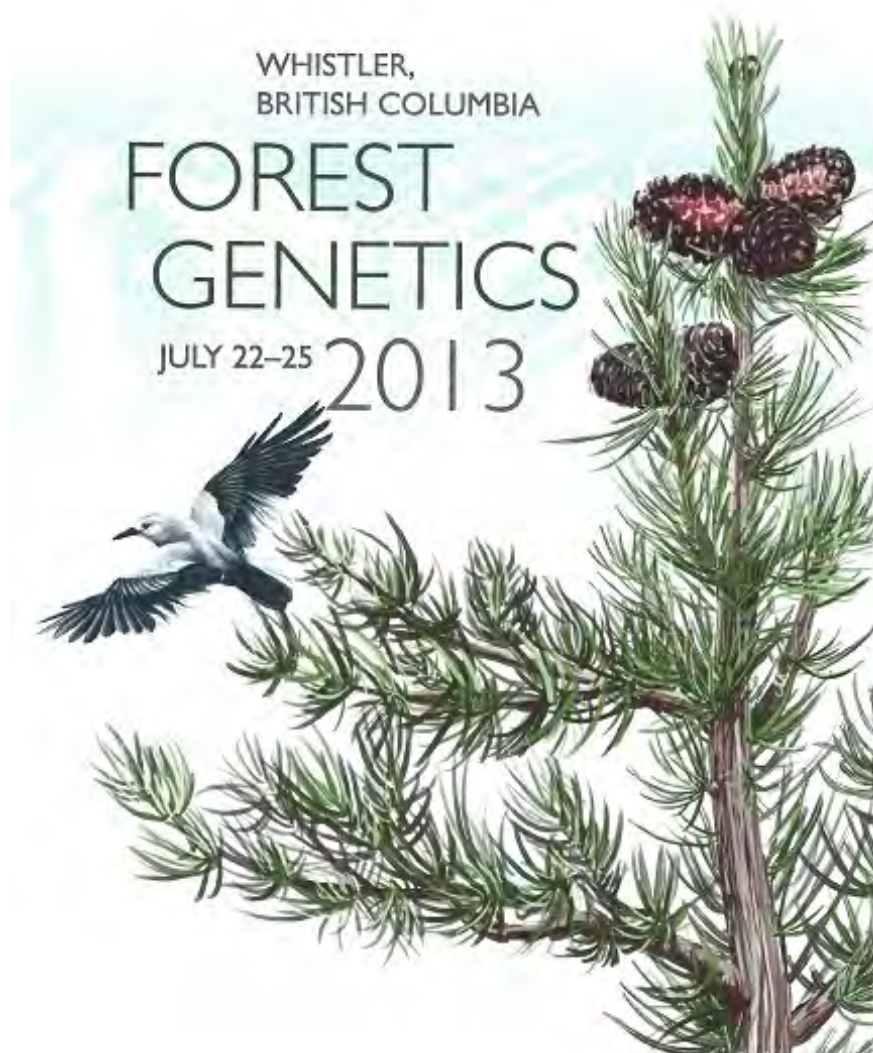


**PROCEEDINGS OF THE THIRTY-THIRD MEETING
OF THE
CANADIAN FOREST GENETICS ASSOCIATION**

PART 1 Minutes and Members' Reports
PART 2 Symposium



**COMPTES RENDUS DU TRENTE-TROISIÈME CONGRÈS
DE
L'ASSOCIATION CANADIENNE DE GÉNÉTIQUE FORESTIÈRE**

1^{ère} PARTIE Procès-verbaux et rapports des membres
2^e PARTIE Colloque

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Canadian Forest Genetics Association. Meeting (33rd : 2013 : Whistler, BC)

Proceedings of the Thirty-third Meeting of the Canadian Forest Genetics Association

Includes preliminary text and articles in French.

Contents : Part 1. Minutes and Member's Reports. Part 2. Symposium.

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3. Forest genetics – Canada – Congresses.
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Comptes rendus du trente-troisième congrès de l'Association canadienne de génétique forestière

Comprend des textes préliminaires et des articles en français.

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**ADVANCES IN SPRUCE GENOMICS AND QUANTITATIVE GENETICS AT
THE CANADIAN FOREST SERVICE, CANADIAN WOOD FIBRE CENTRE,
QUEBEC REGION**

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Keywords: white spruce, black spruce, *Picea glauca*, *Picea mariana*, adaptation, wood quality, open-pollinated families, pedigree, SNPs, association mapping, genomic selection, somatic embryogenesis, assisted migration

This report summarizes the progress made by the Canadian Forest Service (CFS) - Canadian Wood Fibre Centre (CWFC) in forest genetics, genomics, molecular ecology, and biodiversity for the 2011–2013 period. Our research activities were mainly focused on spruces. This work was made possible through grants obtained from the NRCan–Forest Innovation Program, the CFS–Genomics R&D Initiative, and through our participation in the SMarTForests project (J. Mackay, J. Bohlmann, J. Bousquet, K. Ritland, and collaborators), a major initiative funded by Genome Canada, Genome Quebec, and Genome BC to develop molecular breeding in softwood trees. This research was also partly supported by the Fonds de recherche du Québec - Nature et Technologies (FRQ-NT), thanks to a grant awarded to J. MacKay, J. Beaulieu, and collaborators. We also continued investing considerable time and effort in population genetics studies of forest tree species as part of our participation in the research activities of the Canada Research Chair in Forest and Environmental Genomics (Tier 1). Other research activities on the genetics and physiology of white spruce (*Picea glauca*) seedlings and clones were also conducted in collaboration with Hank Margolis of Université Laval and Mohammed Lamhamedi of the Ministère des Ressources naturelles (MRN), with funding received from FRQ-NT.

Research accomplishments were made possible thanks to the generous contribution of dedicated staff, graduate students, postdoctoral fellows, and collaborators who deserve our recognition. These are among others: Julien Prunier, Alain René Atangana, Sylvie Carles, Nadya Wahid, Brian Boyle, Cuauhtémoc S.-Romero, Sylvie Blais, France Gagnon, Daniel Plourde, Éric Dussault, Philippe Labrie, Esther Pouliot, Isabelle Lamarre, and Pamela Cheers.

SPRUCE GENOMICS

During the last two years, grants were obtained from the CFS–Genomics Research & Development Initiative (GRDI) and the NRCan–Forest Innovation Program to carry out association studies and genomic selection of wood traits in white spruce. These grants were used in conjunction with funds awarded to the SMarTForests project, a major initiative funded by Genome Canada, Genome Quebec, and Genome BC to develop molecular breeding in softwood trees and co-led by J. Mackay and J. Bohlmann (U. Laval and UBC, respectively).

A discovery population of about 1,700 white spruce was assembled by sampling trees in a 30-year-old provenance-progeny test. Increment cores were collected on these trees and phenotypic values were assessed for a variety of wood traits and properties at FPInnovations–Paprican’s facilities in Vancouver. Radial profiles of over 25 wood physical properties were obtained for each of the 1,700 trees. A high-density SNP genotyping array was built to genotype the population using the Illumina Infinium iSelect genotyping bead chip (Pavy et al. 2013). Thus, trees were genotyped for more than 6,300 SNPs mined in

2,660 gene sequences. Association mapping as well as genomic selection studies were carried out to estimate genomic estimated breeding values (GEBV) and estimate the gains expected from selection based on DNA marker information. The number of SNPs associated with wood traits was rather limited when a false discovery rate correction was applied to control for multiple testing despite the large population size and large number of SNPs involved. Moreover, as expected for quantitative traits following Fisher's theory, the percentage of variation in wood traits explained by each significant SNP was very low (less than 5%). While association mapping makes it possible to identify genes that are involved in the control of economically important traits, the information obtained cannot be easily used by breeders to help them develop more efficient breeding strategies. Genomic selection, on the other hand, rather than seeking to identify loci significantly associated with a trait, uses all marker data to predict the performance of individuals or their breeding value. Selection can then be based on genomic selection predictions. We thus carried out genomic selection studies on the white spruce discovery population assembled using all 6,300 SNPs or subsets of SNPs identified after association studies, but without applying a correction for multiple testing. The discovery population was divided into training and validation datasets in order to estimate the accuracy of predicted GEBV. The models showing the most accurate estimates were those built with subsets of SNPs having the largest effects on wood and growth traits. The accuracy of GEBV was over 0.5, and was slightly higher than that achieved through traditionally estimated breeding values. The accuracy of GEBV obtained after cross-validation with unrelated individuals was also in the same range, which was unexpected and which suggests the presence of shared ancestry in extant white spruce populations. Genetic gains per unit of time obtained with genomic selection were up to twice those expected using traditional approaches. These results indicate that genomic selection could likely be successfully implemented in this economically important species.

In collaboration with the Canada Research Chair in Forest and Environmental Genomics (CRC-FEG) (J. Bousquet), a scan involving over 1,100 SNPs from about 700 expressed genes was used to assess the potential impact of artificial selection for height growth on the genetic diversity of white spruce. Two case populations of different sizes simulating different family selection intensities ($K = 13\%$ and 5% , respectively) were delineated from the Quebec breeding population. Their genetic diversity and allele frequencies were compared with those of control populations of the same size and geographical origin to assess the effect of increasing the selection intensity. The two control populations were also compared to assess the effect of reducing the sample size. As a result of all population pairwise comparisons, we found that genetic parameters were comparable and no alleles were lost in the case populations compared with the control ones. The only exception was for few rare alleles in the large case population. The distribution of allele frequencies did not change significantly between populations, except for about 10 SNPs showing allele frequency changes between case and control populations of the same size. Globally, the results indicate that neither the reduction in sampling size nor the increase in selection intensity was sufficient to induce any significant change in the genetic diversity of the selected populations. Apparently, no loci were under strong selection pressure, confirming that the genetic control of height growth in white spruce involves many genes with small effects (Namroud et al., 2012).

The genomic architecture of adaptive traits in black spruce (*Picea mariana*) was also examined using SNPs developed in the context of other projects carried out with the CRC-FEG as well as provenance-progeny tests comprised of seed sources from the province of Quebec and a backcross family clonally replicated on two sites. Outlier detection methods as well as association studies and QTL mapping were used to identify genome regions and gene SNPs associated with these adaptive traits. QTL mapping made it possible to identify 22 unique genomic regions that were related to budset timing and tree height assessed over multiple years and sites. Similarly, outlier detection and bulk segregant analyses made it possible to identify 52 candidate SNPs associated with the same traits. These SNPs were subsequently tested in genetic association studies. This research highlighted the fact that some genomic regions as well as some significant SNPs were shared between budset timing and tree height in black spruce. This is likely an indication of the presence of pleiotropic effects of some genes (Prunier et al. 2013). Another study addressing the issue of adaptation to climate in boreal black spruce as well as the potential effects of historical events on the geographical distribution of adaptive genetic polymorphisms had also been carried out previously using 41 populations distributed across its natural range. Forty-seven candidate gene SNPs were tested for relationships with climatic factors using an outlier method and regressions between allele frequencies and climatic variations. It was possible to identify 23 adaptive genetic polymorphisms that were related to temperature and/or precipitation. Two main intraspecific lineages related to glacial vicariance were also detected at the transcontinental scale. It was possible to conclude that the standing genetic variation in the potentially adaptive nature of black spruce was modified by historical events, leading to different adaptive routes between both intraspecific lineages.

Thanks to collaboration with the Canadian Space Agency (CSA-ASC) and the NASA, we carried out a study on the effect of weightlessness on gene expression in white spruce. Hence, 18 seedlings produced through somatic embryogenesis (SE) at J.D. Irving, Limited facilities in New Brunswick were grown both at the Space Life Sciences Laboratory (SLSL) of the Kennedy Space Center and at the Destiny Laboratory of the International Space Station (ISS) for 30 days. Plants were placed in closed environment incubators under controlled light, temperature, humidity, and CO₂. At the end of this period, the leading shoot from three plantlets of each of the three lines tested were sampled and pooled in Kennedy Space Center Fixation Tubes containing a RNA stabilization solution. Samples grown at the ISS were brought back to Earth to the Kennedy Space Center by space shuttles and then transported to Quebec City along with samples collected on plants raised at the SLSL. Transcript levels were determined by quantitative real-time polymerase chain reaction for 27 candidate genes and three reference genes on the nine seedlings grown in each environment. About two-thirds of the 27 genes produced a larger number of transcript molecules in microgravity conditions. However, only three genes showed significant differences between the two environments, and all of them were upregulated in microgravity. These genes appear to be involved in important processes such as cell propagation, plant development and response to stress, and their upregulation likely contributed to influencing seedling growth patterns (Beaulieu et al. 2013).

QUANTITATIVE GENETICS

Quantitative Genetics of Wood Properties

In collaboration with J. Mackay (U. Laval), we assessed acoustic velocity as a selection trait for the improvement of mechanical properties in two 15- and 32-year-old white spruce genetic tests (Lenz et al. 2013). We found that individual heritability of acoustic velocity was moderate and of the same magnitude as that of wood density (≈ 0.40), and that considerable genetic gain could be expected when selecting for this trait or for a combination of wood density and acoustic velocity. The relationship between the latter trait and cellulose microfibril angle (MFA) was strong at the genetic level and selection for acoustic velocity could effectively improve MFA, which is an important determinant of wood mechanical properties. The relationship between acoustic velocity and tree height, although positive, was weak. Both traits could nevertheless be simultaneously improved. Overall, juvenile acoustic velocity appears to be an appropriate trait to select for wood quality in a white spruce breeding program.

Other Quantitative Genetics Studies

In collaboration with H.A. Margolis (U. Laval) and M.S. Lamhamedi (MRN), and with funding obtained from FRQ-NT, genetic variability of morphological and physiological characteristics of containerized white spruce seedlings of 75 open-pollinated families was investigated to determine whether genetic selection for improved seedling root systems was possible. Seedlings were grown for two years in a forest nursery and various traits, including height, diameter, shoot and root dry mass, and root to shoot ratio, were measured at the end of the second growing season. Genetic control of these various traits varied from 0.21 to 0.97 at the individual level and from 0.34 to 0.88 at the family level (Carles et al. 2012). The strong and positive genetic correlation between root collar diameter and root dry mass (0.68) suggests that indirect selection for a stronger root system could be made using root collar diameter.

To evaluate the influence of white spruce seed orchard material on seedling growth and morphology, seedlings produced from seeds originating from the 10 most commonly used first-generation white spruce seed orchards in Quebec were raised under similar nursery conditions. Seedlings from each seed orchard were destructively sampled at two-week intervals during the second growing season. Height and root collar diameter of each seedling was measured, and the above- and below-ground portions of the seedlings were oven-dried to determine shoot and root dry masses. Growth curves for height and diameter for each seedling were fit to a logistic model and were used to compare the growth rhythm of the seedlings from the 10 seed orchards. Results made it possible to show that two-year-old white spruce seedlings produced with seed from geographically and genetically distinct seed orchards and raised under similar nursery conditions differed in height and shoot dry mass, but not in diameter or root dry mass. Two groups of seed orchards could be distinguished based on above-ground seedling traits, namely those located in the sugar maple bioclimatic domain, which produced taller seedlings, and those located in the balsam fir bioclimatic domain. These results support the idea that the present seed zones could be

broadened and aligned with the two breeding zones delineated for white spruce in Quebec (Carles et al. 2011).

There is renewed interest for clonal forestry in Eastern Canada because it can lead to significant increases in forest productivity and help respond to the demand of the global market that is continuously increasing. It is in that context that Quebec's MRN has recently built new facilities to produce several million white spruce SE-derived seedlings every year. The use of SE in clonal forestry requires an accurate assessment of genetic parameters and of the performance stability of clones in plantations. Studies were thus carried out to estimate genetic parameters and clonal variation in growth and nutritional traits of containerized white spruce somatic seedlings in the nursery and performance stability in clonal tests using seed from selected full-sib families (Wahid et al. 2012a,b, 2013). Results showed highly significant clonal effects for most growth traits analyzed at the end of two growing seasons in the nursery as well as for the seedlings' mineral status at the end of the second growing season. The broad-sense heritability of seedling height was 0.60 at both age 1 and 2. At the end of the nursery experiment, the two-year-old containerized somatic seedlings were planted in two clonal tests in abandoned bare-root beds at two provincial forest nurseries. Four years after establishment of the clonal tests, ramets were measured for several growth and morphological traits. The results showed that the SE clones exhibited large variability in growth and branch characteristics, particularly for branch length and diameter. Heritability estimates were lower than those observed in the nursery, presumably because a planting shock effect was still present. Although there was a significant site effect for most traits, the genotype x site interaction was low. Consequently, the correlation between the two sites for the same characteristic was high. There is a clear indication that the performance of SE seedlings is stable between sites and that there is some opportunity for the selection of clones that adapt and perform well over different ecological regions in Quebec.

Quantitative genetics studies were also carried out in collaboration with Professor Cuauhtémoc Sáenz-Romero during his sabbatical stay in Quebec City. Fourteen *Pinus patula* populations were sampled in the state of Oaxaca, Mexico, along an altitudinal gradient (2,400 m to 3,050 m). Seedlings were grown in growth chambers at the Laurentian Forestry Centre under two temperature treatments aiming to simulate current temperature (average of 17°C) and a future global warming scenario (average of 21°C). Seedlings were evaluated after a second growing season (between 8 and 12 months of age). It was possible to show that the populations differed significantly for seedling height and aerial dry weight, and that they exhibited a significant although weak altitudinal clinal pattern, with populations from lower altitudes having on average taller and heavier seedlings than populations from higher altitudes (Sáenz-Romero et al. 2011). However, despite the fact that the mean height and dry weight of the aerial part of the seedlings growing under simulated climate change temperatures was, on average, 4.6% higher than that of seedlings growing under current temperature, the differences were not statistically significant.

OTHER RESEARCH

Assisted migration or colonization is a strategy envisioned by forest geneticists to mitigate the negative effect of climate change on tree species. In a paper published in *The Forestry Chronicle* (Pedlar et al. 2011), we outlined what we consider to be the major steps that should be involved in the implementation of assisted migration and assessed the capacity to carry out each step in Canadian forests. In a second paper, we discussed the concept of assisted migration, showing that implications clearly diverge when it is applied to species rescue than when it is applied to the maintenance of forest productivity in the context of forest operations (Pedlar et al. 2012).

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