyzed the data in the manner of Cockerham and Weir (1977) using SAS' PROC GLIMMIX. In addition to estimating general and specific combining ability effects, the C&W model also estimates extranuclear paternal and maternal effects and general and specific reciprocal effects. However, because of imbalance in our data, reciprocal effects could not be estimated. Additive genetic variation was ~80% of the total genetic variation and narrow-sense heritability ~0.35 in the 2011 and 2012 evaluations; non-nuclear maternal effects were 8% of genetic variances. We regressed bi-parental expected values against family means. In the 2011 data, this relationship was linear. However, in the 2012 data, the relationship was quadratic, showing underdominance and with families from the best parents deviating positively from their expected values. In spite of this, only three families deviated outside the 90% confidence interval for individual values. Thus selection can be largely based on general combining abilities. These results present opportunities for combining PR with single gene resistance to develop long term, durable resistance.

Wednesday July 24, 11:50 - Boardroom

Prediction of wood quality based on age-age correlation for wood density components

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Wood density is considered one of the most important characteristics of timber quality, therefore, the possibility of its formation through population selection can be fundamental for wood quality improvement. Trees need a long time to achieve a proper age to be considered appropriate for use, therefore it is difficult to quickly assess the efficiency of the process of population selection. The observed high correlation between the properties of juvenile wood and mature wood allows for the acceleration and improvement of efficiency of selective actions. To the studies, run by the State Forests in Poland, aimed at selecting the main forest trees species, the studies on genetic aspects of wood structure were integrated. For conducting studies on heritability and genetic correlation of components of wood density of European larch we chose generative progeny of plus trees from a seed orchard. Such a choice was dictated by the requirements of analysing trees with a confirmed origin. The studies included eight families of European larch aged 25, however, in the age-age correlation analyses, the annual rings corresponding to age 8-19 were also taken into account. This allowed for the observation of the relations between juvenile and mature wood and for the assessment of the possibility to predict the properties of mature wood based on the values observed in juvenile wood. This is particularly important in the case of European larch growing in plantation conditions characterized by rapid growth rate. Components of wood density for each annual ring were determined using the X-ray densitometer. Analyses were preceded by the preparation of wood samples including the extraction of resins. Genetic correlation coefficients were estimated on the basis of MANOVA option of SAS PROC GLM. For most wood density components, including the overall density of the annual ring, high correlation coefficients were observed (>0.8). These results were confirmed in previous studies conducted on other species of trees (Fujimoto et al. 2006, Hannrup et al. 1998, Hannrup et al. 2001, Hyllen 1999, Kumar et al. 2002). The highest values of the age-age genetic correlation were observed between the age of 8 and 19, however the high correlation coefficients were observed also for the later age. These results indicate the possibility of making efficient decisions on the selection of individuals promoted during a selection aimed at improving the properties of wood after the first 10 years of the tree growth.

Family reforestation using portfolio theory to improve yield stability and reduce risk in black spruce

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Family forestry, here defined as the deployment of family mixtures into plantations, is becoming an attractive option for black spruce (*Picea mariana* (Mill.) BSP) in New Brunswick, Canada. While there are many elite families of black spruce available, there is a knowledge gap regarding how to determine a mixture of families that optimally balances the objectives of increased yield and reduced risk. This study, based on real field test data, investigates the application of a model based on modern portfolio theory to optimally balance yield and risk when selecting a portfolio (mixture) of black spruce families to deploy in reforestation. The risk was expressed as the variance of the family portfolio, an effective indicator of yield stability. This approach was applied for the first time in forestry practice, and it is compared to the currently used method, truncation-deployment, i.e. the equal deployment of seed of selected families. Results show that the portfolio theory technique searched for the combination of yield and stability and produced family portfolios maximizing yield at a given stability, or conversely minimizing instability at a given yield. By comparison, the new portfolio theory approach was never inferior in maximizing yield to the truncation-deployment approach when yield stability was the concern. While this study used portfolio theory to determine family portfolios for family forestry, the results may be applicable to other deployment strategies where stability is a concern, such as clonal forestry.

Using acoustic velocity for genetic improvement of mechanical wood quality in boreal conifers: An example of early screening in eastern white spruce

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There is a growing interest to use acoustic sensors for selection in tree breeding in order to ensure high wood quality of future plantations. We assessed acoustic velocity as a selection trait for the improvement of mechanical wood properties in two 15- and 30-year-old white spruce (*Picea glauca* [Moench.] Voss) genetic trials in eastern Canada.

Previous studies – mostly conducted in fast-growing pine species such as radiata pine, loblolly pine and slash pine – identified strong correlations between acoustic velocity and mechanical wood traits. This led to the general conclusion that sound velocity can be used for cost-effective indirect selection of mechanical wood traits such as microfibril angle (MFA) and stiffness (modulus of elasticity, MOE). But at the start of this current study, it was not known how a combination of slow growth, small diameter stems and a high incidence of live branches along the whole length of the stem would influence the accuracy of the velocity measurements and, consequently, the suitability of acoustic tools for use in breeding programmes for slow-growing boreal conifers.

For the young trees, genetic analyses included 1534 trees from 59 half-sib families from a genetic trial repeated on two sites. Tree height and breast height diameter (DBH) were recorded for each tree. Acoustic velocity was measured in each stem and increment cores served to determine wood density, ring width and MFA. Individual heritability of acoustic velocity was moderate (h^2=0.38) and of the same magnitude as heritability of wood density (h^2=0.32). However, genetic control of cellulose microfibril angle (MFA) was rather low (h^2=0.22) and similar in magnitude to genetic control of early wood width and DBH (h^2=0.22 and 0.23, respectively). As a result, considerable genetic gain was estimated for acoustic velocity and a measure combining velocity and wood density. The relationship between acoustic velocity and MFA was strong on the genetic level (r_G=-0.84) and selection based on velocity could effectively improve MFA, which is one of the most important determinants of wood mechanical properties. Phenotypic and genotypic correlations between velocity and growth traits were low, which is in contrast to the moderate negative relationship between growth and other wood traits such as density (DBH and density r_G=-0.41, r_P=-0.40). Although low, the positive relationship between acoustic velocity and tree height (r_G=0.17) presents an interesting opportunity for the mutual improvement of both tree growth and wood quality.

On the phenotypic level, MFA was more strongly correlated to acoustic velocity in mature trees than in young trees (R^2=0.54 and R^2=0.24, fixed velocity effect only). The difference was probably related to the presence of reaction wood in the juvenile stem. The evolution of the link between both traits with tree age suggests that age-age correlations should be more closely monitored in future studies. Multivariate regression models showed that velocity was the most important trait for prediction of MFA or wood stiffness, which is in agreement with previous studies. The addition of other easily obtainable traits such as DBH, height-to-diameter ratio as well as wood density to velocity could slightly improve models of MFA at the young and the mature age. We concluded that juvenile acoustic velocity is an appropriate trait to indirectly select for wood quality in a tree breeding context.

Wednesday July 24, 12:35 - Boardroom

Novel remote sensing phenotyping platform and genomic selection will boost the delivery of genetic gain of radiata pine in New Zealand

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Radiata pine breeding has at least 60 years of history in New Zealand. The national programme is based on phenotypic data collected through on-site assessment of genetic improvement trials. Two new initiatives will ensure a step-change in the speed of delivery of improved genotypes to the forest industry: the utilisation of remote-sensing to phenotype individual trees and the integration of genomic selection techniques.

Developments in the types and resolution of remote sensing technologies are creating opportunities for researchers. Satellite images can capture spectral information that can detect areas of poor forest health. LiDAR (Light Detection And Ranging) captures spatial information and is being rapidly adopted for developing spatial surfaces of forest height. These technologies have obvious applications in forest inventory systems and have the potential to be applied to phenotype radiata pine both in replicated field trials, and in the forest itself. We plan to evaluate the application of data collected using remote sensing technologies to phenotype radiata pine in large-plot genetic trials and large-scale forests. In-forest data will be interrogated to determine the effectiveness of LiDAR and RapidEye relative to field-based measurements. The ability to identify outstanding individuals or groups of individuals (e.g. healthy trees in areas under stress) and apply mass selection in the forest estate will also be investigated.

Genomic selection and the utility of single nucleotide polymorphisms (SNPs) have tremendous potential for increasing the speed of delivery of genetic gain to the forest. In New Zealand, we believe that we will be able to halve the time (from 25-30 to 10-15 years) it takes to establish genetically improved trees in the forest by reducing the breeding cycle and facilitating the use of forwards selection in the breeding program. Clonal breeding populations will be trained for key traits, and non-key traits and SNP assays developed for selection on seedlings, with the eventual likelihood of skipping the testing phase altogether. Early selection will finally be possible, and traits that are expensive to measure can have assays developed at a fraction of the cost of conventional phenotyping (e.g. wood quality traits). Increased selection intensities are also possible, assuming sufficient diversity and SNP assays will be cost-effective.

These two programs will come together with the identification of outstanding phenotypes through remote sensing. In order to take advantage of these phenotypes, we intend to identify their parents using a SNP assay developed from the initial stages of genomic selection programme. Once good parents are known, they will inform further breeding and deployment. Final SNP assays for breeding traits will also be applied once available, again, to inform the breeding and deployment of radiata pine genotypes.

The SNP resource created for the development of genomic selection is being undertaken as a partnership between Scion and the Radiata Pine Breeding Company. SNPs will be extracted from DNA sequence data currently being generated to build the first genome sequence of radiata pine, in collaboration with the University of California, Davis and likely to include the University of Concepción, Chile.

These new directions will see an acceleration of delivery of genetic gain for radiata pine in New Zealand and an operational-level integration of molecular and quantitative genetics in the breeding cycle.

Population Genetics and Genomics – Session 8

Wednesday July 24, 10:35 - Cheakamus

Landscape genetic consequences of bird, gravity and wind dispersed valley oak propagules

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We have invested heavily in studying propagule dispersal over the last decade, on the premise that it determines both the amount and pattern of genetic variation across the landscape. We have learned that pollen generally moves farther than seed, and dispersal asymmetries should have implications for male & female gametic affinity patterns across that landscape. We have examined natural recruiting patches in *Q. lobata*: (1) canopy shadow patches (CS) of maternal plants (gravity & small rodent-vectored); (2) open patches (OP) (bird-vectored. These vector differences impact the patterns female gametic affinity. Using microsat assay techniques, we have genotyped both the seed coats and embryos of multiple seeds in both types of patches, and have determined male and female gametophytic genotypes. We have reported seed and pollen dispersal outcomes elsewhere. The point is that the open patches involve recruits dispersed further from their maternal sources. We anticipate more rapid decay of mega-gametophtic relationship with increasing distance for canopy patch than for avian-dispersed open patch recruits. What to expect from the male gametes is a bit unclear. We computed autocorrelograms for both male and female gametes for both types of patches. Female gametes show striking patterns of IBD. For (CS), the progression was ($r = 0.45$) at close quarters → ($r < 0.04$) at 500m. For (OP), the same progression was ($r = 0.27$) → ($r = 0.07$). Male gametes, by constrast, show virtually no difference between patch types and no real decay with distance: for (CS), we found ($r = 0.03$) → ($r < 0.01$); for (OP), we found ($r = 0.03$) → ($r < 0.02$). For each patch type, we also computed within patch (α), among patch (β) and total (γ) allelic diversities. For (CS) and female gametes, the partition is ($\alpha = 1.54$, $\beta = 1.53$, $\gamma = 2.36$). For (OP), the same partition is ($\alpha = 2.18$, $\beta = 1.31$, $\gamma = 2.86$). Greater seed dispersal increases a. decreases b, and increases g). For male gametes, the corresponding partition is ($\alpha = 3.29$, $\beta = 1.08$, $\gamma = 3.56$) for (CS) and ($\alpha = 3.27$, $\beta = 1.10$, $\gamma = 3.56$) for (OP), no meaningful difference, but larger α, smaller β, and larger γ than for female gametes. In overview and to a first approximation, seed flow determines IBD; pollen flow represents the homogenizing glue that holds the species together.

Wednesday July 24, 10:50 - Cheakamus

Population genomics in *Populus trichocarpa* (black cottonwood) reveals a candidate gene for local adaptation

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Black cottonwood (*Populus trichocarpa* Torr. & A. Gray) is an ecologically and economically important forest tree distributed throughout western US and Canada. Along its range, poplar exhibits variation in several adaptive traits including growth, phenology, and disease susceptibility. To understand the genetic basis of this adaptation, we scanned the genomes of *P. trichocarpa* accessions from across its range for signatures of selection. Despite low levels of population genetic structure at most genetic markers, COMT1 (encoding caffeic acid 3-O-methyltransferase), an enzyme involved in phenylpropanoid metabolism, showed strong levels of population differentiation. Here we analysed 302 COMT1 DNA sequences based on whole genome resequencing in individuals from 29 locations in the north, center, interior and south of BC, spanning over 14° in latitude, to ascertain whether increased population differentiation could be driven by adaptation. From 17 COMT1 SNPs (Single Nucleotide Polymorphisms), three were found to be

nonsynonymous (nsSNP) and two of these were located in the O-methyltransferase domain (amino acids 158 and 287 respectively). The geographic distribution of haplotypes revealed that alleles with glutamine at position 287 (Q287) are restricted to northern and interior populations, while alleles with proline at this position (P287) occur at all locations. These results were supported by further population genetic analyses that showed relatively low levels of COMT1 allele exchange between southern and northern populations (F_{st} = 0.193) and between southern and interior populations (F_{st} =0.151). The F_{st} between northern and interior populations was not different from zero. Although statistical tests for selection revealed that COMT1 overall is under purifying selection, certain amino acids may have adaptive significance. While COMT1 E287 is restricted to northern and central populations of *P. trichocarpa*, it is common across the range of *P. balsamifera*, a closely related boreal species. COMT1 P287, on the other hand, is exclusively found in *COMTs* from Arabidopsis, tobacco, *Medicago* and other *Populus* species, as well as in the *P. trichocarpa* COMT2, a gene involved in developmental lignification. We hypothesize that Q287 causes changes in COMT1 enzyme activity, and affects *P. trichocarpa* phenotype, resulting in a competitive disadvantage of this allele at lower latitudes, where both temperature and precipitation are higher. To test if the introgression of COMT1 from *P. balsamifera* to *P. trichocarpa* is being selected against at southern locations, we will perform further selection analyses and phenotypic studies of individuals with different COMT1 genotypes. We are also functionally characterizing COMT1 by transforming an Arabidopsis comt mutant with different poplar COMT1 and COMT2 alleles.

Wednesday July 24, 11:05 - Cheakamus

Genomic signatures of late Pleistocene range dynamics and divergent selection revealed by re-sequencing of the black cottonwood exome

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Temperate and boreal tree species have undergone repeated range expansion and contraction coincident with glacial cycles during the late quaternary. For northern hemisphere species, refugial populations typically existed south of the maximum extent of glaciation, though there has long been interest in the degree to which additional refugia were present in northern ice-free areas, for example, on coastal islands or interior boreal deserts where low precipitation limited ice formation. High phenotypic differentiation among contemporary populations implies rapid local adaptation along these migration routes, but these adaptations may have been hindered by re-colonization bottlenecks and central-peripheral structure. *Populus trichocarpa* (black cottonwood or ‘poplar’) is a temperate species with a wide contemporary latitudinal range that extends from northern California, USA north through coastal southeast Alaska, USA. We recently reported exome re-sequencing of a rangewide sample of poplar genotypes. Here we use these data to elucidate how neutral and adaptive forces have shaped genomic variation within and among populations of this species. The most parsimonious solution to population structure in our range-wide sample of 47 genotypes resulted in three groups arrayed latitudinally (which we refer to as north, central, and south). Mean nucleotide diversity was highest in the northern population and lowest in central population, while linkage disequlibrium was highest in center and lowest in the north. These patterns were consistent across the genome. Estimation of mutation-scaled effective population sizes (N_e) revealed that the north had the largest N_e, followed by the center and south populations. Taken together, these results are consistent with a glacial refugium in the vicinity of the northern population, which is supported by the paleobotanical record indicating the presence of cottonwoods in Beringia during the last glacial maxima. Genome scans of population differentiation reveal a very narrow distribution of F_{ST} for intergenic regions, with much broader distributions for different classes of sites (synonymous, replacement, promoter, UTR) within genes. Promoter regions harbored significantly more outliers than other classes of sites within genes, suggesting the role regulatory variation in adaptation to climate.

Wednesday July 24, 11:20 - Cheakamus

Towards understanding the genomic basis of chemical defense traits in European *Populus* species

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Trees are long-lived organisms that cannot move to escape environmental challenges such as herbivores, pathogens or climate change. Their survival and fitness thus depend critically on adaptive responses to biotic and abiotic stressors. Tree adaptation to changing environments is likely to involve genetic variation supplied by migration within species and introgression from related species, although these hypotheses have rarely been tested in a rigorous manner.

We studied patterns of expression of key secondary metabolites involved in biotic and abiotic responses (salicinoids, flavonoids, and chlorogenic acids) in three large natural hybrid zones and a common garden composed of open pollinated families of two ecologically divergent *Populus* species, *P. alba* and *P. tremula*[1]. The same plant material is also being genotyped with mapped microsatellites[2], Restriction site Associated DNA (RAD) sequencing[3] and related methods, and population samples of each species have been subjected to whole genome re-sequencing.

Uni- and multivariate analysis of metabolomic profiles in recombinant hybrids reveals the potential and limits of wide recombination to generate novel combinations of functionally important chemical traits[1]. By combining admixture mapping of chemical traits in natural hybrid zones with common garden measurements, we provide first insights into the genomic architecture of chemical defense traits that differ between these ecologically divergent, hybridizing species.

We will discuss the implications of our findings for the genetics and chemical ecology of phenotypic species differences with potential community and ecosystem effects. We will highlight the benefits of combining genomics, metabolomics, and ecology in fundamental and applied studies of forest tree biology, using *Populus* as an example.

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Wednesday July 24, 11:35 - Cheakamus

Comparative phylogeography and genetic structure of the eastern North American birches: *Betula alleghaniensis*, *B. papyrifera*, and *B. lenta*

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We examined the phylogeography and genetic structure of birches (*Betula* spp.) in eastern North America, with a specific focus on the widespread, ecologically and economically important species, *B.*

papyrifera Marsh. (4x=56, 5x=70, 6x=84), *B. alleghaniensis* Britt. (6x=84), and *B. lenta* L. (2x=28). Chloroplast DNA (cpDNA) and nuclear microsatellite (nSSR) markers were used for inferring patterns of population structure in relation to historic glacial and postglacial population dynamics as well as contemporary gene flow. Six chloroplast microsatellites and the *psb*A-*trn*H intergenic spacer region were analysed for *B. papyrifera*, *B. alleghaniensis*, and *B. lenta* sampled from 65, 80, and 12 populations, respectively. A subset of these populations was used for examining the population genetic structure at eight polymorphic nSSR markers. Genetic diversity within and among species was compared using rarefaction analysis and haplotype richness maps. Haplotype networks, spatial analysis of molecular variance (SAMOVA), and comparisons of N_{ST} and G_{ST} values were used to evaluate phylogeographic structure at cpDNA markers, while tests of isolation-by-distance (IBD) and Bayesian analysis were used to examine population structure at nSSR markers. *Betula papyrifera* and *B. alleghaniensis* demonstrated relatively high allelic richness at cpDNA and nSSR markers, while *B. lenta* was fixed for a single cpDNA haplotype and demonstrated low nSSR diversity. The highest cpDNA and nSSR diversity for *B. alleghaniensis* was detected in the western Great Lakes region and *B. papyrifera* also had high cpDNA diversity in that region. Populations of *B. papyrifera* and *B. alleghaniensis* were strongly differentiated at cpDNA markers (G_{ST}=0.526 and 0.601, respectively), and the SAMOVA revealed two main phylogeographic groups for these species corresponding to populations of eastern and western refugial origin. *Betula papyrifera* and *B. alleghaniensis* showed extensive regional haplotype sharing indicating that introgression within glacial refugia or during postglacial colonization may have contributed to the shared phylogeographic pattern. In contrast, no phylogeographic structure was observed in *B. lenta* at cpDNA markers. No clear pattern of population genetic substructure was found for any of the species at nSSR makers. Populations were weakly differentiated (F_{ST}=0.008-0.015, R_{ST}=0.011-0.032) and differed significantly only over large geographic scales. The relatively high genetic diversity of *B. papyrifera* and *B. alleghaniensis* in the western Great Lakes region might be explained in terms of haplotype mixing in this region which is a postulated zone of biogeographic contact between eastern and western glacial lineages. The high level of cpDNA-based population differentiation in *B. papyrifera* and *B. alleghaniensis* could be attributable to long-term isolation within glacial refugia leading to differentiation through genetic drift. In contrast, the low level of nSSR-based population differentiation observed in birches could be due to high rates of contemporary gene flow via pollen. High levels of genetic diversity observed in *B. alleghaniensis* and *B. papyrifera* could be attributed to relatively large effective population sizes in glacial and contemporary landscapes facilitated by their relatively broad ecological amplitude or their polyploid origins, which may contribute to high heterozygosity at nSSR loci. The low genetic diversity detected in *B. lenta* could be due to its relatively narrow geographic distribution and lower cold tolerance, which may have led to population bottlenecks during the last glacial maximum. The low genetic diversity at both chloroplast and nuclear genomes suggests that *B. lenta* may possess low adaptive potential to the changing climate, and should potentially be given a high priority in conservation and management programs. In contrast, the relatively high genetic diversity of *B. papyrifera* and B. *alleghaniensis* suggests that they may have maintained relatively large effective population sizes during past glacial/interglacial periods and may possess genetic resilience to climate change.

Wednesday July 24, 11:50 - Cheakamus

Geographic clines in nuclear microsatellite diversity and structure and cone characteristics variation of *Pinus densiflora* natural populations in Japan

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Pinus densiflora (Japanese red pine) is both an economically and ecologically important conifer whose widespread natural distribution in mainly semi-mountainous area constitutes one of major Japanese forest landscapes. Since this species is suffering from serious reduction in population sizes in Japan due to the introduction of pine wood nematode, design of programs to conserve or manage its regional genetic resources and breeding zones are essential. Despite that the levels of genetic diversity and genetic clustering have been evaluated for populations of many other major tree species in Japan (e.g., Takahashi et al. 2005 for *Cryptomeria japonica*; Hiraoka and Tomaru 2009 for *Fagus crenata*), almost no information has been obtained on its current population genetic structure based on molecular markers as well as variation in its adaptive traits. In the present study, the geographic pattern of genetic diversity and structure of 62 *P. densiflora* natural populations (a total of 1,883 trees) across their distribution in Japan was examined using eight nuclear microsatellite markers. The geographic variation in cone characteristics was also investigated for 28 out of the 62 genotyped populations (a total of 628 trees).

We found that the allelic richness was significantly lower in both northern and eastern marginal populations. Analysis of recent bottlenecks detected a significant heterozygosity excess more frequently in the northern and eastern populations. The overall value of the standardized measure of population differentiation (G'_{ST} = 0.122) was similar both to other widespread Japanese tree species and to continental *Pinus* species. STRUCTURE software analysis revealed a gradual cline in the genetic structure, with three main clusters corresponding to the western, central and northeastern populations; the northeastern cluster showed the highest *F* value. We also found that the cone size (length, width) as well as the seed-ovule ratio per cone of trees was significantly larger in both northern and eastern populations.

The microsatellite results indicated strong genetic drift through rapid population expansion of *P. densiflora* in northeastern Japan, associated to recent changes in distribution suggested by anthropological and paleoecological studies. The geographic cline in cone characteristics might also be related with the climatic or environmental conditions of populations such as temperatures. Results obtained on these neutral genetic and morphological variations would provide fundamental information for designing conservation units and breeding zones, also with a light of evaluation of adaptive genetic variations in future studies.

Wednesday July 24, 12:05 - Cheakamus

Using ancient DNA from lake sediments to examine forest responses to Holocene environmental changes

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Paleoecological reconstructions based on fossil pollen records have given us much insight on Holocene shifts in forest community composition in North America. Such long-term records are necessary to make better prediction of forest responses to future climatic changes. However, pollen analysis also has well-understood limitations including long-distance dispersal and similarities in pollen morphotypes in some species. Ancient DNA (aDNA) is a potentially useful complementary source of information to pollen records in understanding historical vegetation shifts. Apart from providing links between Quaternary paleoecology and fields such as evolutionary biology and population genetics, aDNA might be able to provide higher taxonomic resolution than fossil morphology. This potential requires overcoming three obstacles: (1) Ancient DNA must be preserved and recoverable in lake sediments; (2) molecular variation in these aDNA assemblages must contain information of high taxonomic resolution (e.g. identification of species or subspecific lineages); and (3) the bioinformatics problems of assemblages of damaged aDNA must be resolvable. We addressed these issues in one of the most common contexts in Holocene paleoecology: inference about changing forest composition from lake sediments. Working in Upper Michigan lakes with excellent fossil pollen and macrofossil records, we extracted chloroplast DNA (cpDNA) from macrofossils and bulk sediments as old as 6,000 yr BP (Before Present). We used novel sequence capture method and high throughput sequencing to extract and sequence large numbers (on the order of 10^7) of DNA fragments per sample. We are currently addressing bioinformatics challenges of these assemblages of fragmented and damaged DNA using a new bioinformatics pipeline and assembly algorithms. Preliminary results indicate we

can identify cpDNA at a level equivalent to that provided by fossil morphology, with higher confidence at the genus level. We can also identify interspecific variation - species that are not resolved via macrofossil morphology (e.g. species of *Betula*) as well as intraspecific variation unavailable via fossil morphology (e.g. distinct lineages of *Fagus grandifolia*). These results, when combined with paleoecological data (e.g. pollen and macrofossil analysis), provide a better and more comprehensive signal for reconstruction of past shifts in forest community in response to environmental changes.

Wednesday July 24, 12:20 - Cheakamus

Using RNAseq to characterize gene expression in lodgepole pine and interior spruce

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Understanding the genomic basis of how forest trees adapt to climate will be important to making management decisions to prepare for climate change. The AdapTree project aims to characterize the genomic basis of local adaptation to climate in *Pinus contorta* (lodgepole pine) and *Picea glauca, Picea engelmanii* and their hybrids (interior spruce), which are key species of economic importance in British Columbia and Alberta. Plasticity is one important component of how species cope with variable environments, and studying gene expression provides one approach to characterize which genes are most involved in responding to climate. Here, we explore how gene expression varies among 48 accessions of lodgepole pine and 41 accessions of interior spruce, each from a single population. Each individual in the experiment was grown under one of seven treatments in growth chambers, representing a range of moisture, temperature, and light regimes. We then used RNAseq methods to sequence RNA extracted from root, stem, and leaf tissue, yielding approximately 3.8 Gb of data per library. To assemble a reference transcriptome for each species, we combined one library from each of the seven treatments and ran the combined data on Trinity. We used RSEM and EdgeR to estimate expression levels for each gene in our transcriptome, and used WGCNA to group these genes into clusters based on similarity of expression profiles. We identified 8894 and 11618 differentially expressed genes in spruce and pine, respectively, at an FDR = 0.01. Our cluster analysis identified 13 groups, which correspond to genes that have similar patterns of expression across the different treatments. These characterizations of gene expression will provide a basis to test hypotheses about the importance of plasticity in adaptation. Subsequent work in the AdapTree project aims to characterize which genes are involved in local adaptation to climate. Using this data, it will be possible to test whether the most plastic genes also tend to be the most or least likely to be involved in local adaptation.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Wednesday July 24, 12:35 - Cheakamus

Finding the genes for local adaptation: Trimming to account for population structure

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The genes responsible for local adaptation are likely to have more genetic differentiation among populations than do neutral genes. In principle, a good clue that a gene is affected by evolution to local conditions is that it has a higher F_{st} than the rest of the genome. However, testing this statistically is challenging, because some population samples are not evolutionarily independent of other samples. Typical F_{st} outlier approaches have high false positive rates. We introduce a new method of F_{st} outlier analysis--called LookWell-- that adjusts for non-independent sampling. We show through simulation that LookWell gives fewer false positives and much more reliable inference.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

CONFORGEN Symposium – Session 9

Thursday July 25, 8:30 – Mt. Currie South

Ecological genomics of eastern white spruce (*Picea glauca*) in relation to climatic variation

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White spruce is a dominant species of the Canadian boreal forest and it is also important at the economic level. Previous studies reported significant quantitative trait differentiation among populations from eastern Canada for traits related to growth and phenology as well as local adaptation at the molecular level, suggesting climate-driven adaptation. Because of ongoing climate changes, identifying genetic polymorphisms related to climate adaptation becomes critical for the management and conservation of genetic resources and for genomic-assisted tree improvement programs in a rapidly changing environment.

The objective of the study was to detect genes carrying polymorphisms (SNPs) significantly associated to climatic factors in eastern white spruce. To do so, 41 populations from eastern Canada distributed along climatic gradients of temperature and precipitation were sampled. Their genomes were scanned for 11,085 SNPs located in 7,819 expressed genes, which represents nearly one third of the known transcriptome. Two outlier-detection and two correlation methods were used to identify SNPs showing selection signatures where allele frequency variation was related to variation in mean annual temperature and total precipitation or both.

The union of the four detection methods resulted in around 10% of the genes that carried SNPs significantly differentiated or associated with climatic factors. A sizeable proportion of the genes were related to more than one climatic factor, suggesting pleiotropic effects. Sequence alignments with gene ontologies (GO) and protein domains (Pfam) databases revealed that these genes represented a great diversity of putative molecular functions and gene families. Gene families such as kinase domain, caspase domain, lipoxygenase or ferredoxin were significantly enriched with genes carrying significant SNPs. A few genes carried SNPs detected independently by the four methods used, providing very strong evidence of the adaptive role of these genes. One such gene putatively codes for a tubulin/FtsZ GTPase domain, for which expression has been shown to be repressed by cold in Arabidopsis thaliana. Work on identifying potential epistatic effects and gene networks is underway.

Thursday July 25, 9:00 - Mt. Currie South

Conservation strategies under climate change: Accounting for adaptive potential and migration capacity in tree species.

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A number of assumptions underpinning the use of species distribution models to predict biological responses to climate change are violated for temperate and boreal tree species that are widespread, long-lived, and genetically adapted to local climate conditions. To address this situation we propose a methodology to account for the potential effects of genetic structure, adaptive potential and limited migration capacity.

For 24 North American trees, we calculate geographic distances between similar climate habitats in the past, present, and future, with a k-sample nearest neighbor algorithm. Secondly, we employ biological response scenarios to evaluate the potential effects of genetic adaptation to local environments and the capacity of species to adapt and migrate.

In a two case studies for western Canada, we demonstrate how the approach can be used to evaluate the adequacy of a protected areas system, and reforestation guidelines to ensure the maintenance of forest genetic resources. We find that between 35% and 85% of locally adapted populations in protected areas are maintained under a median climate change scenario until the end of the century.

Nevertheless, we find that on average populations already lag behind their optimal climate niche by approximately 130km in latitude, or 60m in elevation. For the 2020s we expect an average lag of approximately 310km in latitude or 140m in elevation, with the most pronounced geographic lags in the Rocky Mountains and the boreal forest.

We propose that approach can provide important information to effectively allocate limited resources available for conservation of genetic resources, and to guide seed transfer in reforestation programs.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Thursday July 25, 9:30 - Mt. Currie South

Genetic conservation in the Anthropocene: The case for assisted gene flow in forest trees

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Assisted migration is being evaluated and in some cases already being used as a tool for maintaining resource production or conserving species as climates change. However, there is a lack of scientific consensus on this subject. Much of the debate arises from a lack of a common definition for assisted migration. To some, it primarily refers to the human movement of seed or individuals within existing species ranges, which we define here as *assisted gene flow (AGF)***.** To others, assisted migration primarily refers to species introductions outside of their historical range. In order to evaluate the risks and benefits of assisted migration, it is necessary to consider AGF and assisted migration outside of native ranges separately. AGF has greater genetic implications for existing native populations than assisted migration outside of native ranges as existing recipient populations will be altered, while assisted migration into novel areas has greater ecological implications than AGF as species will be introduced to ecosystems. Here we focus on the genetic effects of AGF.

To provide a more nuanced perspective on the potential for AGF to facilitate adaptation to climate change, we have evaluated genetic risks and potential benefits through a review and synthesis of the theoretical and empirical literature in combination with simulation modeling of populations. AGF can increase average fitness in a population through introducing or increasing the frequency of pre-adapted alleles or genotypes. However, AGF may result in outbreeding depression due to the disruption of co-adapted gene complexes, called intrinsic outbreeding depression. AGF may also disrupt local adaptation to non-climatic factors such as soil properties, photoperiod, or the presence of other organisms such as competitors, pathogens or mutualists through hybridization of residents and immigrants, termed extrinsic outbreeding depression. AGF may result in lineage swamping and the loss of local populations; however, it may also maintain genetic lineages from rear-edge populations that are likely to be extirpated if left *in situ.*

Many tree populations have large effective population sizes, experience relatively high levels of gene flow among populations, and are locally adapted to climate, thus are ideal candidates for AGF. Intrinsic outbreeding depression is unlikely to occur unless populations have been long isolated, and evolution should resolve weak outbreeding depression due to epistasis in a few generations. Similarly, natural selection should resolve mild extrinsic outbreeding depression due to adaptive differences in large populations. To weigh the risks of maladaptation to climate change against the risks of AGF for a given species requires information on the extent of local adaptation to climate versus other environmental factors, and the patterns of gene flow. We conclude that AGF should be a powerful tool for the management and conservation of many widespread tree species that show historical adaptation to local climatic conditions.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Reference:
Aitken, S.N. and M.C. Whitlock. 2013. Assisted gene flow to facilitate local adaptation to climate change. Annual Review of Ecology, Evolution and Systematics Vol. 44. In press.

Applications of Genomic Tools – Session 10

Thursday July 25, 10:30 - Mt. Currie South

Dissecting the genetic component of insect resistance in spruce

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Here we aim to identify both DNA markers and patterns of gene expression that correspond to interior spruce trees (*Picea glauca x engelmannii*) that are resistant to insect pests, specifically from attacks by the white pine weevil (*Pissodes strobi*) and the western spruce budworm (*Choristoneura occidentalis*). We are using an association genetics approach to map insect resistant phenotypes to DNA markers within the spruce genome, which has now been sequenced within the wider SMarTForests Project (http://www.smartforests.ca). The discovery population is located near Salmon Arm, BC, Canada and represents the F1 generation of the Prince George breeding population, these trees have been derived from control crosses made between the top 144 parents that were selected based on growth. From this F1 population we have sampled one tree per cross (n=251), plus seven genotypes from control seed lots. To disentangle the multifaceted mechanisms of insect resistance in spruce, we performed phenotyping of monoterpenes and sesqiterpenes (using gas chromatography–mass spectrometry), diterpenes and phenolics (using high-performance liquid chromatography), histology (using tissue sectioning, staining and light microscopy), as well as growth measurements and natural field attack by *P. strobi*. By sequencing the messenger RNA (using Illumina RNA-seq) of each individual in the population, differences in gene expression have been identified between trees with contrasting phenotypes, and these differentially expressed transcripts are good candidate genes underlying resistance phenotypes. Applying a "genotyping by sequencing" analysis to the same RNA-seq data, 331k Single Nucleotide Polymorphisms (SNPs) were identified, located within 23k genes. Association testing was performed in TASSEL 4.1.8 using all SNPs and target traits, both the General Linear Model (GLM) and the Mixed Linear Model (MLM; which corrected for kinship and population structure). Associations between the SNPs in several terpene synthase genes and the monoterpenes myrcene, 3-carene, as well as the sesquiterpene β-caryophyllene will be presented. We will also demonstrate how panels of SNP markers can be used to calculate Genomic Estimated Breeding Values (GEBV) that predict the target traits from the DNA sequence, opposed to traditional Breeding Values (BV) calculated from observed phenotypes.

Acknowledgement: This work is part of the SMarTForests Project (http://www.smartforests.ca) funded by Genome Canada, Genome British Columbia, and Genome Quebec, funding was also received from the British Columbia Ministry of Forests Lands and Natural Resource Operations. We would also like to thank Kermit Ritland and Joel Fillon for their contributions to the project.

Thursday July 25, 10:45 - Mt. Currie South

A genomics approach towards selection for biotic resistance in Western Redcedar (*Thuja plicata*)

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Thuja plicata silviculture is hampered by two quite different biotic stressors; deer browsing of planted seedlings render reforestation efforts inefficient and expensive, while fungal heartwood rot reduces yield at the time of harvesting. Resistance to both stressors has been linked to production of monoterpenoid compounds. High levels of thujone deter browsing of foliage, whereas high levels of tropolones, in particular thujaplicins, correlate with rot resistance in living trees. In addition, lignans contribute heavily to rot resistance of wood in service, which in turn is one of the main reasons behind the high price and popularity of lumber from this species. While selection for high foliar content of thujone is well under way, generational selection for individuals with high lignan and especially thujaplicin content in heartwood is not yet feasible due to the long time (10-20 years) it takes before the levels of these compounds can be scored and compared reliably. Thus, there is a need for early prediction of lignan and thujaplicin content, as well as simultaneous scoring with other traits such as thujone content and growth.

Recent advances in DNA sequencing technology now provide avenues for large-scale gene discovery as well as genotyping. In addition, Genome-Wide Association Studies and more recently, Genomic Selection technology have shown that breeding values for allele and allele combinations be assessed in species with large and unknown genomes such as *T. plicata* and be applied directly in the selection of superior genotypes.

Towards this goal we are identifying large sets of genes that are expressed at sites and times of thujone, thujaplicin and lignan biosynthesis. We have used a natural phenotypic variant that lacks foliar resin glands to identify by Illumina tag profiling > 600 genes whose expression is associated with foliar resin glands producing and storing thujones and other terpenoid compounds (Foster et al. 2013). In situ RNA hybridization showed that a putative monoterpene synthase-encoding gene is expressed in the epithelium of foliar resin glands. Furthermore, in vitro enzyme assays showed that the corresponding protein converts geranyl diphosphate into sabinene, a known precursor of thujone. The 600 genes also include candidate genes for the conversion of sabinene into thujone, as well as potential orthologs of proteins involved in the induction of biotic defenses. We have also identified ray cells in the sapwood-heartwood transition zone that secrete hydrophobic, possibly resin and thujaplicin-containing, droplets into adjacent xylary parenchyma cells. The red color of these secretions also indicates that it contains plicatic acid. High concentration of this lignan correlates with decay resistance in lumber (Morris and Stirling, 2012). We have used Illumina RNA sequencing to identify > 7000 mRNA transcripts that are expressed in this transition zone. This large set contains transcripts with predicted open reading frames for putative terpene synthases and other enzymes that may be involved in thujaplicin biosynthesis, as well as dirigent proteins and other enzymes that may be involved in lignan biosynthesis. We are currently comparing this population of transcripts to those that we found were expressed in sapwood fractions that lack thujaplicin and lignans, to identify a subset of candidate genes.

Together, the foliar gland and heartwood-sapwood transition zone-specific populations of transcripts will provide the core of candidate genes for genotype-phenotype association studies. We have generated 36 transcriptome libraries from foliage of trees with a range of thujone levels that will be used for SNP and indel discovery, and are making similar plans for genes expressed in the sapwood-heartwood transition zone.

Foster et al. 2013. Plant Physiology, 161:1993-2004

Morris P.L., and Stirling R. 2012. Wood Science and Technology. 46:991-1002

Thursday July 25, 11:00 - Mt. Currie South

Sequence capture in lodgepole pine and interior spruce

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Local adaptation is common in widespread conifer species and current reforestation policy reflects this through local seed sourcing and breeding programs. However, as the climate changes local tree populations may become maladapted to their environments. Our goal is to identify the genes responsible for climatic adaptation in western Canada's two most economically important conifers, lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca, P. engelmannnii,* and their hybrids). As the genomes of these species are very large (>20Gb), in the Adaptree Project, we are using sequence capture methods to target our sequencing efforts to regions of interest. To identify these regions, we developed a *de novo* transcriptome for each species and conducted an RNAseq expression study. We retained a single isoform of each gene that was expressed in our RNAseq study, or had gene ontology terms potentially related to climate. We also included candidate adaptive loci identified in previous studies and loci currently being mapped in white spruce (Arborea project). To avoid reduced hybridization efficiency resulting from probes spanning intron/exon boundaries, we aligned the transcripts to the draft white spruce genome (SMarTForests Project) and the draft loblolly pine genome (PineRefSeq Project) and identified these boundaries. We removed repetitive sequences as well as mitochondrial and chloroplast genes to avoid capturing sequences that have many copies within a single cell. We were able to identify 70,834 exons from 28,437 genes in pine (6,732 sequences with unidentified exon boundaries) and 75,799 exons from 35,957 genes in spruce (10,531 sequences with unidentified exon boundaries) for resequencing. We also identified non-coding, non-repetitive sequences from the draft genome of white spruce and from low-coverage whole genome shotgun sequence of lodgepole pine. These putatively neutral target regions will allow us to control for demographic history during our search for loci under selection. Our preliminary test of the NimbleGen sequence capture protocol was run on 12 interior spruce and 12 lodgepole pine. More than 90% of the target sequences had reads aligning to them and 40-60% of the reads were on target, demonstrating that the sequence capture protocol is enriching our libraries for the regions of interest. From these data we were able to identify ~600,000 SNPs in each species. These SNPs will be used for analyzing allele-environment and allele-phenotype associations. We will select ~25k of these SNPs and genotype a further ~5000 individuals per species. The objective of this project is to improve seed-transfer policy in response to climate change by comparing the adaptive genetic portfolio of seedlots from seed orchards and breeding programs to the climatic distribution and landscape genomics of natural populations.

Funding acknowledgement: This research is part of the AdapTree Project, funded by Genome Canada, Genome BC, Alberta Innovates Bio Solutions, the Forest Genetics Council of British Columbia, the BC Ministry of Forests, Lands and Natural Resources Operations, Virginia Tech, the University of British Columbia, and the University of California, Davis.

Thursday July 25, 11:15 - Mt. Currie South

Identification of genes involved in the strobili developmental regulation using an aberrant bisporangiate system of *Pinus tabuliformis* Carr.

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Reproductive control is an important part of the conifer species genetic improvement and seed production process that has a lot of practical experience accumulated; however, there is still a considerable lack of molecular biology research on the development and regulation of conifers male and female cones. Conifer cones are typically of restricted growth and either exclusively pollen-bearing (male) or exclusively ovule-bearing (female), and male flowers are borne in clusters at the base of the twig bud while the female flowers are borne in one conelet at the bud apex. However, several reports indicate that bisporangiate cones (also called bisexual cones or hermaphroditic strobili) occur in gymnosperms which possess both male and female structures, but the cause of this anomaly is not well understood. Chinese pine (*Pinus tabuliformis* Carr.) is a widespread indigenous conifer species and an economically and ecologically important hard pine in northern China. Recurrent bisporangiate cones were detected on six trees in Chinese pine seed orchard and this trait was constant during at least three years. Based on the Roche 454 GS-FLX Titanium pyrosequencing platform, the first *P. tabuliformis* transcriptome from a normalised cDNA library of multiple tissues (xylem, phloem, vascular cambium, needles, cones and strobili) and individuals was sequenced in a full 454 run, producing 911,302 sequencing reads. The high quality overlapping expressed sequence tags (ESTs) were assembled into 46,584 putative transcripts, and based on these information, microarrays were designed. Through the microarray analysis of large scale gene expression profiles between bisporangiate and normal cones, we found that the transcriptome of bisexual cones were undergone a great remodeling compared with the normal male cones and the cones adjacent to them in the same clusters. Interestingly, the correlation analysis of gene expression between different cones showed that the transcriptome of male structures at the below of the bisexual cones are more like female cones, however, male and female functions are not altered in bisporangiate structures. Based on a very strict cut-off criterion as $P < 0.001$ in the t tests and fold change (FC) ≥ 5, 1059 differentially expressed genes were obtained. When a 50 fold change (FC) was used to filter our gene list, 157 genes were obtained, 30 genes of them were upregulated, while the other 127 genes were downregulated. This result indicated that the feminization of male cones is primarily by suppressing the expression of specific genes. Three up-regulated genes and three down-regulated genes with unknown function were chosen from differentially expressed genes to analyze their expression pattern in male and female cones at the different developmental stages. The results show that all these genes have a similar expression in female and male strobilus at early developmental stages and have a relatively stable expression in female cones at different developmental stages, but were drastically changed in male cones at late developmental stages. These results indicate that the disorder of genes expression regulation at early developmental stages in male cones result in the feminization and the ultimately formation of bisexual cones. These genes may play a crucial role in sex determination and developmental process of the male and female cones. This study gain insight on the transcriptome remodeling of aberrant bisporangiate cones, and more than one hundred candidate genes were identified that associated with sex determination and developmental of strobili. These data will facilitate future molecular biology studies on the development of male and female cones of *P. tabulaeformis* and other related species.

Thursday July 25, 11:30 - Mt. Currie South

Resistance of Sitka spruce against white pine weevil: A genomic and histological approach to decipher stone cell development

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The white pine weevil (WPW; *Pissodes strobi*) is a major pest of Sitka spruce (*Picea sitchensis*) and interior spruce (*P. glauca x engelmannii*) in British Columbia and also destroys Norway spruce (*P. abies*) plantations in eastern Canada. Breeding naturally resistant trees has proven to be the most effective control for WPW. High densities of cortical sclereids (stone cells) were found to have the strongest correlation with resistance to WPW among the traits examined in resistant Sitka spruce populations. Stone cells are highly lignified cells, which occur in clusters and are hypothesized to serve as a constitutive physical defense against WPW attack. However, the precise mechanism by which they affect WPW is not currently known, and the genes and mechanisms that control sclereid development are also unknown. Identification of genes, metabolites, and molecular processes involved in the formation of stone cells will lead to a better understanding of natural resistance mechanisms against WPW, and can support breeding for resistance. Building on the genomics resources developed in the Treenomix (http://www.treenomix.ca) and SMarTForests Project (http://www.smartforests.ca), we are using transcriptome and metabolite profiling of developing stone cells isolated via laser-microdissection (LMD), as well as fluorescence and electron microscopy to decipher the mechanisms governing stone cell development in WPW resistant spruce genotypes.

Selection of growth and wood density in radiata pine using a panel of single nucleic polymorphisms associated with candidate genes

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Molecular technique advancement has made it possible to incorporate DNA variation into the design of breeding programs to increase the accuracy of selection. Genomic selection has been proposed as an approach to accelerate plant genetic improvement. It is especially valuable to reduce generation interval in tree breeding. The accuracy of genomic selection heavily depends on the number of single nucleotide polymorphisms (SNPs) used in the development of prediction equations. But with the increase of the number of SNPs included in a genomic selection panel, genotyping cost can be a limiting factor. This may limit the application of genomic selection on a large scale. A SNP panel with a small number of SNPs with acceptable accuracy may be a solution. The objective of this analysis is to examine the possibility of developing an inexpensive SNP panel for marker-assisted selection of growth and wood density in radiata pine.

Sixty-seven SNPs from 47 genes were selected from a SNP discovery resequencing programme. Two breeding populations of the Radiata Pine Breeding Company, one control-pollinated progeny trial and a clonal trial, and their parents were successfully genotyped with these SNPs. Fifty-one SNPs remained after removing SNPs with minor allele frequency less than 0.05. The progeny trial had 1849 individuals from 44 parents and was planted at three sites. The clonal trial had 520 individual genotypes from 63 parents planted at two sites with ten clones per family, six ramets per clone planted in six replicates on each site.

Breeding values of diameter-at-breast-height (DBH) and wood density (WD) were estimated separately within each trial series using an individual tree linear mixed model, implemented with ASREML (Gilmour, Cullis et al. 2009). Effects for site and replicate were fitted as fixed effects and sets-within-replicate effects, additive genetic effects and residual effects were fitted as random effects in the statistical model for both the progeny trial and the clonal trial. Clonal effects were also fitted for the clonal trial. The heritabilities of DBH and WD were 0.15 and 0.52 in the progeny trial and 0.25 and 0.22 in the clonal trial, respectively.

In this analysis the clonal population was used as the calibration population and the progeny population as the validation population. The estimated breeding values (EBVs) were deregressed using reliability and the amount of information available from relatives (Garrick, Taylor et al. 2009). The prediction equations of the 51 SNPs were developed with Bayesian ridge regression (Pérez, Campos et al. 2010) using the deregressed EBVs of the calibration population. Marker-assisted EBVs (MEBVs) were calculated as the summation of the products of the prediction equations and their genotypic values. The accuracy of MEBVs, which was equal to the correlation between deregressed EBVs and marker-assisted EBVs, ranged from 0.60 to 0.66 in the calibration population and ranged from 0.36 to 0.46 in the validation population. The percentage of genetic variation explained by the panel in the validation population ranged from 13% to 21% for DBH and WD. One application of this SNP panel is to conduct pre-selection in the nursery. Seedlings can be screened at large scale in the nursery to select the best individuals for planting in the field.

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Thursday July 25, 1:00 - Mt. Currie South

Genomics-assisted selection for growth and wood traits in white spruce could be a reality in the near future

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Spruces are the most important forest resource for the Canadian forest industry and their natural ranges extend on most of Canada. With sizeable gains having been obtained for growth, spruce breeders are now putting more emphasis on wood quality. Given the time delays and high costs for evaluating these traits, genomics-assisted selection is expected to contribute positively in increasing gains per unit of time. To test the potential of genomic prediction for wood and growth traits in white spruce, a discovery population of about 1700 white spruce trees has been assembled from a 17-year-old progeny test replicated on two sites and comprising 59 full-sib families belonging to two unrelated sublines of a breeding population. Increment cores were collected and wood density and microfibril angle (MFA) were assessed for each tree. Tree height and DBH were also analyzed. A genotyping assay with one SNP per gene locus yielded genotypes for 6900 genes segregating in the population.

First, SNP-by-SNP association tests were carried out using a mixed-linear model approach and indicated that between 500 and 700 SNPs were significantly associated ($P \leq 0.05$) with single traits related to wood quality or growth before correction for false-discovery rate. Next, genomic selection models for growth and wood traits were built using either all available SNPs or only those that were significant at $P \leq 0.05$ after association tests. The predictive value of the models was estimated by cross-validation with within and between sublines sampling and assuming same site or different sites. The accuracy of genomic prediction for within-subline selection varied from 0.67 to 0.84 depending of the trait when considering all SNPs, and from 0.61 to 0.78 when using only the subset of significant SNPs. When predicting genomic values across sublines, the accuracy of prediction of genomic values was much lower, except when using only the significant SNPs (for instance 0.58 and 0.49 for wood density and MFA, respectively). These results suggest that significant gene SNPs cover a major part of the QTLs involved and that shared ancestry might be present between sublines. When cross-validation was made across test sites, the accuracy estimates were also in the same range, reflecting the small genotype-by-environment interactions usually seen with eastern white spruce. Gains per unit of time were also estimated, and their amplitude suggests that economically useful gains could be obtained if selection delays are reduced. For instance, when assuming a breeding cycle of 30 years for the traditional approach versus 10 years when using genomic selection, everything else being equal, the gains per unit of time for a selection intensity of 5% obtained from genomic selection where 1.7 to 2.6 times higher that those from traditional selection. These positive results indicate that genomics-assisted selection could likely be implemented with success in white spruce.

Thursday July 25, 1:30 - Mt. Currie South

Mining spruce genomes for defence genes and functions

The SMartForests Project Consortium
Presentation by Jörg Bohlmann[1,2]
[1]Michael Smith Laboratories, Department of Forest and Conservation Sciences, Department of Botany, University of British Columbia, Vancouver, Canada, [2]For a complete list of contributing team members and affiliations see http://www.smartforests.ca
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Draft genome sequence assemblies of two spruce species, white spruce (Birol et al., 2013, *Bioinformatics* 29: 1492-1497) and Norway spruce (Nystedt et al., 2013, *Nature* 497: 579-584), have recently been reported. Birol et al. reported innovative strategies for building sequence assemblies of a very large (~ 20 gigabase) conifer genome sequence and provided insights into the tree’s gene space. Biological insights from the Norway spruce genome, for example insights into the nature of large introns, repeat elements, interpretation of processes that contributed to the very large genome size, and comparison with low coverage genome sequences of other gymnosperm plants are reported by Nystedt et al.. Both studies used the Sitka spruce full length cDNA reference data set (Ralph et al., 2008, *BMC Genomics* 9: 484) to assess the quality and completeness of gene predictions, with conservative estimates suggesting the number of functional protein coding spruce genes to be in the order of 30,000 to 60,000. Here, I will present a summary of the status and challenges of the spruce genome assemblies and initial genome annotations. Other presentations by members of the SMarTForests Project (e.g., see presentations by Drs. Beaulieu, Bousquet, Sexton, and Whitehill) will address aspects of the integration of the genome sequence with genetic mapping as well as application of spruce genomics tools in spruce tree breeding. In my talk I will add to these presentations with information from the identification of genes and gene functions involved in spruce resistance against spruce budworm or white pine weevil. This will include the characterization of diterpene oleoresin defenses as well as a newly discovered glucosyl hydrolase gene function that is important for spruce budworm resistance.

Acknowledgement: This work is part of the SMarTForests Project (http://www.smartforests.ca) funded by Genome Canada, Genome British Columbia, Genome Quebec, and Genome Alberta; funding was also received from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the British Columbia Ministry of Forests Lands and Natural Resource Operations.

Thursday July 25, 2:00 - Mt. Currie South

POPCAN: linking trait variation with whole genome variation in *Populus* to facilitate accelerated tree improvement.

Jürgen Ehlting[1], Shawn Mansfield[2], Robert Guy[2], Yousry El-Kassaby[2], Quentin Cronk[3], Peter Constabel[1], Richard Hamelin[2], Carl J. Douglas[3]
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Black cottonwood and balsam poplar are two closely related *Populus* species with large north-south and east-west ranges in western and northern North America. *P. trichocarpa* and *P. balsamifera*, along with their hybrids, are well suited for high yielding biomass plantations. We are investigating the reservoirs of natural genotypic and phenotypic variation in these species using range-wide collections assembled by the BC Ministry of Forests and Agriculture and the Agri-food Canada Agroforestry Development Centre. The primary aim is to identify allelic variants that could be used for accelerated domestication of poplars including their potential use as bioenergy feedstock. We carried out extensive population wide transcriptome and whole genome re-sequencing of *P. trichocarpa* individuals to identify SNPs that were used to develop a 34K SNP genotyping array covering 3,500 candidate genes, and SNP genotypes were obtained for 450 trees grown in common gardens. Next, in order to reveal the full extent of allelic variation across the genome, we re-sequenced the genomes of approximately 300 accessions of each species from across their ranges. In parallel, the same genotypes were subjected to extensive phenotyping for a suite of wood chemistry and ultrastructure traits and for multiple traits related to growth, photosynthesis, phenology, foliar chemistry, and pathogen susceptibility. These large phenotype and genotype datasets have allowed to us to assess population structure, identify candidate genes related to adaptation, and to use association genetics to identify sets of SNP markers with potential utility in marker-assisted breeding and genomic selection for population improvement.

Thursday July 25, 2:30 - Mt. Currie South

Protecting Canada's forests: The TAIGA project

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Forest health is under a continuous threat from attacks by exotic invasive pathogens, apart from a combination of climate change and anthropogenic mismanagement. A pro-active strategy of disease prevention can help avoid new outbreaks and ensure continued forest health. This approach for forest disease prevention must involve early detection and identification of the causal organism, followed by mitigation measures and pathogen surveillance on local, regional, national and global scales. Additionally, identifying the infection source is essential for discovering the dissemination pathways and preventing further introductions of potential emerging diseases. Canada's regulatory system requires that all imported plant material and wood products be inspected. Plant health monitoring and pathogen detection is widely based on visual inspections. A significant limitation is a particularly high rate of false negatives, as asymptomatic plants with latent infections cannot be detected. Our Genome Canada funded TAIGA (Tree Aggressor Identification using Genomics Approaches) project proposes to address many of these challenges in pathogen detection and identification by harnessing the power of genomics. A new generation of genomics-enhanced pathogen tools will be developed and deployed to detect and monitor different classes of pathogens. In addition to a robust and specific detection of known pathogens, our method will allow for the discovery of lesser known and latent fungal pathogens. An inbuilt redundancy mechanism in the assays will enable a higher confidence in the test results. With a minimal turn-around time for establishing a pathogen profile, TAIGA project will serve a valuable role in keeping the Canadian forests healthy.

Genetic Conservation – Session 11

Thursday July 25, 10:30 - Cheakamus

Genetic impacts of forest management practices in Southeast Asian forests

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The major threat to genetic viability of the Southeast Asian forest species is commercial logging and fragmentation to a lesser extent. Species' vulnerability to the threat of genetic viability posed by commercial logging is highly correlated with its abundance in a particular forest management unit. Tree density for the species can be a useful indicator in reflecting the risk of genetic viability rather the overall disturbance level based on reduction in basal area of all trees. Mating and gene flow patterns tend to be similar in species with similar ecological characteristics. Therefore, information on the mating system, gene flow and inbreeding depression relating to major species could be relevant to closely related species, and used to determine the minimum number of mother trees to be retained for successful breeding. Knowledge of the biological attributes of species including the main pollinators, flowering phenology and synchrony of individual species can be used to develop field guides in harvesting operations to ensure genetic quality of residuals left behind in the post-harvest stands. There is a general lack of understanding on structure of genetic variation and on the evolution of adaptability of the affected populations. Though genetic diversity of a number of tropical tree species has been determined, the critical levels of the genetic parameters that render a population more susceptible to extinction are known for a few species only. DNA-based systems for the analysis of gene expression for the understanding of gene expression in different tissues and in response to biotic and abiotic stresses have not been deployed in genetic viability studies of tropical forest species. Genetic mapping of quantitative trait loci (QTL) will enable rapid assessment of ecosystem integrity for sustainable management of ecosystems.

Thursday July 25, 11:30 - Cheakamus

Genetic impacts of forest management practices in North American boreal and temperate forests

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Genetic diversity is the basis of all biodiversity because it provides the raw material for survival, adaptation and evolution of all organisms, especially under changed environment, climate and disease conditions. Conservation of genetic diversity in forest trees is especially critical for the stability and functioning of forest ecosystems because forest trees are normally the keystone species of many ecosystems, and many faunal and floral associations depend on their existence. Therefore, genetic diversity of forest trees could be viewed as the foundation of ecological and economic forest sustainability and ecosystem stability. Forest management practices based on partial and clearcuts followed by natural or artificial regeneration systems can impact genetic diversity, population structure, mating system, gene flow, and effective population size. Thus, adaptation, fitness, survival and productivity of forest trees may be adversely affected. Therefore, knowledge of the genetic impacts of forest management practices is crucial for the conservation and management of forest genetic resources.

North America has about 17% of the world's forest resources as boreal and temperate forests. The boreal forest region is the largest of all forest regions in North America extending from Alaska to Newfoundland. Boreal forests are usually managed by clearcut harvesting followed by natural and/or artificial regeneration. Forest trees in other North American temperate forest regions are managed by both clearcut and partial-cut harvesting followed by natural and artificial regeneration systems. Several forms of partial-cut systems, such as high-grading, shelterwood, seed tree, patch cut and group selection, are implemented.

Of these, shelterwood and seed tree methods have been commonly used. The harvesting and tree retention intensities in partial-cut systems vary from species to species and regions to regions.

I will discuss synthesis of the results and conclusions from the studies conducted on genetic impacts of forest management practices on boreal and temperate forest trees of North America. The extent of genetic impacts depends upon the population characteristics and reproductive biology of the species and the harvesting and reforestation practices used. For example, early successional, widely distributed conifer species with serotinous or semiserotinous cones and good seed bank on the ground, such as black spruce (*Picea mariana*) and lodepole pine (*Pinus contorta*), will likely have very little or no genetic effects of forest harvesting and reforestation practices. On the other hand, late successional or species that do not regenerate well after clearcuts or do not have good bank of viable seeds on the ground, such as white spruce (*Picea glauca*) and eastern white pine (*Pinus strobus*), can have significant genetic impacts of forest harvesting and reforestation practices. Therefore, the genetic impacts of forest management practices cannot be generalized to all boreal and temperate conifer species. This is evident from the mixed patterns observed so far. Silvicultural practices should take the population and reproductive biology and silvics of a species into account to ensure that the genetic diversity and structure and evolutionary processes are maintained in forest populations. The predominantly outcrossing mating system, severe inbreeding depression and long distance pollen dispersal in North American conifers can help buffer the negative genetic impacts of harvesting, such as bottleneck, genetic drift and inbreeding. More than one molecular marker type should be used for assessing genetic impacts of forest management practices as different markers may provide different results. Allelic diversity measures are more suitable than expected heterozygosity (H_e) in assessing the genetic impacts of forest management practices because H_e is not that sensitive to bottlenecks and perturbations in populations.

Thursday July 25, 11:15 - Cheakamus

High genetic diversity of a demographically vulnerable rainforest tree in an urbanized landscape

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Over the last 150 years, Singapore's primary forest has been reduced to less than 0.2% of its previous area, resulting in extinctions of native flora and fauna and leading to an expectation of genetic erosion in primary forest species. We surveyed >95% of the remaining primary forest in Singapore and used eight highly polymorphic microsatellite loci to assess genetic diversity indices of 179 adults (>30 cm dbh), 193 saplings (>1 yr), and 1,822 seedlings (<1 yr) of the canopy tree *Koompassia malaccensis* (Fabaceae). We tested the hypotheses that (1) the *K. malaccensis* population underwent a genetic bottleneck, and (2) recruits may exhibit genetic erosion and inbreeding. Contrary to expectations, we detected neither a population bottleneck nor a reduction in effective population size, and high genetic diversity in all three age classes. Genetic diversity indices among age classes were not significantly different; we detected overall high heterozygosity (mean $H_e > 0.850$), high allelic richness (mean $R = 19.7$), a large proportion of rare alleles (30.1%), and a low Inbreeding Co-efficient (mean $F_{IS} = 0.068$). However, long-lived species such as trees might need several generations for detrimental genetic effects of deforestation and/or fragmentation to accumulate. The spatial genetic structure (SGS) showed higher relatedness of saplings and seedlings compared to the adult cohort, as well as increasing SGS intensity in younger cohorts. In addition, demographic factors for this population (i.e. <200 adult *K. malaccensis* trees) are a cause for concern, as rare alleles could easily be lost due to stochastic factors. The high outcrossing rate (calculated from seedlings) ($t_m = 0.924$) may be an underlying factor for maintenance of genetic diversity, and supports the hypothesis that effective pollination may increase resilience to acute habitat loss.

Genetic diversity and differentiation of Indian (*Azadirachta indica* A. Juss. var. *indica*) and Thai neem (*Azadirachta indica* A. Juss. var. *siamensis*) varieties

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Information about genetic diversity within and among populations is important prerequisite to develop an efficient conservation strategy for a target species. In most of the tropical countries, fragmentation of forest areas is growing rapidly, which is threatening the maintenance of genetic diversity and fitness of ecologically and economically important forest trees. Neem (*Azadirrachta indica*) is one of the popular multipurpose trees in tropical areas. Genetic diversity and differentiation of 17 accessions of Indian neem and seven accessions of Thai neem varieties were investigated covering south Asia, south-east Asia and Africa by employing eight moderate to highly polymorphic microsatellite markers. Overall genetic diversity was higher in Indian neem than in Thai neem variety. Genetic differentiation measured by F_{ST} among 24 neem accessions across the two varieties was 15%, while it was almost 20% between the two varieties. Significant positive correlation between pairwise genetic and geographic distances was observed. Several unique alleles for Indian and Thai neem varieties were detected. Both Bayesian and non-Bayesian cluster analyses grouped the accessions from Thai and Indian neem varieties in separated clusters. The data presented in this study have implications for the conservation and management of genetic resources of both neem varieties.

Ponderosa pine evolutionary history and genetic variation: Results from range-wide mitochondrial and nuclear marker studies

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Ponderosa pine (*Pinus ponderosa*) is the most broadly distributed pine species of the Western Hemisphere, where it has considerable ecological and economic importance. It exhibits complicated patterns of morphological and genetic variation, suggesting it may be in the early stages of differentiation into multiple species. These patterns have created confusion about evolutionary relationships within the ponderosa pine complex, while the near absence of paleoecological data for the species during the Pleistocene obscures important phylogeographic processes that have influenced the evolutionary history of the complex. To clarify ponderosa pine evolutionary history and phylogeography, we amplified the highly polymorphic mitochondrial DNA second intron minisatellite region in 3,100 trees representing 104 populations across the range of the species. We estimated population-level haplotype diversity and determined diversity partitioning among varieties, races and populations. After sequencing all length variants and aligning sequences of minisatellite repeat motifs, we evaluated evolutionary relationships among haplotypes. The results suggest a complex phylogeographic history not revealed by other genetic and morphological data, or by the sparse paleoecological record. The geographical structuring of the 10 haplotypes corresponded with division between Pacific and Rocky Mountain varieties. Pacific haplotypes clustered with high bootstrap support, and appear to have descended from Rocky Mountain haplotypes. These results appear consistent with long-term divergence between the Pacific and Rocky Mountain varieties, along with more recent divergences not well-associated with race. Pleistocene

refugia may have existed in areas of high haplotype diversity (the southern Sierra Nevada, northwestern California, and southern Nevada), as well as in the Great Basin, the Southwestern United States/northern Mexico, and the High Plains. Separately, we used six nuclear microsatellite markers and 19 allozymes to assess patterns of genetic variation across the range of ponderosa pine. Preliminary results show low levels of inbreeding within populations and moderate differentiation among populations. When individual trees are separated based on mitochondrial DNA haplotypes, those in the core of the species range have somewhat higher nuclear marker genetic variation. The results of both the mtDNA and nuclear marker studies should assist in management decision-making and conservation planning relating to ponderosa pine subordinate taxa and populations in the face of multiple threats, including those associated with climate change. For example, each haplotype may represent an evolutionarily distinct unit that may respond differently to climate change because of differences in adaptation to climatic conditions. Measures such as seed archiving, silvicultural treatments, and prescribed fires should take into consideration the presence of rare haplotypes or nuclear alleles and of high haplotype or nuclear diversity.

Plenary Session 12

Thursday July 25, 3:20 – Mt. Currie South

Genetic changes accompanying post glacial colonization in outcrossing perennials: Lessons from *Pinus* and *Arabidopsis*

Outi A. Savolainen
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All northern plants have undergone considerable evolution during colonization, partly due to demographic effects such as bottlenecks, partly due to selection to new conditions. The consequences of this process are compared between *Pinus sylvestris* (very large populations in Eurasia) and *Arabidopsis lyrata* (a short-lived perennial with a scattered distribution in Europe and in North America). In both cases, local populations show evidence of local adaptation, and there is high phenotypic divergence for example in photoperiodic reactions and timing of life history events. The underlying genetics will be compared in the two cases.

Thursday July 25, 4:00 - Mt. Currie South

Back to the future: Evolutionary genomics of hybridization

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One of the longest running debates in evolutionary biology concerns the role of hybridization in evolution. During the previous century, many botanists argued that new gene combinations generated by hybridization contributed importantly to adaptive evolution and speciation. In contrast, others accorded little evolutionary significance to hybridization, viewing it as a primarily local phenomenon with only transient effects, a kind of "evolutionary noise". These views became more nuanced in recent decades as theoretical and empirical evidence showed that hybridization could have a broad range of evolutionary consequences, including the persistence of hybrid zones, the reinforcement or breakdown of reproductive barriers, the origin and transfer of adaptations, and the birth of new hybrid lineages. However, the frequency and importance of these different outcomes remains controversial.

Here, I will discuss what we have learned about the role of hybridization in evolution from genome-scale analyses, focusing on studies of sunflower (*Helianthus*) species. I will (1) report on patterns of genomic divergence between populations or species that vary with respect to levels of gene flow, and discuss the causes of so-called "islands of speciation; (2) describe a likely genomic signature of "speciation by reinforcement" that might be might be useful for establishing the frequency of this evolutionary mechanism; (3) present the results of a long term selection experiment that validates a textbook example of adaptive introgression and identifies the traits and candidate genes targeted by selection; and (4) show that hybridization may be incidental to adaptive evolution in newly arisen hybrid species. I will conclude by evaluating the overall importance of these different consequences of hybridization in the plant and animal kingdom and discuss how their evolutionary role may depend, in part, on the genetic architecture of adaptation.

Posters

Located in Cheakamus Room

Adaptation and Climate Change

P.1

The role of symbiotic association between mycorrhizal fungi (AMF) and willows on copper uptake and storage: A hydraulic perspective

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A vast concern is emerging worldwide related to the management and restoration of natural and industrial sites affected by the accumulation of complex mixtures of trace-metals, toxic inorganic and organic compounds through mining, agricultural and industrial activities. Soil and water remediation using plants and their associated microbes has become a lower cost, versatile and non-invasive alternative for moderated decontamination of those sites, in which shrubs of the genus *Salix* (willows) are widely used. In the case of plants growing under trace-metal polluted soils, different plant mechanisms have been described for overcoming stress when metals are uptaked and accumulated in plant tissues, including the production of intra-cellular metal-binding compounds and their storage into the vacuole or apoplast. In willows growing in copper (Cu) polluted soils, accumulation of Cu has been observed in various tissues, with roots accumulating the most. In order for plants to maintain their cellular homeostasis while accumulating trace-metals, changes in their water relations must occur, which should have an impact on their Aquaporin (AQP) activity. In this study, we investigated the effect of symbiotic associations between mycorrhizal fungi (AMF) on the uptake and storage of Cu and on hydraulic traits and AQP expression patterns in roots of *Salix purpurea* (Fish creek) saplings. Rooted saplings that were either inoculated or not with *Rhizophagus irregularis* were grown under three Cu treatments: no-Cu (Control), low (66.7 ppm of free Cu) and mild concentrations of Cu (156.78 ppm of free Cu). We found that root hydraulic conductance (*Lp*) of small roots was gradually reduced in plants exposed to Cu when compared to controls in non-innoculated plants. In contrast, plants inoculated with the fungus showed a steady *Lp* within the different levels of Cu, suggesting that the symbiotic relationships might be beneficial for maintaining root homeostasis during trace-metal stress. *Lp* was reduced when exposed to an AQP inhibitor in all cases (up to 92%), showing a physiological role of AQPs in root hydraulics. A phylogenetic analysis of functional *Salix* AQPs was conducted, comparing them with their closest related heterologous genes from *Populus trichocarpa* for further classification within the subfamilies of the Major Intrinsic Proteins. In addition, *AQPs* belonging to two subfamilies, Plasma Membrane Intrinsic Proteins (PIPs) and Tonoplast Intrinsic Proteins (TIPs), were transcriptionally profiled in roots to identify *AQPs* candidates involved in homeostasis balance in *Salix* during copper accumulation.

P.2

Genomic architecture of apical bud formation in *Picea glauca* Moench. Voss: An eQTL mapping approach

Sylvie Carles[1, 2], Walid El Kayal[3], Betty Pelgas[1,2], Elie Raherison[2], Sébastien Caron[2], Julien Prunier[2], Jean Bousquet[2], John MacKay[2], Janice E. K. Cooke[3], Nathalie Isabel[1, 2]
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Apical bud formation is an important adaptive trait which permits conifer trees to synchronize their phenology to seasonal changes and may determine the fitness of individuals under variable climatic conditions. Recently, 4,500 genes that were differentially expressed during bud formation were identified by comparing gene expression profiles of unrelated individuals of white spruce (*Picea glauca*) (Kayal et al. 2011). Among these genes, 108 were differentially expressed exclusively in developing buds and were expressed at higher levels in buds than in other tissues (Kayal et al. 2011). Several of these genes fell within robust QTL clusters obtained for bud burst and bud set across pedigrees, years, and environments (Pelgas et al. 2011). We have undertaken an eQTL mapping approach that integrates expression profiling experiments and our most recent genetic maps to gain a deeper understanding on how genomic architecture influences bud phenology and related regulatory pathways. First, we isolated total RNA from terminal spruce shoots for two biological replicates at two developmental stages: 1) first sign of bud formation, and 2) midpoint of bud development. Samples were collected from 145 full sib progeny that were a subset of cross C94-1-2516 (Pavy et al. 2012; Pelgas et al. 2011). Transcript levels were quantified using the spruce large-scale oligonucleotide microarray which contained 25,045 oligonucleotide probes representing 23,853 unique genes (Raherison *et al.* 2012). An analysis of variance (ANOVA) of the normalized expression data identified 10,985 gene-probes with significantly different expression levels between the two developmental stages ($P \leq 0.05$). In parallel, we genotyped 1,800 full sib progeny for 16,720 single nucleotide polymorphisms (SNPs, Arborea PgLM3 array) with an Illumina Infinium bead array and assembled these data into two parental genetic maps (Pelgas et al. in preparation). We are currently using these genetic maps to screen the expression profiling data of a subset of the mapped gene-probes from the spruce oligonucleotide microarray (8,839, of the 25,045 gene-probes), to identify expression quantitative loci (eQTL) using a composite interval mapping approach (Michaelson et al. 2009). Ultimately, we hope to use this integrated QTL mapping approach to identify genes and/or network of genes directly (*cis*-regulatory genes) or indirectly (*trans*-regulatory genes) involved in bud set.

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P.3

Utilizing the intra-specific variation in growth to develop management guidelines for Douglas-fir under climate change: A proposed study in Austria and Bavaria

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Climate change poses serious challenges to sustainable management of forests throughout the world. Planting of alternative and also non-native tree species or different provenances better adapted or having a higher potential for adaptation to future climate conditions has been discussed as an important silvicultural measure to adapt forests to climate change. Douglas-fir (*Pseudotsuga menziesii var.* (Mirb.) Franco) is considered as one of the most promising species in Austria and elsewhere, because it exhibits superior productivity, high wood quality and low risks for storm damage. Particularly it grows well under warm and dry conditions where currently native conifers such as *Picea abies* are considered as vulnerable to an array of insect and desease organisms. Douglas-fir is known to have a wide natural range and occurs under varied environmental condition. Therefore a detailed study on provenance specific growth performance in response to climate is crucial. In the present study, we will utilize 63 Douglas-fir provenance trials throughout Austria and more than 20 trials from Bavaria, Germany to understand the intraspecific variations in growth in response to climate.

The aims of the project are:

- To understand the interaction of intra specific genetic variation and climate on growth of Douglas-fir
- To identify the most important climatic factors that pose risk for growth of Douglas-fir in the study area
- To integrate Douglas-fir provenances into the dynamic forest ecosystem model PICUS in order to assess forest management strategies for different sites and provenances

The methods include:

- Multivariate analyses to determine the climate related growth and stress factors that constrain the growth of Douglas-fir in the study area
- Developing provenance specific transfer and response function and ultimately combining these two functions into a *Universal response function*.
- Parameterization and evaluation of the forest ecosystem model PICUS (e.g. Lexer and Hönninger 2001, Seidl et al., 2005) with several Douglas-fir provenances in order to simulate and assess forest management strategies for different sites and provenances.

Preliminary analysis from 33 provenance trials in summer-dry eastern regions of Austria reveals strong correlations of provenance specific growth of diameter at breast height (dbh) to climate of the site where introduced. The expected project outputs (e.g. suitability maps, guidelines etc.) will help to define management guidelines and provenance recommendations for Douglas-fir as an alternative conifer species in Austria.

P.4

Genetic resource management and climate change: Growing healthy forests for the future

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Providing seed for operational reforestation and restoration has long been the principal focus of the U.S. Forest Service's Genetic Resource Management Program. Although this work will continue into the future, climate change predictions will require changes in the ways these needs are met.

The guiding principle for managing the genetic resources of National Forests has been through the use of local seed sources in reforestation and restoration. The advent of a rapidly changing climate, however, means that a new paradigm will be required to maintain healthy and productive vegetation on National Forests and to preserve at-risk species and populations. At a minimum, the current practice of relying on seed sources that were best suited to the past climate will need to shift to allow consideration of the source, or sources, of seed that will be best suited to predict future climates. In many cases, species and seed sources that may be optimal under climate change scenarios have not received adequate research or management attention and thus lack basic genetic information as well as sufficient representation in forest seedbanks. More aggressive gene conservation programs, especially ex situ seed collection, will also be needed for species and populations most vulnerable to climate change impacts.

At present, there is no generally applicable national guidance for incorporating climate change impacts into the management of National Forest genetic resources. In the spring of 2010, Forest Service and university geneticists convened to share background information and develop consensus for revising National Forests System genetic resource management guidelines. The goals of the meeting were to 1/ provide information on climate change scenarios and potential effects on vegetation and forest genetic resources, 2/ facilitate the interaction and exchange between climate scientists and geneticists to develop strategies for responding to climate change in Forest Service genetic resource management programs, 3/ identify genetic options for responding to climate change and its effects on vegetation and genetic resources, with an emphasis on the next 5-10 years, 4/ identify gaps related to information, resources, research, and tools needed to manage effectively within a changing climate. A whitepaper on key genetic issues, guidelines, and program gaps/needs in light of climate change has been written and published.

P.5

Inferring parental genetic effects and genetic control of trembling aspen (*Populus tremuloides* Michx.) for growth and adaptive traits in a hybrid population under a warming climate

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Although parental genetic effects and their temporal variation in plants were recognized for long time, their ecological and evolutionary roles remain unclear for forest tree species in terms of early growth, natural and artificial selection responses under rapid changing climates. Recent studies underscore an accelerating mortality of *Populus tremuloides* natural stands that are widely- distributed in North America with high potentials in biomass and biofuel production as well as various ecological values such as carbon sequestration and animal shelters.

Here, we evaluated the maternal and paternal additive genetic effects of *Populus tremuloides* in growth and adaptive traits based on progeny trials with factorial mating designs, within which ten common garden trials were established with 58 female and 64 male half-sib families as well as 100 full-sib families. Ratios of additive genetic variances for both parental sources were compared within sites and the age effects of these ratios were also investigated. General combining ability and specific combining ability, narrow-sense heritability, and genetic correlations of these traits were estimated for single site and combined age groups of trees.

We found discernible decreasing trend of inferred maternal genetic effect of tree height and fall senescence by ages while the ratios of paternal effect maintained or rose; the maternal genetic effect of spring bud break had an opposite increasing trend during developmental stages. The varying weights of maternal effect were revealed by the proportions of parental variances in multiple cohorts. Within the same cohort, site effect was significant for tree height and adaptive traits. Dominance genetic effect decreased when tree grew older. Also high additive genetic correlation existed between height growth and fall adaptive trait.

The diminishing trend of inferred maternal genetic effect during juvenile tree growth had important values for predicting the tree performances under natural and artificial selection. For such a hybrid population, decreasing maternal genetic effect combing with pollen flow from the southern population would potentially raise frost risks because of the delayed the senescence timing and extended growing season length.

P.6

Predicting the risk of cedar leaf blight (*Didymascella thujina*) in British Columbia under future climate change

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Cedar leaf blight (*Didymascella thujina*) is considered to be the most important disease of western redcedar in British Columbia. The disease is most prevalent in warm-moist coastal low-elevation environments causing mortality among seedlings and significant loss of incremental growth and branch death among mature trees. In this study we used a principle component regression model to spatially project the disease risk under observed climate (2003 to 2008) and multiple future climate scenarios for the 2020s, 2050s, and 2080s. We found that while the majority of future climate scenarios predicted coastal environments will continue to favour occurrence of the disease, intensity is predicted to decrease towards the 2080s. Projected reductions of available summer climate moisture (cumulative precipitation – potential evapotranspiration), corresponding to the time of ascospore discharge and germination, contribute significantly to this finding. Disease intensity is however, projected to increase under moderate temperature and precipitation increases for the 2020s. We therefore recommend current reforestation efforts deploy disease resistant western redcedar seedlots in high risk environments common to hypermaritime coastal regions such as Haida Gwaii and northern Maritime, to avoid significant mortality and growth reduction.

P.7

Common garden experiments in contrasting climates reveal adaptive variation in growth, phenology and physiology of *Alnus rubra*

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To explore adaptive variation and genotype × environment interactions in red alder (*Alnus rubra*), we examined the pattern and range of variation in growth, phenological and physiological traits among 59 families of red alder. We related this variation to the climates of family origin to identify the climatic variables associated with adaptive variation. Red alder families from six regions in coastal BC were grown in common garden experiments at two sites with contrasting climates. We found significant variation among regions in growth, canopy cover, cold hardiness and nitrogen nutrition of red alder families. Differences in continentality and available moisture of the climate of origin explained most of the among-family variation in phenology and cold hardiness, whereas temperature and growing season length was associated with among-family differences in cold hardiness and growth. Red alder families from the north coast had earlier bud burst at the southern test site, and less autumn canopy cover and higher nitrogen fixation rates at both test sites. A trade-off between growth and cold hardiness was clearly evident. Families vary in the degree of phenotypic plasticity; however, most families are relatively tightly adapted to their climate of origin and may perform sub-optimally in contrasting climates.

P.8

Risk and benefit analysis of intra-specific assisted migration of white spruce (*Picea glauca* [Moench] Voss) in Ontario

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The 410-series of white spruce range-wide provenance tests, cooperatively established by the Canadian Forest Service and the Ontario Ministry of Natural Resources in the 1970s – 1980s, provided a good source of information for assessing risk and benefit associated with intra-specific assisted migration (ISAM) with the species. The 410-series of experiments included 245 range-wide provenances of white spruce at 16 Ontario field sites, representing the species' growing environments in Ontario. Trial measurement data at ages of 19 – 28 years (in 2001) were analyzed for adaptation (indicated by tree survival) and growth potential (height, DBH and volume). Results indicated that while most white spruce provenances survived reasonably well across the 16 Ontario sites, those performing better were usually from milder environments (i.e., lower latitude), especially those from south-central Ontario and southern Quebec. Southern provenances were more responsive to improving thermo-conditions of planting sites and were significantly superior to northern provenances at warmer sites. Southern provenances performed better or equivalent to local provenances at northern Ontario

sites under the climate of past 30 – 40 years. In light of climate change, ISAM seemed to be a good strategy to enhance genetic diversity and climatic adaptation of white spruce in northern Ontario, which may bring about genetic gain in growth equivalent to that achievable from Ontario's first generation tree improvement programs with boreal conifer species. Future work is to re-measure the 410-series trials and determine the safe distances of ISAM by incorporating data from both long-term provenance trials and short-term farm field tests with the assistance of climate-based software like Focal Point Seed Zones.

P.9

Toward more resilient forests and ecosystems: BC MFLNRO forest stewardship action plan for climate change adaptation

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Adapting BC's forest practices to a changing climate is both an immediate imperative and long term proposition that must begin now. In February 2012, the BC Ministry of Forests, Lands, and Natural Resource Operations released a Forest Stewardship Action Plan for Climate Change Adaptation. This Action Plan signals an important new policy direction for climate change adaptation within the sphere of forest management in British Columbia. It identifies goals, objectives and initial actions the ministry will take between now and 2017 to adapt BC's forest management framework to a changing climate. This is the first step in a broader adaptation strategy for the natural resource sector that will grow and evolve as we move forward.

The Forest Stewardship Action Plan aims to reduce risks, capture opportunities, and improve the ability of BC forests to withstand, recover from, and adapt to the full amplitude of climate change and variability. The plan outlines actions underway and planned to adjust BC's existing forest management framework to foster resilient forests and improve the sustainability of BC forests, communities and industries.

The ministry's Climate Change Adaptation Team sought input from a broad array of partners, including e.g. industry, government, and community forest managers. The most promising opportunities were identified through four regional workshops in Creston, Kamloops, Nanaimo and Prince George.

Actions were identified under three goals: 1) Foster Resilient Forests, 2) Maintain Future Options and Benefits, and 3) Build Adaptive Capacity. Actions to foster resilient forests will also enhance other values such as water quality and wildlife. For more information, visit Adaptation Action Plan (http://www.for.gov.bc.ca/het/climate/index.htm); and, the new BC MFLNRO Adaptation Knowledge and Tools web site (https://www.for.gov.bc.ca/het/climate/knowledge/index.htm).

Executive Sponsors: Dave Peterson, BC MFLNRO A/Deputy Minister, Chief Forester and Tom Ethier BC MFLNRO ADM, Resource Stewardship Division.

Climate Change Steering Committee: Tom Ethier (chair), Rory Annett, Brian Barber (former), Normand Bilodeau, (CAS rep), Alec Dale, Glen Davidson, (TOPFND Rep), Paul Knowles, Lynn Kriwoken, Allan Lidstone, Madeline Maley, Leslie McAuley, Diane Nicholls, Albert Nussbaum (former), Jim Sutherland (former), (IROD Rep), Andrew Wilson.

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P.10

Tree ring analysis indicates genetic component in drought response of Douglas-fir

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Douglas-fir is among the most important economical and ecological conifer species in western North America. These functions may, however, be jeopardized by recent and projected warming. The expected increase in the frequency and severity of drought events may exceed the adaptive capacity of local populations. To mitigate the negative effects of an expected increase in drought, the transfer of tolerant seed sources has been proposed. We used a dendro-ecological approach to identify drought tolerant genotypes using samples from a provenance trial in British Columbia.

The study site, located approximately 60 km east of Vancouver, was hit by a severe drought event in the summer of 1985. We selected two provenances from British Columbia, one provenance from Washington, and two provenances from Oregon according to latitudinal, altitudinal and summer drought gradients. A total of 304 increment cores on 76 trees were sampled from a completely randomized block design with four replications. As a measure of drought tolerance, we used growth depression and subsequent post-drought recovery as well as correlation coefficients of annual growth to summer temperature and precipitation.

The drought event of 1985 caused reductions in increment ranging between 40 and 60 %, as compared to a two year pre-drought reference period. Counterintuitively, we found that a cold, high-elevation provenance from Washington was least affected by this drought event. The second least affected provenance was a source from southern Oregon, which also showed the fastest post-drought recovery. A provenance from the wet area of northern Vancouver Island and a provenance from northern Oregon were the most highly affected, as indicated by reduced increments for several years. These results were mirrored by the climate-growth correlations. The southern Oregon and the high elevation source from Washington exhibited lower correlations with summer precipitation and temperature. In contrast, the provenances from British Columbia and the source from northern Oregon showed moderate correlations with these two variables.

We could not fully verify that provenances from the southern coastal range of Douglas-fir are more drought tolerant than provenances from the north, as we have found a northern Oregon provenance strongly affected by drought and a high elevation seed source from Washington with superior drought tolerance. Both drought-tolerant provenances were among the below-average performers in the studied trial, with the southern Oregon source being the least productive. The provenance from Washington might, however, represent a practical compromise between risk avoidance and productivity.

P.11

A proposal to explore responses of Douglas-fir populations to current and future climates using data from provenance tests established worldwide

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Douglas-fir is a valuable tree species that is grown in temperate regions around the world. A large number of provenance tests have been established worldwide with the primary objective of identifying those provenances that do well in a particular region. With increased interest in climate change, however, researchers are revisiting questions of appropriate provenances and are beginning to take a fresh look at provenance test data to explore the relationship between population performance and climate. With this in mind, we began gathering Douglas-fir provenance test data from around the world with the goal of developing response functions to predict the performance of populations given information about the climate of seed sources and planting locations. Response functions will be used to predict productivity of Douglas-fir stands in current and future climates, and to evaluate management options for maintaining or enhancing productivity by choosing populations appropriate to future climates. We began by putting together a list of people and organizations that might currently be responsible for Douglas-fir provenance tests, particularly those established in the 1970s as part of the collections undertaken and organized by IUFRO beginning in 1967, and used the list to solicit data beginning in 2010. To date we have data from provenance tests established in the United States, Canada, Belgium, France, Germany, Netherlands, and Spain. Much of the data collected so far has been incorporated into the Center for Forest Provenance Data, a centralized, web-based data management and information system designed to archive and make available data from provenance tests with the hope of encouraging collaboration among researchers for evaluating the data in new ways.

P.12

Is there a relationship between climate and soil factors and genetic structure in different tree species on the Sierra Madre Occidental, Mexico?

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Climate and soil factors are likely to impose strong directional selection in many plant populations, which must therefore adapt if they are to survive. Within populations, microgeographic genetic differentiation of individuals with respect to climate and soil suggests that populations may adapt to certain types of climate and soil through changes in gene structure. Such adaptation may also apply to tree species in the structurally rich Sierra Madre Occidental, Mexico. In this study, several populations of *Picea chihuahuana* Martínez, *Pinus durangensis* M., *Pinus cooperi* Blanco, *Pinus engelmannii* Carr. and *Pinus ayacahuite* Ehrenb. ex Schltdl. in Durango State, Mexico were examined with regard to adaptive genetic differentiation, which was presumably caused by different altitude, temperature, aridity and concentrations of essential elements in the topsoil. Needle samples were analyzed using modified AFLP technology. Outlier loci were detected using the genotype differentiation δ among populations and its components, D_j, and permutation tests considering false discovery rates. The covariation C was used to measure how the relative frequency of the recessive genotypes was related to altitude, temperature, aridity and element concentrations. On the basis of the results obtained, we conclude that an evolutionary response to climate and soil factors is detectable in the studied populations, and also that interactions between soil factors and genetic structure are of potential interest for future research related to forest trees.

P.13

Evolutionary history and speciation of *Pinus armandii* and its Chinese relatives: Influences of the uplift of Qinghai-Tibetan Plateau and quaternary climate changes on the high plant species diversity of China

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Tertiary geological event and Quaternary climate change are two major drivers of speciation and evolution. In this study, we used *Pinus armandii* and other ten Chinese white pines (subsection *Strobus*) as a system to explore the relative influences of uplift of the Qinghai-Tibetan Plateau (QTP) and Quaternary climate changes on the exceptionally high plant diversity in the south of China. We conducted a chloroplast and mitochondrial DNA survey of these species with range-wide population sampling of *P. armandii*, an ecologically and economically important species with the broadest geographical distribution in Central and Southwest China. Four geographically and genetically distinct subdivisions were detected in *P. armandii*, i.e. Qin-Daba Mountains (QDM), Hengduan Mountains (HDM), Yungui Plateau (YGP) and Taiwan. Contrast to the strongly structured cpDNA of *P. armandii*, the mtDNA genetic structure was surprisingly much lower at the rang-wide scale. Coupled with the distribution patterns of the chlorotypes and mitotypes, we inferred two eastwards migratory routes during the glacial periods, one along the Qing Ling-Daba Moutains in the north and the other along Nanling Mountains in the south, probably extending as far as to East China and Taiwan. Repeated range shifts during the Quaternary would promote the speciation of the white pines in Eastern (*P. dabeshanensis*) and Southern China (*P. fenzeliana*, *P. kwantungensis*, *P. wangii*) and Taiwan (*P. armandii* var. *masteriana*, *P. morrisonicola*). The recent divergence time was consistent with their genetic similarity and

close phylogenetic relationship to *P. armandii*. Chloroplast introgression would occur among the Taiwanese white pines, *P. pumila* and *P.sibirica* as well as the HDM populations of *P. armandii* and *P. bhutanica*. Molecular dating estimates showed that Asian white pines originated in the early Miocene (~20.04 Ma), but the initial diversification took place until the late Miocene (7.41 Ma), suggesting the important role of the rapid uplift of the QTP in the allopatric speciation.of Asian white pines. Within *P. armandii*, the divergence of the north and south lineage was estimated to the Pliocene. Divergent time partly explained the contrasts in the strong intraspecific variation between the two lineages of *P. armandii* and low interspecfic differentiation among *P. armandii* and Southern and Eastern China white pines. Our study suggested that the uplift of the QTP and Pleistocene climate change played a complex role at different spatial and temporal scales in the speciation and diversification of Asian white pines.

P.14

Exotic plantations of forest trees in British Columbia: A valuable source of information for assisted migration

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Assisted migration of tree species and genotypes has been suggested as a method for climate change adaptation in forestry. To realize and implement this kind of strategy on larger scales, information from migration trials is needed for all stages of tree growth, from seed germination and seedlings to mature trees. Regarding mature trees, provenance trials including 'exotic' species and genotypes were established throughout Canada during the last century, in various efforts searching for improved productivity, pest resistance, etc. There is a potential to 'repurpose' these kinds of plantation experiments for studying climate adaptation and potential implications for assisted migration. We studied a series of 'XP' exotic plantations established by CFS in British Columbia starting in the 1930s, in many cases using stock originating from provenance experiments started in the late 19th Century that were eventually incorporated into CFS experiment stations. We assessed migration distances versus tree survival for 146 provenances (56 endemic and exotic species) that were migrated within North America to 55 plantation sites. Genera included *Abies*, *Acer*, *Betula*, *Chamaeocyparis*, *Fraxinus*, *Juglans*, *Larix*, *Liriodendron*, *Picea*, *Pinus*, *Populus*, *Prunus*, *Pseudotsuga*, *Robinia*, *Quercus*, *Salix*, *Sequoia*, *Thuja*, and *Ulmus*. For the overall dataset, linear regression analysis only showed weak trends. There was a nearly significant reduction in survival vs. increasing latitudinal migration distance (P=0.056), but the trend only explained a minor part of the variance (R^2=0.0251) and there were no significant effects versus total (P=0.1112) or longitudinal (P=0.1884) migration distance. More northerly migration tracks (mean migration latitude) generally had increased survival (P=0.0486), but again the trend only explained a very small amount of the variation (R^2=0.0267). For particular genera, *Quercus* spp. (*Q. macrocarpa*, *Q. rubra*) collectively showed decreasing survival with increasing total (P=0.0041, R^2=0.83) and longitudinal (P=0.0009, R^2=0.90) migration distance. *Robinia pseudoacacia* also showed decreased survival with total migration distance (P=0.0009, R^2=0.90), while *Pinus* spp. (*P. banksiana*, *P. contorta*, *P. echinata*, *P. engelmannii*, *P. flexilis*, *P. jeffreyi*, *P. monticola*, P. *ponderosa*, *P. pungens*, *P. radiata*, *P. resinosa*, *P. strobus*, *P. sylvestris*, *P. taeda* x. *rigida*) collectively showed a nearly significant, slight (P=0.0592, R^2=0.08) reduction in survival with increasing longitudinal migration distance. Regarding the distribution of tree survival results, there was a spike in mortality at 7-8 years after planting, nearly double that of other age categories. When the distribution of mean migration latitudes was compared in trees with less than 9 years of survival versus those with greater survival, no clear patterns emerged; trees with poor survival were often represented at shorter migration distances, while trees with good survival were often represented at the greatest migration distances. These results indicate that trees may generally have a highly variable tolerance to assisted migration that only partly depends on migration distances. More extensive studies incorporating other long-term field data and differences in climates at sources and destinations are needed before we can determine how these trends apply to assisted migration of particular species and genotypes.

P.15

Case study: Impact of climate change on industrially relevant wood traits determined with SilviScan^{TM}

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Global climate is expected to change further with intensifying weather extremes due to an increase in greenhouse gas concentration. Climate change affects not only tree growth and survival (Andalo et al. 1999, Rehfeldt et al. 1999, Reich and Oleksyn 2008), but also wood quality (Ceulemans et al. 2002). It is well-known that wood quality traits are strongly influenced by growing conditions including climate conditions. There is a lot of information about the response of tree growth and survival to climate change; however the impact of climate change on wood quality traits is not well studied. Given the importance of wood quality and resulting product performance to the economic values of our forests, it is imperative to understand how wood quality responds to climate change. Advanced technologies such as SilviScan are now available for research into impacts of climate change on industrially relevant wood traits.

SilviScan rapidly and cost-effectively determines multiple wood quality traits on the same increment core or wood disc sample. It offers high-resolution pith-to-bark measurements of fibre dimensions, wood density, ring width and microfibril angle, from which modulus of elasticity, cell wall thickness, fibre coarseness, and other industrially relevant wood traits can be determined. Compared to conventional densitometry, SilviScan technology integrates growth-ring orientation information and automated sample-stage rotation. This provides precise and distinct densitometric and diffraction measurements from early-wood and late-wood fibres within a sample, resulting in sharp definition of growth-ring boundaries.
In this case study, transfer models were developed to establish the relationship of wood quality traits with transfer distances for 17 annual climate variables. The wood quality traits were measured with SilviScan on wood discs from 3 planting sites and 21 provenances in Québec. Quadratic regression analyses showed that variation in wood quality traits between provenances is large, reflecting the significant genetic control of wood traits. The study also revealed a significant impact of climate change on wood quality traits, even with the restricted range of climate values and small number of provenances available in the study. For example, total annual precipitation alone accounted for 18% of the variation in fibre coarseness, one of the key fibre attributes impacting pulp and paper product performance.

In summary, SilviScan efficiently measures wood quality traits that are of crucial importance to the forest sector, and is a valuable tool for providing measurements for various aspects of forest genetics, genomics and climate change related research.

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Breeding Pacific Northwest Conifers

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Western hemlock forest genetics program for British Columbia

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Western hemlock is the commonest tree on the British Columbia coast. It is roughly 30% of the annual volume harvested and estimated to be about 60% of timber inventory for the maritime region. As a consequence of its potential, tree improvement activities commenced early on with parent tree selections starting in the late 1950's. First progeny trials were established in the early 1970s and a provincial breeding program was launched in the second half of that decade under Mike Meagher. With the recession of the early 80s and consequential budget restrictions, activities were restricted to maintenance of existing sites and provenance seed collections placed in the hands of the Douglas-fir breeder, Jack Woods. The hemlock program revived with the economy, as forest industry put weight behind their investment in the species requesting, in the words of Western Forest Product's John Barker, that if they were to have seed orchards they should be tested. John King was engaged as breeder, and seeing that few parent trees were tested to that point, set out with others, to develop the Hemlock Tree Improvement Co-operative (HEMTIC) in the 1990s. Its course is well described by Jayawickrama and Ye in their description of endeavors in the US Pacific Northwest.

For hemlock in BC unstable economics of the new millennium combined with the fecundity of the species led to reliance on natural regeneration. Planting numbers have fallen from a high of about 9 million a year in the early 1990s to as little as 10% of that recently. As a consequence, it was decided by the guiding committee for tree improvement for the province, the Forest Genetics Council of BC, that the program should be put on hold. This has meant once again activities are limited to securing long term research installations, and finishing planned measurements of field trials.

The current program has several facets. Gene conservation status has been assessed and as expected for a prolific, invasive, shade tolerant species, risk of loss of genetic variation is relatively low. For genecology research there is a broad network of almost 40 field installations covering most of the environments in which hemlock occurs in BC. Measurements to age 10 years are fairly complete for the trials and reporting on features of the genetic architecture and adaptation is planned for the near future. Preliminary analysis suggests a latitudinal cline of decreasing growth capacity from South to North. With elevation, at least for coastal seed sources, there appears to be a step cline with a fairly abrupt differentiation of low and high altitude genotypes. Longitudinally trends within the coast, transition and interior regions are not detectable, but movement of seed from one of these regions to another does not have positive outcomes.

For investigation of growth and yield of improved genotypes versus wild type plants, 3 series (9 test sites) were established. These tests confirm gain estimates from single tree progeny trial plots by area based 0.1 ha plots. As well, 6 of the trials have varied planting densities in order to predict effects from competition. Results to date confirm expectations for gain. In addition, comparison of wild stand progeny to controlled-cross orchard mean lots suggests inbreeding depression results in a loss of about 5% in early height growth.

Breeding to identify genotypes superior in desired traits ceased almost a decade ago, but progeny tests established then are just now yielding reliable data. Poly-cross (Px) trials of high elevation parents age 12 years are due for measurement this fall, with new breeding values subsequently. Similarly, measurements of polycrosses of backwards selections from the HEMTIC program have been carried out recently, with final rankings due to be tabled this fall. Forward selections from HEMTIC second generation testing have been ranked and the best 120 genotypes grafted for inclusion in clonebanks, but planned Px trials to confirm the breeding values are not to be carried out. Also this fall, tests of best BC parent trees not ranked in time to be included in the HEMTIC second generation will have a final measurement. Unlike the earlier second generation trials, the parents were cloned to allow for more effective within family selection.

Prospects for the future of the hemlock forest genetics program are likely limited. Despite 2 year delays for natural regeneration versus planting, inbreeding depression, patchiness, clear evidence that local seed is surpassed by more southerly sources and increasing genetic gains in seed orchards, the method is inexpensive and often fairly successful. Perhaps declining rotation lengths, or rising genetic worth of seed, either from our orchards or those of the Pacific Northwest, will lead to resurgence of this program once again.

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Operational deployment trials of foliar terpene enhanced western redcedar (*Thuja plicata*) to deter ungulate browse damage

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Western redcedar (*Thuja plicata*) is an ecologically and economically important tree species in coastal British Columbia. Post-harvest establishment of western redcedar seedlings in areas that are densely populated with mule deer (*Odocoileus hemionus*) is an operational challenge because the seedlings are preferentially browsed by ungulates. Browse damage is most severe early in the season of seedling establishment. Existing strategies to mitigate seedling growth losses in newly established forests include barrier installations, chemical repellant applications, planting with large stock, enhanced fertilization regimes, late season plantings, and obstacle plantings. These applications can be costly and marginally effective. Previous research trials show that ungulate browse preference is negatively correlated to increased foliar monoterpene concentrations, particularly the monoterpenoid α-thujone. Testing of western redcedar planting stock with enhanced monoterpenes commenced in six operational reforestation trials established by Western Forest Products on Vancouver Island in Spring 2013. The objective of this study was to assess ungulate browse of western redcedar A-Class seedlots having low to average terpene concentrations compared to trial seedlings and rooted cuttings that were produced from pedigreed seed and selected for mid and high foliar terpene concentrations. Each of the six replicates consisted of two minimum 5ha areas that have similar aspect, slope, mature and immature adjacent stands, accessibility, and predicted mule deer browse pressure. Seed orchard seedling stocks were randomly mixed with mid-level seedlings and high-level terpene clones in each respective area. Terpene enhanced stocks were marked, and 50m^2 plots were staked and numbered. At least two months after establishment, seedlings and cuttings at each plot will be assessed for extent of ungulate browse. We hypothesize that mid-level and high-level terpene stocks will be browsed less than A-Class orchard stock. Further operational studies are needed to determine the long term effects of enhanced terpene stocks on browse resistance. Browse measurements will be taken annually to monitor the operational deployment trials.

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Progeny testing, orchard seed production and deployment of noble fir in Oregon and Washington

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Introduction

Noble Fir (*Abies procera* Rehd.) is a conifer species native to western Oregon and Washington, and northern California. While definitely one of less important timber species compared to the dominant coastal Douglas-fir, it has some interesting features that have led to tree improvement work in the Pacific Northwest (PNW): (1) it is an important component of higher elevation stands, and with its short stout branches is adapted to handling frequent wet snows (2) forest practices rules require successful reforestation of harvested land and natural seed crops are unreliable (3) lumber from this species forms part of the "white wood" component of PNW timber production (4) foliage is harvested in an economically important bough production industry and (5) Noble Fir Christmas trees are highly valued (Landgren et al. 2003) and generate about US $80 million sales annually from these two states. Yearly, plantings in Oregon since 2004 average 3.2 million seedlings (NASS 2011).

In addition to lower log values compared with coastal Douglas-fir, cracking in the stem is an unfortunate phenomenon which affects lumber recovery and reduces deployment of this species for timber production. It is widely believed though not conclusively proven that severe or unseasonal cold is the primary factor (low moisture in late summer has also been suggested as a cause).

First-Generation Testing

Lipow et al. (2002) report 1,460 parents put into tests between 1981 and 1987. We present one example:

The Bureau of Land Management (BLM) selected 195 first-generation parents in two Oregon testing programs, breeding units 50 (Cascades, 11 test sites) and 51 (Coast Range, four test sites). It was unusual for first generation programs in PNW conifer species in having extensive cross-testing between the coast range and the Cascades. Test sites were established in 1981 and 1987, between 897 to 1,270 meters elevation. Total height (HT, cm) was assessed at ages 5, 10, and 15. Diameter at breast height (DBH, mm), number of forks, and number of ramicorns were also assessed on each tree at the latest measurement. A total of 19,736 trees (14,724 from BU50, and 5,012 tfrom BU51) was used for analysis. Individual-tree heritabilities for HT10, HT15, DBH15 and VOL15 were 0.28±0.03, 0.34±0.04, 0.26±0.03 and 0.26±0.03, respectively. Type B genetic correlations among sites were relatively high (0.74~0.82), indicating good stability of families despite sites being located both in the Coast Range and the Cascades. Parents originating from the northwest and higher elevation had slightly higher predicted gain, but this trend was weak ($r < 0.3$). Little stem cracking was seen in the progeny tests, but grafted parents of those progeny showed cracking in a seed orchard. Christmas tree growers have made their own seed collections and tests (Brown 1988, Landgren & Bays 2011). Progeny tests established for the Christmas tree program have also included a number of families from the BLM's program, with focus on BU51. These tests are usually small and established on grower farms, operate on a Christmas tree rotation, and important selection criteria include rapid growth and bud development, foliage traits, and resistance to Current Season needle necrosis. Trees are evaluated for insect problems. Christmas tree growers have strong preferences for seed from certain provenances (Brown 1988 Landgren & Bays 2011). Currently there are approximately 250 parents in test programs (Landgren unpublished data)

Seed Production and Deployment

Seed orchards have been established by the US Forest Service, BLM, Weyerhaeuser Company, the PNW Christmas Tree Growers Association, and Cascade Timber Consulting (CTC). The USFS established the largest extent of orchards (65.8 ha in Lipow et al. 2002), but these are no longer managed because of greatly reduced regeneration harvests on the National Forests. The BLM manages two mature Noble fir orchard blocks (established in 1973 and 1978) at their Horning seed orchard complex. Noble Fir grafts easily and does not need specialized rootstock, although initial growth of the grafted ramets is slow. After 15-20 years sizable orchard crops are produced about every three or four years. Response to stimulation is inconsistent (Landgren et al. 2008). Mature orchards can produce a lot of seed: the BLM orchards produced 309 kg. in 2012 from 5.2 ha. (873 kg. between 2000-2008 even without collecting all the cones). Trees eventually get too large for cost-effective cone collection, leading to re-establishment of new orchards. Noble fir seed are large, running 18,700 – 20,900 per kg., and germination at the outset is moderately high (85%). However, the seed is easily damaged during cone processing, and germination begins to drop significantly after 5-6 years. This means that orchards have to be maintained over long periods since long-term seed storage is not viable. Recent developments in seed processing and stratification, upgrading seed lots with low germination potential, may allow nurseries to make better use of low germination seed lots

Most forest growers in OR and WA with high-elevation land plant some Noble fir. For example on units 760m elevation and above ODF plants 20-30% Noble fir, using 1-0 container stock, while CTC plants about 30% above 910 m. Expected rotations are in the order of 50-60 years.

Future Work

There are no plans to move forward to advanced-generation breeding and testing. The main unresolved questions are the cause and prevention (if possible) of stem cracking and a reliable seed stimulation procedure.

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Breeding western white pine and ponderosa pine in British Columbia: Disease resistance and anticipated range expansion

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The main objective for the western white pine (*Pinus monticola*) breeding program in BC has been resistance to white pine blister rust (*Cronartium ribicola*). Results from provenance testing revealed little population level variation within the species, however, enough variation was observed between coastal (west of the Coastal Mountains) and interior (east of the Coastal Mountains) populations so that two seed zones and breeding populations were established. The program began with the selection of disease-free parent trees from stands that had been exposed to blister rust. Seed was collected from parent trees and seedlings were inoculated with blister rust at the Cowichan Lake Research Station. Surviving seedlings were monitored for several years then selected and grafted into seed orchards for seed production. The apprach taken in the two breeding programs began to diverge. The interior program supplemented local BC selections with 50 trees selected from the Idaho breeding program. The Idaho trees tested very well in BC and formed a strong foundation for the seed production program. Crossing was conducted among the 50 Idaho clones and progeny were screened for disease resistance in inoculation experiments. Surviving seedlings were monitored for several years before being selected for the seed orchard and breeding population. The coastal program began controlled crossing among local parent trees and established several field trails to screen for disease resistance. These field trials are now a source of resistant material for seed orchards. A final series of F1 field trials will be established across both seed zones with material from both breeding populations. The coastal program will continue to rely on the F1 field trials for information and material for seed orchards and the interior program will begin an F1 breeding and field testing program. There is currently one coastal and three interior seed orchards that supply nearly 100% of the demand for white pine seed.

Ponderosa pine (*Pinus ponderosa*) will become a more ecologically important species as the climate changes. Many areas of the province are expected to become hotter and drier and will become climatically more appropriate for ponderosa pine. There is no formal breeding program for ponderosa pine, however, provenance testing has led to the selection of progeny from elite provenances and the establishment of a provenance based seed orchard. We are attempting to stimulate flower production in a mature provenance test in order to collect seed that will form the basis of a formal progeny testing program. This material will be supplemented by wild-stand collections and parent tree selections. A formal progeny testing program will allow us to better understand genotype-by-environment interactions, family variation and calculate accurate breeding values for our orchard and breeding programs.

Genetic Conservation

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Conservation genetics of Baker cypress (*Cupressus bakerii*)

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Cupressus bakerii (Baker cypress or Modoc cypress) is found in appoximately twelve isolated stands in northern California and southwestern Oregon. It has serotinous cones and reproduction is facilitated by fire. Many of the existing stands are declining because fire suppression has allowed other conifer species to encroach into these stands. This species was also rated highly vulnerable to the potential effects of climate change in a vulnerability assessment for southwestern Oregon. To-date no information has been available on the level of genetic diversity or population structure. We used 12 polymorphic isozyme loci to assess genetic diversity and popualtion structure of eight geographically separate populations. Genetic diversity (H_o) was generally high (>0.15) for most populations (7 of 8) but most populations also exhibited a deficiency of heterozygotes (F>0.09) (6 of 8). Over all sites, ~17% of the genetic variation was among sites (F_{st} = 0.171), although this parameter is likely inflated by the northernmost site (Flounce Rock), which also had the lowest diversity. Implications of these results for manangement of the species are discussed.

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Fragmentation of a population and its effect on effective population size for genetic conservation of *Taxus cuspidata* in Mt. Sobaek, South Korea

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Japanese yew (*Taxus cuspidata* var. *cuspidate*) is an endangered species at subalpine mountains and a keystone species at the protected area of forest genetic resources in Mt. Sobaek, South Korea. The yews in this region had grown vigorously until 1960s, but were reducing in number during 1970s to early 1980s due to illegal logging, stealing for ornamental trees, and damages from excess number of climbers. The yew forests designated as the protected area of forest genetic resources in 1990s. However, the yew forests have a problem suffering from very rare seedlings or saplings and poorly growing adults. The region could be divided into three management units according to their legal status as the core zone (BR), the buffer zone (YW1) and two remnant sites (YW2 and KM). They also were geographically fragmented, especially for the remnants. The total number of individual yews in 1980s was reported 1,999 of 15 ha at BR, 1,633 of 119 ha at YW1, 90 of 20 ha at YW2, and 218 of 110 ha at KM, respectively, by the local management office. We collected the needles from 111, 59, 23, and 31 trees per site, respectively, according to their relative densities for genetic analysis using nuclear microsatellite marker system. Total 57 alleles of 8 nSSR loci were acquired from 224 samples. The observed and the expected heterozygosity were 0.269 and 0.532 respectively, and the population was under the Hardy-Weinberg disequilibrium state. The average number of alleles was 5.3. Considering 4 sites, the private alleles were found in KM with high frequency as 0.5. In Bayesian clustering, four sites showed quite different genetic compositions so the proportions of individuals reallocating to the clusters were not similar between the sites. The existence of four sites could explain 9% of total genetic variation in the population from AMOVA. The effective population size estimated from individual genotypes based on linkage disequilibrium was 32.6 with a range of 28.0 to 37.9. When the sites were considered

separately, those were 41.5 at BR, 60.2 at YW1, 28.4 at YW2, and 41.5 at KM, respectively. Genetic patch size from spatial autocorrelation was detected 20 meters at BR and 10 m at YW1, respectively. At the remnant forests, YW2 and KM, there was no spatial genetic patchiness. The differences in the spatial genetic patterns in Mt. Sobaek might be its own attribute according to environmental conditions. We have to consider not only the core or the buffer zone also the remnant forests when making the conservation strategy of the yews in Mt. Sobeak.

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Preservation stands as a form of protection of forest gene resources in Poland

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The protection of forest gene resources has gained significance in the recent decades. All around the world, numerous programs to preserve the most valuable populations of native species have been initiated. Autochthonicity of population becomes the determinant of its genetic value. At the same time, diverse methods of forest management and the stand composition prevent the formulation of a uniform definition of old stands. At present, overmatured stands cover the area of 277522 ha (as of January 1, 2008), which represents 4% of the total forest area in Poland. This share is likely to increase in the upcoming years. The major part (60%) is covered by pine stands. The share of seed stands in relation to the entire surface of protected forest stands reaches 42%. The selected stands are not subject to logging when fulfilling their basic function - *i.e.* to provide a valuable seed base. These stands are also, according to current knowledge, the best populations in terms of phenotype and probably also in terms of genes. However, doubts appear more and more often as to the native character of these stands.

When choosing preventive stands one of the main selection criteria is the age of the population which enables the determination of a native character of the population. For coniferous species the minimum age of stand was determined as 150 years. For deciduous species, which seeds have not been traded in the past, the age was determined as 200 years. The continuity of presence within the area results in the formation of species best adjusted to local habitat and climate. Preservation stands, which are the remnants of the natural populations and which adapted to local conditions through centuries of selection, are the most valuable objects for the protection of forest gene resources. There are, however, no studies on the recognition of the breeding value and the genetic structure of such populations.

Results of studies on the variability of traits in seeds and seedlings of selected preservation populations of Scots pine presented in this work serve for the first and only attempt to determine the level of variability in those stands at the anatomical and morphological level. A crucial element of the presented study is the analysis of traits of seeds and seedlings in relation to the age of the parental stands and the verification of a generally accepted approach about the decrease of seed life and of the quality of seedlings along with age.

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A preliminary evaluation of genetic variation of *Litsea cubeba* (Lours.) Pers. in northern part of Thailand

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Litsea cubeba (Ta krai ton) is one of the citral source and volatile oil tree that occurs widely range 700-1,600 m above sea level in lower montane coniferous forest in the northern part of Thailand. According to the survey, two varieties of L.cubeba were found in Chiangmai province namely *L.cubeba* var.cubeba and var.formosana. However, in Phitsanulok, L.cubeba var.cubeba was only found. For utilization, volatile oil can be developed for mosquito (*Aedes aegipti*) repellent fluid with 6.33 hours repellency duration. Due to the high demand of the agricultural land, forest in northern part of Thailand has been degraded and deforested which affects the habitat loss and gene pool of this species. Therefore, it is important to investigate the status of genetic resources of this species.

The preliminary results showed that the three out of seventeen ISSR (Inter Simple Sequence Repeat) primers can be used to evaluate genetic diversity in those two varieties. Forty ISSR loci were identified and use to evaluate genetic diversity in nine natural populations of L.cubeba in the northern part of Thailand. A hundred and five samples of nine populations from two provenances, Chiang Mai (8) and Phitsanulok (1) were evaluated. The results showed that Nei's gene diversity (He) was 0.24 and Shannon Index (I) was 0.38 whereas Gst was 0.34. The UPGMA dendrogram based on Nei's genetic distance illustrated that the nine populations were divided into two groups. Based on these results, the suggestion of futher study and the conservation management of this species is discussed.

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Assessing range-wide genetic variation and structure in subalpine larch (*Larix lyallii*)

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Subalpine larch (*Larix lyallii*) populations are already restricted to a relatively small geographical and altitudinal range. Changing climate could further reduce available habitat, either by increasing levels of late-summer desiccation or by encouraging the upward migration of more competitive timberline species. Recent studies have also suggested that subalpine larch is poorly equipped to adapt to changes in its environment: in the northern part of its range, *L. lyallii* is genetically depauperate, most likely due to the combined effects of recent bottleneck events and reproductive isolation among spatially discontinuous populations. To examine genetic diversity in this species, we are collecting foliage samples from populations distributed throughout the species' natural range. Variation in the maternally inherited mitochondrial genome, the paternally inherited chloroplast genome and the biparentally inherited nuclear genome will be assessed. By evaluating levels and patterns of genetic diversity in *L. lyallii*, we hope to identify genetically unique populations, which may be of particular importance for planning future management and conservation efforts.

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Relationship between tree species diversity and genetic structure in *Picea chihuahuana* Martinez

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Genetic structure is the distribution of the genetic variation determined by diverse factors such as mutation rate, genetic derivatives, natural selection and genetic flow. Genetic diversity and species diversity are two basic components of biodiversity. Populations evolve within a community context and the diversity of the species in the community can influence the genetic structure of a population via selection. However, until now, the selective effect of the diversity of species within a community on the genetic structure in the population or individual species has not been explored. The objective of the present study was therefore to identify any relationships between tree species and genetic structure in *Picea chihuahuana* Martinez. With this aim, 14 populations of *P. chihuahuana* located in the Sierra Madre Occidental (states of Chihuahua and Durango, Mexico) were analyzed. Within each population, data from a permanent field plot covering an area of one quarter ha (50 x 50m) were used to measure the diversity of tree species. Needles of *P. chihuahuana* were collected (50 samples per population) in the plots for analysis by AFLP (Amplified Fragment Length Polymorphism). The tree species diversity was calculated by the well-known measure ν_2 ($\nu_2 = 1/\sum p_i^2$), which is frequently referred to as the "effective number" of variants. This measure is one of an infinite family of measures, ν_a, where a is a real number ranging from zero to infinity. The results revealed significant relationships between the tree species diversity and genetic structure in *Picea chihuahuana* Martinez. On the basis of our findings, it appears that an interaction between genotype and diversity of tree species may be an important mechanism shaping the composition of plant communities. The present results may help us understand the interactions between ecological and evolutionary processes that determine community structure and dynamics.

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Genetic differentiation of the endemic Mexican species *Picea chihuahuana* Martínez

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A primary goal of empirical population genetic studies is the identification, quantification and comparison of genetic differentiation among loci, individuals, populations, species and studies. Genetic differentiation is the accumulation of differences in allelic or genotypic frequencies between populations and individuals due to evolutionary forces. The distribution of genetic variation among populations is known as the genetic structure and is determined by genetic factors such as rate of mutation, genetic drift, natural selection, gene flow and mating systems, as well as by ecological factors including life history, geographic distribution and the dispersal mechanisms of the species. The adaptation of species to different ecological conditions under strong selection pressures has led to changes in genetic structure, morphology and physiology of populations. The analysis of genetic differentiation enables us to find out more about the influence of evolutionary factors in the genetic structure of the species. The most widely used means of genetic differentiation has been Wright's fixation index, F_{ST}, which was developed as part of a set of hierarchical parameters (F_{ST}, F_{IS}, and F_{IT}) to assess how genetic variation is hierarchically partitioned in natural populations. However, the frequently used fixation index, F_{ST}, and its derivatives produce errors as descriptors of genetic differentiation, principally when applied to highly variable genetic markers. Moreover, this index is not applicable at the genotypic level. Hence, the use of other parameters has been suggested for accurate measurement of genetic differentiation. *Picea chihuahuana* Martínez is endemic in Mexico and is considered endangered, according to the Mexican Official Norm (NOM-ECOL-059-2010). The species is therefore considered fundamental for determining the degree of genetic differentiation between populations. The principal aim of the present study was to determine the genetic differentiation of 14 populations of *P. chihuahuana* located in the State of Durango, Mexico. Needles were sampled from about 700 randomly chosen individuals of *P. chihuahuana* from these 14 populations, and DNA data were obtained through AFLP technology. A data matrix was constructed according to the presence or absence of bands. To measure the genetic differentiation, parameters such as genotypic differentiation δ and its components D_j were determined for each AFLP locus, thus enabling indirect, accurate measurement of the presence of direct forces that increase or decrease the differentiation among populations. A value of zero indicates that collectives are genetically identical, and conversely, a value of one indicates that collectives are genetically completely different. Significant genotypic differences between populations of *P. chihuahuana* were found that are probably caused by effects in small isolated populations and by selection.

Genetics of Wood Quality

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Coexpression network analysis of candidate genes related to wood physical traits in white spruce

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The molecular functions and biological connectivity of candidate genes related to wood formation have rarely been explored. The aim of this study was to conduct a network analysis of gene expression data and identify sets of functionally related candidate genes based on correlated patterns of expression. The anticipated results should help highlight the nature and biological role of candidate genes, determine what are the main expression profiles represented among candidate genes and verify if there is an over-representation of specific profiles. To identify coexpression modules, a gene correlation network for 2660 candidate genes was generated using gene expression data from seven white spruce tissues obtained with microarray measuring the accumulation of transcripts of 23,853 genes (Raherison et al. in preparation). The data was submitted to the weighted gene coexpression network analysis (WGCNA) package (Langfelder and Horvath 2008). We carried out gene ontology (GO) enrichment analyses for all coexpression modules obtained to identify genes containing particular GO terms that are over-represented in these modules.

A total of nine modules of correlated genes were detected in the WGCNA correlation network analysis. The modules varied in size from 62 to 551 genes and had an average size of 280 genes. The largest modules were composed of genes whose expression was contrasted between needles and secondary vascular tissues (both xylem and phloem). The network analysis also showed that genes associated with wood traits had diverse expression profiles. For example, module 2 consisted of 511 genes, of which 367 genes had high secondary vascular tissue expression and 144 had low secondary vascular tissue expression. The functional enrichment showed that genes that were highly expressed in vascular tissues were enriched with gene products that target the Golgi apparatus (GO:0005794), plasma membrane (GO:0005886) and endoplasmic reticulum (GO:0005783). The distribution of genes significantly associated with wood traits in these modules might be the key to understanding the genetic architecture of these complex traits.

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Inbreeding in mid-rotation coastal Douglas-fir

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The effects of inbreeding on growth traits are fairly well understood in young conifers. However, in trees approaching mid-rotation, this information is not widely available. To evaluate inbreeding in coastal Douglas-fir trees, a 9-clone founder population was used to produce 20 outcross, 66 half-sib, 36 full-sib, 17 parent-offspring and 9 selfed families. The trees were planted on two farm-field sites in coastal BC and were re-assessed at age 26 from seed for this study. Height, diameter, survival and wood density were recorded and single-tree volume calculated. Previous results obtained from this test population showed negative linear effects with levels of inbreeding in seed production, nursery growth performance and growth traits in the field assessed at age 10. At age 26, inbreeding depression was highest in survival, ranging from 20% to 80% for f=0.125 and f=0.5, respectively. In contrast, the most severe inbreeding depression among the three levels of inbreeding was only 4% for wood density at f=0.5 (selfing). Inbreeding depression in height, dbh and volume increased linearly from f=0 to f=0.25 then leveled off. Founder genotypes had varied responses to inbreeding as parental breeding values across inbreeding levels were inconsistent (in magnitude, sign and trait). Differences in levels of inbreeding depression were also found between full-sib matings and parent-offspring matings, despite having the same level of inbreeding (f=0.25). These differences also depended on the trait and field site. Heritability estimates varied among traits and inbreeding levels.

P.29

Genomics-assisted selection of white spruce benefits from SilviScanTM: Advanced phenotyping technology for measuring industrially relevant wood traits

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With the rapid advancement in genotyping technologies, genotyping is becoming more affordable and will no longer be a limiting factor in forest genetics research. Consequently, to keep pace with these developments, there is an increasing need for precise, high throughput and cost effective phenotyping for wood and fibre traits in genomics research. Advanced phenotyping technologies such as SilviScan can facilitate the acquisition of wood traits accurately, efficiently and cost effectively for large sample sets.

SilviScan rapidly and cost-effectively determines multiple wood traits on the same increment core or wood disc sample. It offers high-resolution pith-to-bark measurements of fibre diameter, wood density, microfibril angle, modulus of elasticity, cell wall thickness, specific surface area, ring width and area, fibre coarseness, and cell population. The data have applications in many fields, such as resource characterization, forest inventory enhancement, forest management decision-making, forest genetics research, tree improvement and adaptation to climate change.

One example of the benefits of SilviScan in forest genetics research is illustrated in a pilot study to test for association between single nucleotide polymorphisms (SNPs) of candidate genes and wood traits in white spruce (Beaulieu et al. 2011). Genomic selection in boreal conifers appears particularly promising for traits that must be assessed at a more mature stage, such as wood quality traits. Preliminary results indicate that genomic selection could likely be applied to spruces using only several tens of gene SNPs found to be associated with wood traits. In this study, SilviScan played a key role in the measurement of industrially relevant wood traits that are significant to the performance of wood, pulp and paper products.

In summary, SilviScan efficiently measures wood quality traits that are of crucial importance to the forest sector, and is a valuable tool for providing measurements for various aspects of forest genetics and genomics research.

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Applications of Genomic Tools

P.30

Genetic purity certification through molecular marker techniques in *Eucalyptus grandis*

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The correct identification in the genus Eucalyptus is hindered due morphological similarities between species. Furthermore, the high capacity of interspecific hybridization in this genus also hinders the correct identification. Molecular techniques have been incorporated into forest improvement programs, as well as used in varietal identification and genetic purity certification. *Eucalyptus grandis* Hill ex Maiden seeds from Coff"s Harbour-New South Wales – Australia, were sowed in Mogi-Guaçu (SP) and Salto (SP), creating a base-population. Some individuals of the base-population were selected and subjected to a progeny test. According to this test a new individual selection was performed and, by vegetative propagation, a clonal seed orchard of E. grandis was created. A new progeny test was performed, in which E. grandis individuals were localized next to individuals of E. urophylla (from Timor). The seeds that derived from open-pollinated between E. grandis and E. urophylla were harvested from E. grandis. These seeds were sowed in nursery, and individuals with characters typical of *E. grandis* were selected and transferred to an experimental field. New selection according to growth and stem form was performed and the selected individuals cloned in clonal bank are localized in Morrinhos farm (Itatinga/SP). However, morphological analysis of the bark suggested that some individuals are not E. grandis. Thus, it is necessary a complementary analysis with molecular techniques. The aim of this work was to confirm E. grandis identification carried out by phenotypic characters (bark) using molecular tools.

Sixteen selected trees were characterized by bark phenotype as E. grandis or E. urograndis. Genomic DNA extraction was based on Ferreira and Grattapaglia protocol, with a reduction of CTAB concentration of 10% to 5%, and by using the volume twice. PCR reactions were performed with specific SCAR (Sequence Characterized Amplified Regions) primer, called Elig, that amplifies in individuals without lignotuber character (99% of E.grandis). The amplicons were analyzed on agarose gel 1.5% (w / v) in Tris-borate (TBE 1X, pH 8.0), stained with ethidium bromide 1% (v / v).

SCAR Elig primer was developed to identify E. grandis individuals with lignotuber absence. More than 99% of Eucalyptus grandis population does not possess lignotuber. However, some individuals can present this structure due to unknown interspecific hybridizations, defining the individual as not pure.

This work aimed to confirm the identification of some individuals from open-pollinated progeny test, whereupon the female parent is E. grandis. Once we sought only E. grandis individuals to compose the clonal bank and some selected individuals presents untypical features, we realized a test with SCAR Elig primer that amplified in E. grandis without lignotuber character.

The test resulted in four situations:
1. Typical bark and band presence: Confirms the identification as E. grandis.
2. Typical bark and band absence: The individual can be a hybrid E. urograndis, which the character bark was inherited from E. grandis parent; and molecular marker absence can be explained by remote and unknown interspecific crosses.
3. Untypical bark and band presence: The individual can be considered E. urograndis hybrid that the phenotypic character (bark) was inherited from E. urophylla male parent, and the genome possess the molecular marker inherited from E. grandis female parent.
4. Untypical bark and band absences: The individual can be a hybrid E. urograndis, with the E. grandis female parent possessing in its genome traces from E. urophylla originated from remote and unknown interspecific crosses.

The capacity of interspecific hybridization with the genome similarities in this genus hinders the exact identification of pure species and hybrids. Even though, molecular tools complement morphological analysis, it is necessary more extensive analysis in order to have a better reliability in the results.

P.31

Validation of SNP-trait associations identified in black cottonwood (*Populus trichocarpa*)

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Populus trichocarpa is a tree species with high economic and ecologic relevance. Hence, it is an important model species in forest tree research. Extensive genomic resources, e.g. the full genome sequence and tools for the identification of single nucleotide polymorphisms (SNP) are available. Furthermore, with the advent of SNP genotyping arrays and more recently, low-priced sequencing technologies, genome-wide SNP profiles from hundreds of individuals can be assayed. We and others are using this information in combination with extensive phenotype data from trees grown in common gardens for genome-wide association studies (GWAS) to study the correlation between genetic variants and quantitative trait differences based on linkage disequilibrium (LD). These studies identify a considerable number of SNPs that that are significantly associated with various traits. However, our GWAS have revealed many SNP-trait associations in genes of unknown function or genes not previously known to be related to the associated trait. Also, many SNPs appear not affect protein function or are located non-coding regions. Major questions thus include: How can we corroborate the allelic effects of these SNPs? Which strategies can be applied to identify causal SNPs (QTN) that directly impact trait variation? In our work we are analyzing selected genes and SNPs identified in two *Populus* GWAS that focused on wood properties and adaptive traits with respect to these questions. We will discuss our strategies and initial results for experimental validation of the SNP-trait associations including analysis of expression level variation and/or transgenic expression of the target alleles.

P.32

Western white pine quantitative resistance to blister rust fungus: Understanding the role of key proteins to facilitate tree improvement

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Western white pine (*Pinus monticola*) is a commercially and environmentally important species in North America that is being threatened since early 1900s by an introduced pathogen, white pine blister rust fungus (WPBR, *Cronartium ribicola*). Natural Resources Canada (Pacific Forestry Centre) in collaboration with the BC Ministry of Forests and USA government has established a breeding program since 1970s which has incorporated qualitative and quantitative resistance mechanisms. However screening trials for quantitative resistance can take up to seven years. The objective of our research is to identify the functional gene candidates and genetic markers associated with resistance to *C. ribicola* in *P. monticola* to expedite this screening process. As quantitative resistance are expected to be multigenic, several defense-related gene candidates were previously identified by our group in *P. monticola* needles using a proteomic strategy including: antimicrobial proteins, pathogenesis-related protein 10, chitinases and thaumatin-like proteins. Chitinases are enzymes hydrolyzing the glycosidic bonds in chitin, which is the major component of cell walls of fungi, subsequently resulting in cell lysis. However, the exact mechanism of thaumatin-like protein action is still unclear. To further investigate the function of these genes in resistance, recombinant proteins were generated for two gene families: class IV chitinase and thaumatin-like protein. Indeed, we overexpressed these genes in an *E. coli* expression system and used affinity chromatography to purify the recombinant proteins to assess their inhibitory activity against different fungal pathogens. We found that proteins of both gene families have antifungal activity. This data would provide a better understanding of host-pathogen interactions and potential molecular markers for selection of white pine blister rust resistance.

P.33

Identification of SNPs in candidate genes related to water stress, transport and absorption Boron in *Eucalyptus*

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Forestry companies have occupied Northern and Northeastern of Brazil with *Eucalyptus* plantations. However, limitations on water supply and boron element (B) are affecting the biomass production and reducing the yield significantly [5,3]. Tolerant plants develop defense mechanisms like hormone production of abscisic acid (ABA), and osmoprotector glycine betaine (GB) when submitted to drought conditions [2]. Polyol sorbitol with Bor1 carrier are correlated in the boron translocation and absorption [1,4]. Identifying and studying genomic regions related to water stress tolerance, Boron transport and translocation are important for forest breeding programs. We identify SNPs (Singlenucleotide polymorphisms) in four Eucalyptus candidate genes, 9-cis-epoxy-carotenoid dioxygenase (NCED) and choline monooxygenase (CMO) related to drought tolerance and Sorbitol dehydrogenase and Bor1 transporter, related to the boron absorption and trasnport.

Contrasting plants of *E. grandis*, *E. urophylla,* and the hybrid *E. grandis x urophylla* were selected according to their physiology. Specific primers were designed from homology sequences from *Eucalyptus* ESTs databank and amplification products submitted to sequencing which allowed SNPs identification and plants genotyping.

In the four studied genes, were found 110 SNPs, of which 54 in the genes BOR1 and Sorbitol dehydrogenase. At 3860 bp in the BOR1 gene, were found 35 SNPs, of which, 16 located in exons with synonyms mutations in 12 (75%) and non-synonyms mutations in four (25%), and 19 SNPs were located in íntrons. Nineteen SNPs were found at sorbitol dehydrogenase gene in a 2889 bp region, being that 7 are located in exons, with synonyms mutations in 4 (57%) and non-synonyms mutations in 3. Ten SNPs were located in íntrons and two at UTRs regions. In the NCED e CMO genes were found 66 SNPs, being that seven SNPs in a 1230 bp region on NCED gene, from which five (71.5%) were present in codified regions and generated synonymous mutations and two (28,5%) in UTRs regions. For the CMO gene, 49 SNP were identified in 3885 bp region, which 12 were in codified regions, for these codified regions; ten (83,3%) of the mutations were synonymous and two (16,7%) were non-synonymous and 37 in UTRs and íntron regions. For BOR1 gene, were found 16 haplotypes with 0,957 diversity haplotypic and 0,00308 nucleotide diversity Pi(π), averaging one SNP for each 120bp. For the Sorbitol dehydrogenase gene, were found 18 Haplotypes with 0,967 haplotypic diversity and nucleotide diversity Pi (π) of 0,00296, averaging one SNP for each 152bp. NCED gene presented seven haplotypes which generated 15 different genotypes. For the CMO gene, these polymorphic sites constituted 18 haplotypes producing 16 diverse haplotype combinations. Nevertheless, CMO gene showed some unique genotypes for the species *E. grandis* and *E. urophylla*. As a result, the genotyping of individuals by the allele-specific extension technique demonstrated to be efficient; in addition the SNPs primers designed can decrease costs and permit the genotyping of these mutations in large scale of contrasting populations to water deficit and in *Eucalyptus* population studies.

P.34

Genome-wide association mapping and genomic prediction for wood property and quantity of male strobili in *Cryptomeria japonica*

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Genome-wide association studies (GWAS) and Genomic selection (GS) can be a powerful technology in conifer breeding because conifers have long generation intervals, protracted evaluation times, and high costs of breeding inputs. In the present study, we examined the potential of GWAS and GS in conifers using 367 unrelated plus trees of *Cryptomeria japonica* D. Don, which is the most widely planted and commercially important tree species in Japan, and tried to detect significant associations between wood property traits and quantity of male strobili on the one hand, and 5,055 single nucleotide polymorphisms (SNPs) assigned to 5,055 genes on the other. The mixed linear model taking into account kinship relationships and subpopulation structure and Ridge-regression model were used for GWAS and GS, respectively. In total, dozens of SNPs were found to have significant associations with the variations in phenotype. These SNPs were not associated with the positions of known genes and QTLs that have been reported to date, thus they may identify novel QTLs. Eighty percent of these SNPs were found in sequences showing similarities with known genes, although further analysis is required to dissect the ways in which they affect wood property traits and abundance of male strobili. These presumptive QTL loci provide opportunities for improvement of *C. japonica*, based on a marker approach. Genome-wide predictions for GS were accurate at the medium level (0.54) in quantity of male strobili and at low levels (0.2) in wood property traits. The results suggest that GWAS and GS have potential for use in future breeding programs in *C. japonica*.

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P.35

Paternity recovery in a maritime pine polycross trial

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Maritime pine (*Pinus pinaster* Ait.) is the main plantation species in France with nearly one million hectare of cultivated forests in South Western France. A breeding program has been implemented since the early 1960s using a recurrent selection scheme. The breeding strategy combines two successive experimental designs: polymix crossing to evaluate parental breeding values and full-sib crossing from which selections are made for the next generation of breeding. This strategy was efficient both to increase genetic gains (maritime pine varieties reach 30% expected gain in volume and in stem straightness) and to maintain genetic variability in the breeding populations (Bouffier *et al*., 2008).

Future improved varieties must be adapted to environmental evolutions (climate change, development of some pests) and to wood industry diversification. In that changing context, forest tree breeding programs have to integrate more and more selection criteria and to propose different varieties adapted to various environments and diversified uses. Then it appears of first importance to accelerate the selection cycles in order to often renew variety composition. Fast development of molecular genetic tools and their cost drop-off can be of great help to cope with these challenges. Shortening selection cycle could be reached by polymix breeding with parental analysis of progeny as proposed by Lambeth et al. (2001) instead of the current breeding strategy of full-sib breeding and testing.

In that perspective, a maritime pine polymix progeny trial is currently under study. Trees have been highly phenotyped for various traits relative to growth (diameter, height), wood quality (stem straightness, spiral grain, wood density) and adaptation (water use efficiency). We are now genotyping this population with 60 SNPs (Sequenom technology) to recover the paternity identity. The poster will present first results of this study which aims to answer the following questions:
Is there a differential reproductive success among pollen donors?
What is the gain for breeding value accuracy when analyses are carried out with the full pedigree instead of with only the mother identity?
Which new breeding strategies integrated the pedigree recovery can be implemented?

Bouffier, L., Raffin, A., Kremer, A. 2008. Evolution of genetic variation for selected traits in successive breeding populations of maritime pine. Heredity 101:156-165.

Lambeth, C., Lee, B.C., O'Malley, D., Wheeler, N. 2001. Polymix breeding with parental analysis of progeny: an alternative to full-sib breeding and testing. Theor Appl Genet 103:930-943.

Population Genetics and Genomics

P.36

Genomics resources for North American hardwoods

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The hardwood forests of Eastern North America are complex biological systems, covering millions of acres, providing habitat for wildlife, long-term carbon sequestration, fiber, recreational uses, and other essential services. The sustainability of these forests is increasingly threatened by forest health issues such as exotic pests, diseases, invasive plants, climate change and fragmentation. In this project, researchers at seven universities are collaborating to develop genomic resources and genetic tools for a broad taxonomic distribution of hardwood tree species, including northern red oak (*Quercus rubra*), black walnut (*Juglans nigra*), white oak (*Quercus alba*), yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), honey locust (*Gleditsia triacanthos*), sugar maple (*Acer saccharum*), blackgum (*Nyssa sylvatica*), and green ash (*Fraxinus pennsylvannica*). We are producing expressed gene sequence databases, DNA markers, genetic linkage maps, and reference populations with an emphasis on tools for assessing genetic variation in growth, adaptation, and responses to abiotic environmental stresses. These resources are being made available through the project website (www.hardwoodgenomics.org). Sequence data will be deposited in GenBank, and the genetic linkage maps and associated marker data will be available at the Dendrome database (http://dendrome.ucdavis.edu/). This project is supported by grant # TRPGR IOS-1025974 from the National Science Foundation's Plant Genome Research Program.

P.37

The chestnut genome project

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Hardwood trees in the northern hemisphere are under increasing threat from invasive pests and diseases. The devastation of American chestnut (*Castanea dentata*) populations in Eastern North America by the chestnut blight fungus (*Cryphonectria parasitica*) is a well-chronicled environmental disaster. Unlike the American chestnut, the closely related Chinese chestnut (*Castanea mollissima*) evolved resistance to the sympatric chestnut blight fungus. Hence the Chinese chestnut is an important species for decoding the evolution of disease resistance, especially in hardwood species. Thus, we have undertaken a project to sequence the Chinese chestnut genome to provide a high quality reference genome for the discovery of blight resistance genes, and for studying the evolution of disease resistance in forest trees. Using mostly next generation sequencing data, we produced over 61 billion bases of NG DNA sequence data providing 76 X coverage of the estimated 800 Mbp chestnut genome. The sequence data assembled into 41,270 scaffolds, with an N50 scaffold length of 39,580 bp, covering 724.4 Mbp of the estimated 800 Mb chestnut genome. A total of 38,146 genes have been predicted and annotated in the genome scaffolds, using Chinese chestnut and American chestnut RNA sequences. To ensure that all of the blight resistance genes are identified, we also sequenced BAC clones covering the three major blight resistance QTL from the Chinese chestnut physical map. Over 400 candidate disease resistance genes were identified in the QTL sequences, of which 22 have been selected as high priority candidate genes for blight resistance. This project was supported by The Forest Health Initiative (http://www.foresthealthinitiative.org).

P.38

Genetic variation in chloroplast DNA of *Dalbergia cochinchinensis* Pierre in Thailand

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The Siamese rosewood (*Dalbergia cochinchinensis* Pierre) is an economically important tree species throughout Laos, Cambodia, Vietnam, Myanmar and Thailand. This species has been illegally logged since the last few decades which may affect gene pool, genetic diversity and genetic structure. In this study the cpDNA variation of *D. cochinchinensis* was investigated by using cpDNA sequencing to characterize the spatial genetic variation and pattern of haplotypes of investigated populations in Thailand. *D. cochinchinensis* was sampled from 9 populations of the central and northeastern part of Thailand.

The obtained 1,841 bp nucleotide sequences from three cpDNA regions (*trn*S-*trn*G: 469 bp, *trn*V-*trn*M: 664 bp and *trn*C-*pet*N1R:708 bp) of 98 sampled trees of *D. cochinchinensis* were determined. A total of 11 haplotypes with 10 polymorphic sites, including 8 single nucleotide polymorphisms (SNPs) and 2 insertions/deletions were observed. The haplotype diversity (h) for each population ranged from 0 to 0.697 and the nucleotide diversity (π) from 0 to 0.00056. It was found out that Na Dun population in Maha Sarakam Province (NM) had highest haplotype diversity and nucleotide diversity. This may be because this population is the forest community which has been reforested by the different sources of *D. cochinchinensis* materials. However, it is revealed that, Chuen Chom population in Maha Sarakham Province

(CM), Phu Laenkha population in Chaiyaphum Province (PC), Tapraya population in Buriram Province (TB), Nadun population in Maha Sarakham Province (NM) and Muakleknai population in Saraburi Province (MS) have different unique haplotypes. Therefore, the unique haplotype information of the certain mentioned populations can be use as a tool to identify the origin of illegally logged wood in the future.

Therefore, more molecular markers, number of samples and populations of *D.cochinchinensis* in Thailand and Asian region should be collected and investigated for the indicative haplotype for specific populations, sub-regions and countries. Then it can be used to identify the origin of genetic resources of illegally logged wood, since Thailand is facing this problem due to high demand of illegally trade.

P.39

Fine-scale spatial genetic structure of a *Quercus gilva* population in South Korea by microsatellite markers

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Quercus gilva is an evergreen and endangered oak species in Korea. This species distributes in Japan and southern China, but grows naturally at volcanic Jeju Island only in South Korea. We conducted a total inspection of growth performance and analyzed the genetic variation of a *Q. gilva* population using microsatellite makers. A total of 668 individuals were found in the surveyed region (38.5km^2), and the mean height and the diameter at breast height of *Q. gilva* were 9.5 meters and 21.7 centimeters, respectively. Mean distance to the nearest neighbor individual was 41.7 meters and they distributed highly aggregated (R = 0.15***). We sampled randomly 192 individuals for genetic analysis with eight nSSR markers. The number of observed alleles and the effective number of alleles were 10.1 and 4.1, respectively. There was a little bit difference between the observed and the expected heterozygosity (Ho = 0.617 and He = 0.702) so the fixation index was small (f=0.112) as compared to those of other oak species. This population was out of the Hardy-Weinberg equilibrium state. The sampled individuals were spatially aggregated (R = 0.58 ***). From spatial autocorrelation analysis, the size of genetic patchiness was estimated as at 50 meters in diameter. Distribution pattern of genetic variation by the genetical bandwidth mapping method showed that the individuals in southern part of the population were genetically heterogeneous, while others in northern part were homogeneous. This difference of spatial genetic composition might be explained by topographic characteristics and by the history of the land use. The northern part is a foot of a mountain and the southern is flat land which was once used for farming. These two parts also are divided physically into two sites along the road. Acorn distribution by birds or rodents was surmised to be a major factor of spatial genetic structure in the region rather than the dispersal by the gravity.

P.40

Comparative genomics of *P. glauca* and *P. abies*

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Picea glauca and *P.abies* are economically and ecologically important tree species that dominate temperate and boreal forests in North America and Northern Europe. The recent sequencing of their complex and big genomes provides a unique opportunity to study the genome structure evolution in conifer species.

We assessed the levels of synteny and colinearity between *Picea glauca* and *P.abies*. Sequences from 38 fully sequenced bacterial artificial chromosomes (BACs) from *P. glauca* were compared to 450 *P. abies* fosmid-pools (representing aprox. 50% of the complete genome sequences) and diploid whole genome shotgun data (*P.abies* 1.0 assembly, 19.6 Gb).

Data analysis was done using customized perl and shell scripts. Selected fosmid-pools'contigs were correctly oriented and ordered using nucmer and displayed with mummerplot.

We found that the number of BACs with at least one match to *P.abies* varied from 28 (fosmid-pools) to 30 (whole-genome) from a total of 38. The percentage of coverage in the BAC scaffolds by one or several fosmid-pools varied from 0.57 to 47.48%. Significant synteny was found in seven BACs, in which >20% of the BAC sequence was contained in one or several *P.abies* fosmid-pools' contigs. Five of these BACs contain important genes in wood formation or nitrogen metabolism in *P.glauca*.

P.41

Fine-scale transcriptome reconfiguration in response to a climate-induced stress: Testing the naïve host hypothesis

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The current outbreak of mountain pine beetle (MPB; *Dendroctonus ponderasae*) and its associated pathogenic fungi (i.e. *Grosmannia clavigera*) have had devastating impacts on the health of western North American pine forests. Approximately 28 million hectares of lodgepole pine (*Pinus contorta* var. *latifolia*), the historic host for MPB, have been destroyed within western North America [1-3]. In recent years, MPB has undergone marked range expansion into novel habitats northward, eastward and into higher elevations attacking a new host species, jack pine (*P. banksiana*), an invasion facilitated by a hybrid zone between the two pine species [4]. This expansion has, in part, been driven by warmer winters associated with global climate change that have occurred at different stages of the epidemic [5] allowing further spread into novel habitats of the boreal forest. Consequently, understanding the evolutionarily naïve host defense response relative to the evolutionarily co-evolved host may aid in evaluating risk of MPB spread in novel habitats. Given the history of selection and adaptation of constitutive and induced defenses of lodgepole pine and MPB, we expect the defense response of lodgepole pine to differ from that of the novel host, jack pine. Furthermore, as stressed trees are more prone to successful MPB attack under endemic conditions, we expect differential partitioning of resources to defense and other aspects of host suitability in response to environmental stress. We used microarray transcriptome profiling to evaluate the transcriptional response of two-year old lodgepole pine and jack pine seedlings subjected to either well-watered or water deficit treatments in concert with inoculation of *G. clavigera,* as a proxy for MPB attack. RNA was extracted from phloem harvested over a time course of 1, 7, and 28 days post-inoculation (dpi). Loblolly pine cDNA microarrays containing 26,496 elements [7] were hybridized with aRNA according to El Kayal *et al.* [8]. The loblolly pine sequences represented on the microarrays were cross-annotated with the corresponding lodgepole pine and jack pine transcript sequences, in addition to *Arabidopsis* TAIR annotations. Statistically over-represented gene ontology terms categorized from differentially expressed pine gene putative orthologs, were integrated into Ontologizer [9]. Differentially expressed genes were clustered using K-means in MeV [10] across all dpi and treatments. Identification of functional categories or 'bins' were developed using the Mercator Automated Sequence Annotation Pipeline (Lohse et al. unpublished) for loblolly pine sequences. These data were used to visualize functional categorization of differentially expressed genes across dpi and treatments for the two pine species within MapMan [11]. Microarray analyses revealed significant transcriptional differentiation across treatments, including a considerable influence of water limitation between species over time. Several thousand significantly differentially expressed genes were observed across treatment combinations, including defense-related processes such as terpenoid, phenylpropanoid, and phenolic compound biosynthesis, and cell wall modification. These categories were statistically over-represented within the differentially expressed gene set of jack pine, although similar patterns were not observed for lodgepole pine. Cluster analysis revealed similar gene expression patterns between species over time, with the greatest number of differentially expressed genes observed at 7 dpi. However, MapMan fine-scale functional classification of these genes suggests species-level differences in the functional categories represented by those differentially expressed genes. Indeed, we observe a greater number of treatment-induced differentially expressed genes in functional bins associated with defense that are unique to jack pine. These findings identify fine-scale patterns of differentiation in the regulation of defense-related genes. In addition, these data suggest that water limitation modulates the defense response, suggesting abiotic and biotic factors may interact to influence the transition of MPB from its historic host, lodgepole pine to its naïve host, jack pine.

This study identifies functionally important environment and species-specific transcriptional changes during defense. While timing of defense is conserved between species, fine scale differentiation among functional categories of differentially expressed genes point towards species-specific strategies associated with climate-induced stresses. These unique responses to both abiotic and biotic stresses within the native and novel host may influence evaluation of MPB spread into novel habitats and ultimately management of the new host species.

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P.42

Phylogeographical study of *Quercus phillyraeoides* A. Grey in the western part of Japan revealed by microsatellite markers

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Quercus phillyraeoides is an evergreen shrubby oak species growing on the Pacific coasts and the Seto Inland Sea of southwestern Japan. Most famously this oak species has been utilized for producing high-quality charcoal, Bincho-tan, and an important component of the dry hill vegetation of Japanese coastlines. In a previous study we showed some of the isolated populations on the Pacific coast (Kushimoto (Ks), Ashizuri (Az), Sata (St) and Ryugadake (Rd)) were the refugial populations of the last glacial maximum (LGM) using cpDNA non-coding regions.

In this study using 414 individual samples collected from the same 19 populations of the previous study were examined by using 11 mircrosatellite loci. Eight of the 19 populations were located in the Seto Inland Sea and four were in the East China Sea side (Fig. 1). Totally 104 alleles were found (average = 9.45 alleles per locus), and 12 of them were private alleles. Average observed heterozygosity was 0.560 ± 0.021 (SE) and average expected heterozygosity was 0.551 ± 0.017 (SE). The expected heterozyogsity for populations was ranged from 0.422 ± 0.081 in Izena-jima (Iz) in Okinawa to 0.608 ± 0.056 (SE) in Nagahama (Ng) in the Seto Inland sea. AMOVA by Arlequin software showed small, but highly significant FST (= 0.07656, P<0.0001). Pairwise FST showed the easternmost population in Iwai (Iw), the southernmost population in Sata (St) in Kyushu and Iz were found to be the most isolated populations. Neighbor Joining tree was constructed using pairwise genetic distance. The tree showed Iz, the southern and the westernmost population was paraphyletic to the other populations and suggested to be the oldest population. Whereas, Iw, the easternmost population was at the tip of furthest branch showing the most recent population. These observations suggest that Q. phillyraeoides has migrated to the Japanese Archipelago from mainland China through Ryukyu Islands to the east on the Pacific coasts.

We examined population structure using STRUCTURE software. The most probable number of cluster (K) was four. These clusters appeared in a series (i.e. cluster 1 - cluster 2 - cluster 3 - cluster 4) from west in Iz (cluster 1) to the east end population of Iw (cluster 4) on the Pacific coast, but the populations in the Seto Inland Sea were the mixture of cluster 2 and cluster 3. This observation together with the result of the phylogenic tree suggested that migration and expansion of Q. phillyraeoides has occurred in a series producing new clusters one by one. Because at the LGM, when the sea level downed about 150m, the Seto Inland Sea was a dry land and the populations here were the newly migrated ones from the Pacific coast through the opening Bungo and Kii straits, which are dividing Kyushu and Shikoku, and Shikoku and Honshu, respectively. This scenario is also supported by cpDNA data in the previous study. Geological studies have shown that Ryukyu islands was once a land bridge at the end of Pliocene about 1.7 to 2.0 million years ago, connecting the mainland China and the Japanese Archipelago and provided the route of migration of animals and plants from the subtropical area of China. The land bridge was broken by newly opened Tokara gap between the southern part of Kyushu and Okinawa Islands about 1.7 to 1.3 million years ago and was never closed. Iz population has isolated since then, and the southwestern populations in Kyushu had suggested to expanded to further east during the later Quarternary period. Clustering may have occurred at the periodical glacial events.

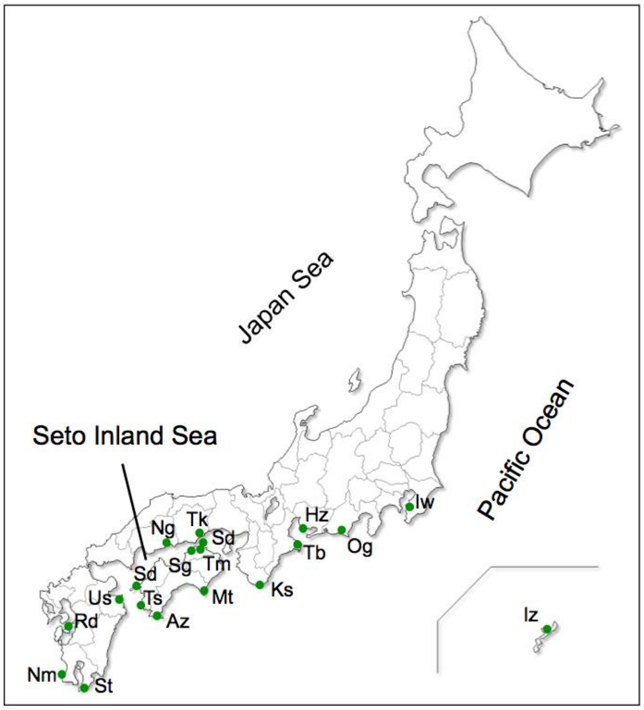

Fig. 1. Collection sites of Quercus phillyraeodes (dots). Name of the locations are shown by tow-letter abbreviations.

P.43

Genetic divergence between populations along the Japan Sea and Pacific Ocean side of Japan in *Cryptomeria japonica* using nuclear genomes

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Genetic diversity and population genetic structure in plant species are influenced by various factors, including their life history and ecological traits (e.g. mating system, modes of seed and pollen dispersal); historical shifts in distribution caused by climatic changes, especially those associated with ice ages. In particular, climatic fluctuations during the Quaternary appear to have caused profound changes in the distribution ranges of many plant species, and thus strongly affected their genetic diversity and structure.

In this study, genetic diversity and structure were examined using 20 nuclear microsatellite markers in 37 natural forests of *Cryptomeria japonica*, the most economically important forestry tree in Japan. *C. japonica* is an allogamous coniferous species with wind-mediated pollen and seed dispersal systems. Natural forests of the species are distributed under variable environmental conditions, from Aomori Prefecture (40° 42' N) to Yakushima Island (30° 15' N) in the Japanese Archipelago. The geographical variation between natural forests of *C. japonica* has been investigated, focusing on both morphological traits (e.g. needle length or curvature) and diterpene components and clonality. The results of these studies suggested that there are two main lines: Japan Sea lineage and Pacific Ocean lineage.

Bayesian clustering analyses provided clear evidence of genetic divergence among four genetic cluster; 1) the populations in north region, 2) the populations in Japan Sea side, 3) the populations in Pacific Ocean side and 4) the populations in Yakushima Island. Each linage was divided into two genetic cluster, this genetic structure was not inconsistent with previous reports of refugia from fossil pollen data. Based on coalescent theory, the DIY ABC analysis suggests that the four genetic clusters diverged at same time in late-glacial period. We also analysed the relationships between genotypes from several SNPs loci and environmental variables to obtain information on the selective pressures acting on individual populations. These results indicate that local adaptation and/or physical barriers to migration and gene flow among refugial populations, have promoted genetic divergence among these populations.

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P.44

Adaptive divergence of candidate insect defense genes in interior spruce

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Plant resistance to insect attack involves a complex mechanism, controlled by many genes. In this study, we selected 210 candidate genes for insect defense, and sequenced 18 individuals from each of three Interior spruce provenances: East Kootney (EK), Fort Nelson (FN), and Prince George (PG). These provenances span the range of Interior spruce in British Columbia. The first objective of our study was to find evidence for positive selection as given by dN/dS ratios (the ratio of non-synonymous codon changes to synonymous changes). We found dN/dS of most genes to be less than one, however it should be noted dN/dS is normally estimated for individual sites across a phylogeny; at least the ranking of dN/dS is informative about which genes are more subject to positive selection. These are presented. Interestingly, four genes had dN/dS values greater than one in EK, five in FN, and four in PG. Moreover, when the three provenances were merged, dN/dS of five genes were greater than unity. These genes are strong candidates for positive selection. A second objective of our study was to detect correlated changes between SNP loci across the three provenances; this is termed "selection covariance analysis". The correlation of SNP frequency changes between adjacent SNPs on the same gene were significant for five genes, showing that SNP changes in response to environmental pressures can be complex.

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P.45

Population structure and migration history of a poplar pathogen, *Mycospharella populorum*

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Due to the need for wood and wood products, the hybrid poplar industry has expanded rapidly over the last 100 years. The fast growing nature of hybrid poplars has enabled rapid breeding of desirable characteristics and bulk up of preferred genotypes via clonal propagation. This has encouraged frequent and massive exchange of poplar material (via cuttings) between Canadian provinces and American states. *Mycosphaerella populorum* Thompson is a leafspot and canker causing pathogen of poplar. The disease affects both quality and quantity of wood produced in hybrid poplar production and is considered the most important disease of poplar where it occurs. Inevitably, the intensification of poplar culture has resulted in intensification of the disease. The latest discovery of this pathogen was in the lower Fraser Valley in BC in 2006. In order to manage disease spread, prevent further incursions and ensure the sustainability of breeding programs, we need to discern the pathways of pathogen movement. We sequenced the genomes of 88 *M. populorum* strains collected from plantations in five Canadian provinces and six American states. Genetic clustering revealed a mid-US center of diversity for *M. populorum* with colonization pathways eastward following the distribution of the native host *P. deltoides*. The best-supported modeling scenario of the spread of the pathogen to BC is a recent introduction from an eastern source via massive or repeated introductions. This suggests that the movement of the pathogen is linked to the distribution of poplar cuttings.

P.46

Genotyping-by-sequencing as an economical method of SNP discovery in *Pinus contorta* and *Picea glauca*

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Genotyping-by-Sequencing is an economical way to reduce the complexity of a genome before sequencing to make the best use of expensive sequencing space. Here we present a modified protocol to produce GBS libraries that reduces hands-on sample preparation time and increases the total amount of library produced. We use the restriction enzyme *Pst1* to cleave the genomic DNA sample,and then we ligate double stranded adapters onto each end of the cleaved fragments. We then amplify the fragments that have the adapters correctly ligated using a high-fidelity polymerase, and perform a final size selection for fragments 400 - 600 bp using magnetic beads. We have sequenced a preliminary plate of 96 lodgepole pine samples using this technique. We aligned the GBS sequences to a draft the draft lobolly genome (PineRefSeq Project) using bwa. After filtering we identified 168,326 SNPs, but only 34,025 SNPs representing 9437 genes, were shared across 50 or more individuals. We plan to fine-tune our modified protocol for interior spruce and lodgepole pine and use the SNPs we discover to complement our sequence capture data to assess the genetic basis of adaptation to climate in those species. This will complement a large sequence capture data set that will capture SNPs across much of the exome. The GBS will allow us to expand our search to other areas of the genome, such regulatory regions or other genes not included in our re-sequencing project. It will also allow us to identify more putatively neutral loci that will allow us to assess the effects of demographic history on the genome.

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Quantitative Genetics and Tree Breeding

P.47

Genetic variation in second generation *Pinus elliottii* var. *Elliottii*

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Genetic parameters were estimated for growth traits in *Pinus elliottii* var. *elliottii* second generation half-sib families. Twenty five families harvested from a first generation seed orchard established in Colombo, PR, Brazil, were used in the study. The experiment was established in a randomized complete block design with thirty two replications of single plant plots. Height and dbh (diameter at breast height) were measured four years after planting. Deviance analysis and genetic parameter estimations were performed by using the best linear unbiased predictor (BLUP) and the residual maximum likelihood (REML) methods. A highly significant family variation in growth traits was detected. Narrow-sense individual heritabilities were estimated at 0.32, 0.14 and 0.37 for total height, dbh and volume, respectively. Average family heritability estimates ranged from 0.54 to 0.76 in dbh and volume, respectively. These results show promising perspectives to obtain substantial genetic gain, especially in wood volume growth, through either individual or family selections.

P.48

Phytopathology of a quantitative trait: identification of potential resistance mechanisms to *Didymascella thujina* in *Thuja plicata*

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Thuja plicata (western redcedar, WRC) is one of the most valuable trees in British Columbia due to the durability, dimensional stability and beauty of its wood. WRC is prone to infection by the foliar fungus *Didymascella thujina* (Cedar Leaf Blight, CLB), an airborne disease that is especially problematic in forest nurseries. Early studies with CLB in Europe noted that another species in the genus, *Thuja standishii*, was completely resistant to the blight, and that hybrids of the two species were equally resistant to the blight. However, all WRC trees studied were susceptible but with varying amounts of the disease between individual trees. More recently, the British Columbia Ministry of Forests, Lands and Natural Resource Operations research staff have shown quantitative variation among iWRC populations and families in CLB disease symptoms and that such potential quantitative resistance is significantly related to population or family origin. Trees originating in either drier, hotter or colder ecosystems tend to exhibit more disease symptoms than those from humid, cool environments.

Most studies on CLB have focused on chemical methods to control the disease, but little research exists on the underlying resistance or tolerance mechanisms. Our objective is to examine the histological characteristics of WRC that may be related to CLB resistance or tolerance. Two groups of WRC F_1 families, one that exhibited less disease symptoms and the other more, were assessed for stomatal density, epidermis thickness, leaf thicknesses, cuticle thickness, and lignin and flavonoid deposition by using compound-specific stains and a combination of light and fluorescence microscopy techniques. Preliminary analysis of the data depicts differences among families in cuticle thickness, lignin and flavonoid deposition. Cuticles were thicker in families exhibiting more disease symptoms, which may

be related to parental origin rather than a defence system against CLB. Differences in lignin and flavonoids deposition have also been seen, which may represent a complex defence system in resistance to CLB.

P.49

Application of joint modeling of competition effects and environmental heterogeneity in Douglas-fir trials using an individual-tree mixed model and Bayesian inference

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Forest genetic evaluation involves the use of mixed linear models to calculate "best linear unbiased predictors" (BLUP) of tree breeding values (BV). As BLUP depends on the values of the (co)variance matrices for the assumed model, the specification of the dispersion parameters should take into account both the negative correlation caused by competition among individuals, and the positive spatial correlation due to the environmental heterogeneity. Both phenomena, in any given experiment, are dynamic and coexist simultaneously. Therefore, modelling only one of these effects may lead to biases in the estimation of genetic parameters and the prediction of breeding values, and a joint model must be fitted to account for both sources of potential bias. As a first step in the analysis, we used several approaches to identify and quantify the competition effects (at genetic and environmental level) or/and environmental heterogeneity. Then, a joint individual-tree mixed model with direct genetic effects, genetic and environmental competition effects and a two dimensional B-spline smoothing surface to account for environmental heterogeneity (competition + spatial model), was applied to three Douglas-fir (*Pseudotsuga meniziessii*) progeny tests. This model was compared to three reduced individual-tree mixed models: a standard model with direct genetic effects only, a competition model including direct genetic and genetic and environmental competition effects, and a spatial model with a smoothing surface and direct genetic effects only. Three growth traits: diameter at breast height (DBH), total height (TH), and volume (VOL), were assessed at two ages (12 and 35 years old). This data set was composed of 78 parents and 165 families arranged within 10 diallels and included in a random complete block design with 4 replicates of 4 tree row plots. The traits DBH and VOL at age 35 years revealed strong competition effects at both genetic and environmental levels. There was also evidence of environmental heterogeneity for these traits. In general, the joint competition + spatial model gave a better fit (lower DIC value) than the simpler models on the three test sites. With strong competition genetic effects (i.e., correlation between direct and competition additive genetic effects higher than -0.3) the standard model yielded additive variance estimates that were smaller (from 39.5% to 50.6%) and residual variances that were higher (from 16.3% to 45.0%) than those estimated from the competition + spatial model. Ignoring the genetic and environmental competition effects leads to overestimating environmental heterogeneity; i.e., the spatial model yielded variance estimates of the random knots effects that were higher than those of the competition + spatial model. Ignoring the environmental heterogeneity leads to underestimating genetic and environmental competition effects; i.e., the competition model yielded direct and competition additive correlation estimates that were smaller (from 6.0% to 71.5%) and environmental competition variance that were higher (from 6.3% to 29.4%) than those of the competition + spatial model. The potential impact that the simultaneously adjusting for competition genetic effects and environmental heterogeneity has on the selection will be discussed with respect to the Douglas-fir genetic improvement program.

P.50

Genome-wide effects of selective breeding on adaptation of reforestation seedlots for future climates

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Climatic change is predicted to cause a lag in the productivity of western Canada's forests that will have negative economic, ecological, and social impacts. Such a lag in productivity is caused by shifting climates that are expected disconnect locally adapted provenances from their established climatic optima. To assess the impacts of climate change on forests in British Columbia and Alberta, the AdapTree project is utilizing a combination of genomic and phenotypic approaches to quantify the genetic architecture of local adaptation to climate and to assess climate-based seed transfer approaches.

The component of AdapTree described here will investigate the effects of selection in tree breeding programs on adaptive diversity and climate-related phenotypic traits in the economically and ecologically important species lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca* (Monech) Voss*, P. engelmannii* Parry ex Engelm. and their natural hybrids). In Western Canada the use of reforestation seedlots from advanced generation selective breeding programs is increasing rapidly and accounts for a majority of reforestation. The genetic effects of selective breeding on long-domesticated crops species are well documented, but the genomic consequences of selective breeding on adaptive forest diversity and divergence, as well as the suitability of reforestation seedlots to future climates remain unclear. We are evaluating how selective breeding affects the adaptive phenotypic and genomic architecture of reforestation seedlots. The primary questions that guide this research are: 1) How much do climatically-linked phenotypic traits differ between natural and seed orchard populations; 2) Do correlated responses to phenotypic selection exist between height as the primary artificial selection trait and other phenotypic traits of importance to local adaptation; 3) What are the effects of selective tree breeding on adaptive genetic diversity in reforestation seedlots; and 4) How much molecular genomic divergence exists among natural and seed orchard populations from the same geographic areas, and does the amount of adaptive genomic divergence reflect phenotypic divergence?

A combination of analysis techniques that dissect the effects of selective breeding on adaptive phenotypic and genomic diversity and divergence in both species are being applied. Seedlots have been sampled across British Columbia and Alberta to obtain representative natural (>250 seedlots per species) and selectively bred reforestation seedlots (~20 orchard lots per species. Seedling common gardens containing N>2,500 individuals per species have been established at UBC and at a field site in the central interior of BC. Phenotypic data is being collected on several climatically relevant phenological and growth traits. All trees will be genotyped for a suite of ~25,000 to 50,000 candidate adaptive SNPs being identified through exome capture and re-sequencing, and analyses including associations with provenance climate or phenotypes and outlier tests in other activities within AdapTree.

This research will allow evaluation of current provincial reforestation seedlot diversity standards in the context of adaptive rather than neutral genetic variation, and prediction of future climatic seedlot transfer ranges. This will contribute vital information to developing provincial policies on assisted genotype migration that aim to maintain forest productivity in British Columbia and Alberta under a changing climate.

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P.51

Genotypic selection in *Dipteryx alata* Vog. in the midwest region of Brazil

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The success of forestry breeding programs is closely linked to the quality of seed-trees selected to constitute their respective base population. The seed-trees selected, according to the evaluations of progeny tests, gathers, among its features, the genetic merit transmitted to subsequent generations. The choice of species to be improved is also one of the important points to think about when conducting a breeding program because it has to present attractive qualities to attract the market. Thus, it was deemed advisable to study the *Dipteryx alata*, but known as baru, which belongs to the family Fabaceae, is an arboreal species of the cerrado and is characterized by possessing excellent qualities of products like wood and chestnuts. This work aimed to select progeny of *Dipteryx alata*, aiming at genetic improvement of the base population, constituted of three provenances. At eight years of age made up the character of the measurement diameter at breast height (DBH). The experimental design was random blocks, with three populations, with 16 progeny to GO, 42 progeny for MG and 39 progeny for MS, five repetitions and six of plants per plot, with spacing 3.0 x 3.0 m. Estimates of variance components and genetic parameters were obtained by REML/BLUP. From these estimates was made a selection of 50% of the best individuals, so we selected 232 plants from GO, 561 plants from MG and 570 plants from MS. The average estimate for the DBH of 463 individuals analyzed was 7.42 cm for GO, 7.80 cm of 1,121 individuals analyzed for MG and 9.45 cm of 1,140 individuals analyzed for MS. Thus, for a selection of 50% the best genotypes the DBH of new populations would become 9.63 cm for GO, 8.93 cm for MG and 10.29 cm to MS, that is, a gain of 29.8% for the new generation for GO, 14.5% for MG and 8.9% for MS. The selection, based on DBH, at eight years of age, in this progeny/provenances test, would result in considerable genetic gains and still keep the populations with sufficient genetic basis for new selections to be practiced in the future in order to produce wood or chestnut high nutritional value.

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Genetic variability in progenies *Dipteryx alata* Vog. coming in three states of Brazil

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Dipteryx alata, known as baru, is an arboreal species of the cerrado characterized by having a relatively fast growth, able to grow and develop in poor soils, producing high quality wood, primarily for use in the construction industry. In his adulthood, the baru fruits annually and produces nuts with high commercial value. By having different qualities, this species has been explored intensively, being endangered. Thus, this study aims to evaluate the genetic variability for diameter at breast height (DBH) and survival in progeny of three natural populations of *Dipteryx alata*, to provide subsidy for conservation programs and breeding. In April 2004 we installed a progeny test with three natural populations of *D. alata* coming from the states of Minas Gerais (MG), Goiás (GO) and Mato Grosso do Sul (MS) in Farm of Teaching, Research and Extension of the Faculty of Engineering of Ilha Solteira/UNESP, located in Selvíria-MS. At eight years of age made up the character of the measurement diameter at breast height (DBH) and survival. The experimental design was the random blocks with three populations, 16 progeny to GO, 42 progeny to MG and 39 progeny to MS, five repetitions and six of plants per plot, with spacing 3.0 x 3.0 m. Estimates genetic parameters were obtained by the method of linear mixed model univariate additive software SELEGEN - REML/BLUP (restricted maximum likelihood / best linear unbiased prediction). The progenies of all populations showed good adaptation, with a survival rate of around 97%. The progenies coming from MS showed the greater DBH, with 9.45 cm, corresponding to a mean annual increment (MAI) of 1.18 cm, which was expected by the test to be installed in the same region. Then were the progeny of MG with 7.80 cm (MAI = 0.98 cm) and GO with 7.42 cm (MAI = 0.93 cm). The variation coefficient was good for all populations ranging from 13:23% (MS) to 17.77% (MG). The accuracy, which represents the ratio between the genetic value true and the estimated was high, ranging from 82.20% (MS) to 92.61% (GO). Based on the likelihood ratio test (LTR) it is noted that the

three populations showed variation for the character DBH in the progenies of *D. alata*. The same is not true for survival in which there wasn't difference between the progenies coming from MG and GO, but had a variation coming from the MS, in other words, at least one of the progenies have survival different from the other. For the character DBH, the coefficient of heritability of individual additive effects, coefficient of heritability of mean progeny and heritability additive within plot were 0.48, 0.70 and 0:49 in provenance MG, 0.72, 0.86 and 0.73 in provenance GO and 0.35, 0.68 and 0:31 in provenance MS, respectively. Comparing the values of heritability, the coefficients of heritability on a progeny mean were higher than within progeny, indicating that the selection level mean is more likely to gain from selection based on plot or individual plants. The coefficient of additive genetic variation individual was greater than the coefficient of genotypic variation among progenies in the three populations, with 24.34% and 12.17% for MG, 36.04% and 18.02% for GO and 17.07% and 8.54% for MS, respectively. In the joint analysis there was a significant difference between the populations, and the population of Brasilândia (MS) contributes to positive genotypic values and Campina Verde (MG) and Itarumã (GO) with negative values. Thus, in a breeding program, should emphasize the importance of provenance of the material to be collected. Thus, it was found that the populations of *Dipteryx alata* have a genetic basis and significant difference between them, which highlights the importance of preserving these as an active germplasm bank, to serve as the base population for breeding programs.

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Investigation of intra-species variation in lodgepole pine (*Pinus contorta*) secondary metabolite synthesis as a defense against *Dothistroma septosporum*

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Over the past century the world's forests have faced increasing pressure from a variety of sources. From a forestry perspective, fungal diseases alone have caused considerable damage in terms of growth loss and costs of control measures. The damage caused by fungal pathogens has been especially severe in British Columbia, where native lodgepole pine (*Pinus contorta*) has suffered high mortality to foliar fungal pathogens such as *Dothistroma septosporum*. While chemical agents can be used to combat these pathogens in small plantations, their use over larger areas is considered impractical, and poses a potential threat to the native ecosystem. The preferred methods of control include enhancement of species and age class diversity, or selection for resistant species.

Resistance to fungal pathogens can be conferred through a variety of mechanisms. For lodgepole pine these have been found to include higher levels of defence chemicals: Greater levels of some secondary metabolites have been correlated with heightened resistance to foliar fungal infection. A high-throughput assay system was developed to determine the toxicity of these compounds to *D. septosporum* in media. Using this system, we have shown that a number of secondary metabolites associated with disease resistance also inhibit growth of *D. septosporum* in broth culture. Current work aims to correlate provenance metabolite profile differences with expression changes of producer synthases, and the disease history of the original provenance locations.

An improved knowledge of the molecular basis for *Dothistroma* resistance will aid in the development of resistant varieties, and future correlation with disease history will improve prediction of outbreaks in a changing climate.

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