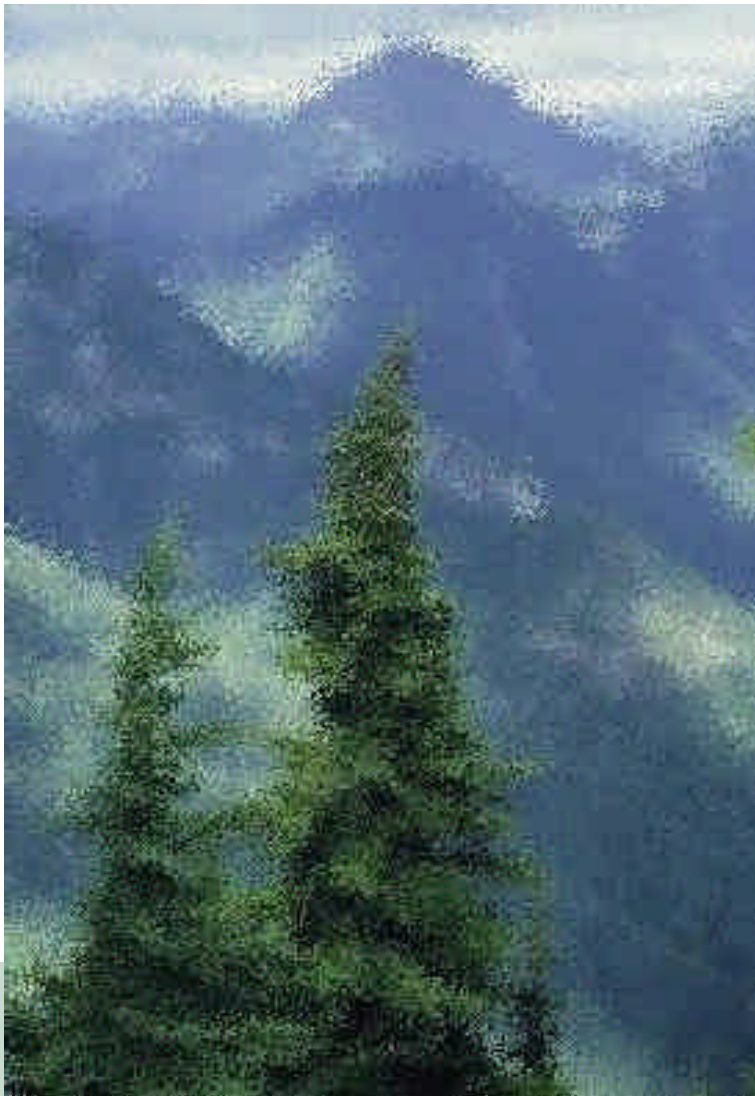


WFGA 2003



Genomics & Adaptation

July 28 - 31, 2003
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PRESENTATION ABSTRACTS

Tuesday July 29

Genomics and population structure

Functional genomics in spruces and poplars: A case study on terpenoids, volatile emissions, direct and indirect defence

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Oleoresin terpenoids and non-resin terpenoids form the predominant chemical defense system of conifers. Resin terpenoids are thought to protect trees against potential herbivores and pathogens, including bark beetles, weevils, and insect associated tree-pathogenic fungi. Airborne terpenoid volatiles have important functions as info-chemicals in host identification by bark beetles and can serve as precursors in bark beetle pheromone formation. Herbivore-induced terpenoid volatiles emitted from conifers and poplars could function as alarm signals of trees in beneficial, tritrophic plant-insect interactions. However, terpenoids (e.g. resin acids) are also problem-chemicals during wood processing and pulping. Volatile terpenoids released from conifers such as isoprene, methyl butenol and monoterpenes are a major source of global, non-anthropogenic reactive carbon emission.

Our research addresses biochemical, molecular, and functional ecological aspects of terpenoids in conifers and poplars. Terpenoid synthases (TPS) are responsible for the chemical diversity and plasticity of conifer terpenoid defenses. We have identified a large family of TPS genes from 5 conifer species (Norway spruce, Sitka spruce, White spruce, Grand fir, and Douglas fir) and discovered a new TPS gene from poplar. We reconstructed a phylogeny of extant TPS biochemical functions, and investigated mechanisms of insect-induced terpenoid accumulation and emission of terpenoid info-chemicals in spruce and poplar. In addition to a family of diverse TPS genes, we have identified by genome mining of more than 30,000 spruce ESTs many cDNAs of the MEP- and mevalonic acid-dependent isoprenoid pathways, prenyl transferases, and cytochrome P450 enzymes of diterpenoid oxidation. These cDNAs are candidates for biochemical, functional characterization. In species of spruce, induced expression of terpenoid defense genes and enzymes is associated with cell differentiation of traumatic resin ducts in developing stem xylem and de novo terpenoid emission from foliage.

Tuesday July 29, 9:15 am

The Floral Genome Project and comparative analysis of sequence and expression of florally expressed genes.

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Project Web Address: <http://www.floralgenome.org>

Much of the knowledge about genes controlling flower development is derived from studies of a few model plants such as *Arabidopsis*, *Antirrhinum*, petunia, maize, and rice, which are members of the eudicot and monocot groups. At the same time, a great deal of floral morphological diversity is found among the basal angiosperm and basal eudicot lineages. How the floral developmental program originated and diversified, and how much of what we already know from models is generally applicable to floral development in other species, remain central questions. The Floral Genome Project (FGP) aims to identify florally expressed genes from 13 angiosperm species that occupy important phylogenetic positions, including the forest tree species *Liriodendron tulipifera* and two gymnosperm species. Thousands of ESTs are being generated from early (premeiotic) floral bud libraries, and a subset of complete cDNA sequences will be determined from selected species. Phylogenetic analysis will be performed with many gene families to identify homologs of known floral regulatory genes and to uncover new floral genes and gene families. Microarray and in situ hybridization will be used to determine their expression patterns. The FGP sequences and expression data will be compared with data from other species to derive a consensus set of floral regulators. At the same time, genes that might be specific to a lineage or responsible for distinctive structural properties can be identified and characterized. Based on the first 25,000 EST sequences sampled from six species, the project has identified homologs of a wide range of known floral regulators in the most basal lineages of angiosperms as well as gymnosperms and basal eudicots. Thousands of additional genes have been detected; most of these are the first representatives of their respective gene family in any basal angiosperm. New statistical analyses have been used to consistently define all gene families from *Arabidopsis*, rice, and other species, and serve as a basis for comparative analysis of plant genes. These results suggest a striking similarity of floral transcriptomes at the gene family level, leading to the conclusion that the ancestral angiosperm probably had a diverse 'tool kit' of floral regulators and other florally expressed genes. Progress during the first 1.5 years of the project will be presented and implications for adaptation of floral development in trees will be discussed.

Detecting heritability in the bush: inheritance of defense chemistry in *Eucalyptus melliodora*

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We used microsatellite-inferred relatedness to study the heritability of defense traits in a natural *Eucalyptus melliodora* population. This method is potentially useful for studying the evolution of quantitative defense traits, due to the limited applicability of common-garden results to natural systems. Alternatives are particularly desirable for long-lived organisms, such as forest trees. Adult chemical profiles appear after several years in *Eucalyptus* and their strong spatial genetic structure makes them ideal candidates for the measurement of heritability in natural conditions, which relies upon the detection of significant actual variance in relatedness. Microsatellite genotypes for 225 adult trees showed significant spatial autocorrelation at a spatial scale of 60m. We examined concentrations of sideroxylonol, the formylated phloroglucinol compound responsible for limiting intake of mammalian and insect herbivores in this species, and 1,8-cineole, the major component of eucalyptus oil whose concentration constrains the rate of feeding. These compounds are highly correlated and patchily distributed within populations. Using Ritland's marker-based heritability method, we detected significant actual variance of relatedness and heritability for each compound, as well as genetic covariance between the traits. These results show that marker-based quantitative genetic methods can be useful tools for studying the evolution of quantitative defense traits in *Eucalyptus*.

Tuesday July 29, 9:55 am

Somatic embryogenesis of loblolly pine is useful as the foundation of a testing system for investigation of wood quality and growth genes

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ArborGen is using somatic embryogenesis of *Pinus taeda* (loblolly pine) for rapid capture of genetic gain. Methods have been demonstrated to be adaptable to most families. From families requested by customers of ArborGen in the United States and Brazil, individual lines captured number in the thousands, and material is being supplied for clonal tests. Among 6000 elite lines tested, 12% produced at least 200 somatic embryos per gram of tissue.

This robust SE system has provided a platform on which to build the use of transformation to test genes that may be involved in wood quality and growth. We improved pine tissue culture, transformation and selection methods such that stable transgenic lines were obtained from an average of 72% of the lines in all of 15 elite families tried, plants are routinely regenerated from transgenic lines even in a recalcitrant elite family, and false positives decreased to less than 1%. Over the past few years almost 1000 transgenic pine trees have been planted in field trials, including tests with lines from only elite families. Both the ortets and cuttings from transgenic loblolly plants have shown comparable survival to non-transgenics, and transgene expression after two propagation cycles is comparable to expression in transgenic ortets.

ArborGen's genomic platform is based on over 22,000 contigs each from pine and Eucalyptus, derived from a database of over 630,000 ESTs. Data from an initial scale-up example comprising about 1700 trees from an elite family being used for testing of genes involved in lignin biosynthesis will be presented, showing how different constructs for a single gene can differ in throughput. The experiments described above laid the foundation for high-throughput gene testing in a gymnosperm. Over 7000 transgenic pine trees are ready for field establishment this year, and more than 3600 trees are already in line for tests next year. Transformation will uniquely enable us to examine metabolites produced by manipulating pathways that differ between hardwoods and softwoods. These trees will be also be used for detection of the effects of specific genes, information that could be valuable in transgenic applications, marker-assisted selection, and combinations.

Deciphering the spruce and poplar genomes: physical, functional and comparative genomics

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The Genome BC Forestry project is taking a combined structural, functional and comparative approach towards understanding the genomics of spruce (*Picea* spp.) and poplar (*Populus* spp.) with an emphasis on forest health and wood formation traits. A suite of specialized cDNA libraries has been developed for both species from a range of tissues (xylem, phloem, bark, foliage, roots) at different developmental stages of seedlings and mature trees, as well as from trees exposed to biotic stress (insect herbivory), chemical elicitors (MeJA), or mechanical wounding. Thus far, we have obtained 40,000 spruce and 20,000 poplar 3' ESTs of the 100,000 planned for each species. cDNA normalization strategies have been implemented to minimize sequence redundancy. Our program is emphasizing the construction of full-length (FL) cDNA libraries so that through a targeted strategy, we will completely sequence ~5,000 spruce and ~10,000 poplar non-redundant FL-cDNAs. This EST and FL-cDNA collection will be the basis for gene- and protein expression profiling, marker development and mapping, phylogenetic comparisons, functional gene discovery, and biochemical gene characterization. We have assembled cDNA microarrays consisting of ~16,700 and ~4,000 unique genes in spruce and poplar, respectively, to examine gene expression profiles associated with stress response and stress adaptation, wood formation and development. Proteomics is being applied to dissect expression profiles during spruce embryo development and stress response. As part of the international effort to sequence the poplar genome, we have constructed a physical map by fingerprinting ~46,000 BAC clones from the *P. trichocarpa* Nisqually-1. Both ends of approximately 44,500 BAC clones have been sequenced, which will allow scaffolding of the poplar whole genome shotgun sequence to the physical map and will facilitate meshing of the physical map to poplar genetic maps. For syntenic comparisons and quantitative trait loci mapping, we have identified 230 simple sequence repeats (SSR) from our spruce EST collection, and together with some published SSR primers, have assembled a battery of 37 SSR primer pairs which give interpretable SSR loci across the majority of 23 spruce species examined. We are also developing a strategy for SNP detection and assay, for the eventual genetic mapping of ESTs. Finally, we are identifying conserved orthologous sequences for comparative mapping over greater phylogenetic distances.

Tuesday July 29, 11:10 am

Clonal diversity, fine-scale genetic diversity, and genetic structure in *Chamaecyparis nootkatensis* populations.

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Layering, a form of clonal propagation, has been documented to occur in *Chamaecyparis nootkatensis* (yellow-cedar), but little is known about the frequency and spatial extent of this phenomenon. Microsatellite markers were used to infer the spatial extent of clonally-derived individuals in three natural populations of *C. nootkatensis* and investigate the potential effects of clonal reproduction on fine-scale genetic diversity and genetic structure in this species. Clonal extent was found to vary greatly between populations and no clear relationship between clonal extent and specific habitats was detected. Clonal diversity showed that on average ~23% of the trees sampled were clonally-derived. Fine-scale genetic structure and the coefficient of inbreeding increased with clonal extent. The greatest effect of clonality on fine-scale genetic structure was detected between trees separated by approximately five meters or less (the average size of clonal patches). Surprisingly, no relationship was observed between clonal extent and genetic variation. Clonal propagation appears to play a crucial role in sustaining tree numbers in this species. Clonality may be buffering the negative effect of extremely low sexual recruitment, potentially caused by the purging of genetic load in the inbred *C. nootkatensis*. Clonality may also be a mean of preserving very fit, ancient gene combinations from recombination.

Genetic diversity and population structure of *Gaultheria shallon* (salal) and its potential biocontrol agent *Valdensinia heterodoxa*

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Valdensinia heterodoxa is the causal agent of leaf-spot disease in *Gaultheria shallon* (salal), a densely growing shrub native to the Pacific Northwest. Once established in an area, salal expands predominantly by vegetative reproduction forming large clones. *V. heterodoxa* is an ascomycete fungus that reproduces mainly via asexual spores. Currently *V. heterodoxa* is being considered as a potential biocontrol agent for salal in forested sites where it competes with regenerating conifers (Shamoun et al. 2000. *Can. J. Plant Pathol.* 22: 192). In order to design an effective biocontrol agent and to assess the risks and challenges associated with releasing large quantities of *V. heterodoxa* into the environment, the population structure of both *V. heterodoxa* and salal must be investigated. This research focused on three populations from Vancouver Island and one from the Sunshine Coast of British Columbia where healthy and *Valdensinia*-infected salal leaves were collected and their locations within these populations mapped. Salal DNA was isolated directly from leaf tissue while *V. heterodoxa* was cultured from the infected salal leaves to obtain single spore cultures prior to DNA isolation. Amplified fragment length polymorphisms were used to generate DNA fingerprints for both the salal and *V. heterodoxa* samples. These data are being analyzed and the initial results will be presented. The findings from this study will help to increase our understanding of the asexual and clonal nature of *V. heterodoxa* and salal, respectively, while providing the framework for a range-wide study. It will also indicate how these factors may play a role in the use of *V. heterodoxa* as a potential biocontrol agent for salal.

Tuesday July 29, 11:50 am

Clonal Growth in Second Growth Stands of *Sequoia sempervirens* (coast redwood)

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This study is the first to use molecular genetic markers on a large scale to identify clonal structures in second growth stands of coast redwood. The primary goal of this study was to determine the importance and structure of clonal growth in these stands, and to test for variation due to site topography. Amplified fragment length polymorphisms (AFLPs) were used to establish a genetic fingerprint for up to 50 contiguous stems on each site. These fingerprints were then used to identify the size and shape of each clone. Clones were found to be very large, with sizes skewed toward a few extremely large genets. On average, 4 clones comprised the vast majority of the stems on a site. Clones were not limited to fairy ring structures, but were found to display a wide range of shapes including, concentric rings, ring chains, disjunct, and linear structures. Nine sampling sites representing upland (north facing slopes, south facing slopes) and riparian zones were selected in an effort to detect differences by site topography. Few were found at this sampling scale. Our results were very similar to those found in a study of old growth sites with the exception of greater ramet numbers per clone on our second growth sites.

Genetic structure among California's red oaks and susceptibility to *Phytophthora ramorum* (Sudden Oak Death)

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A recent outbreak of the disease pathogen *Phytophthora ramorum* (Sudden Oak Death) in California oak woodlands has resulted in high levels of mortality in some oak species belonging to the section Lobatae (red oaks). Concerns over the future health of these woodlands, has raised the need for a better understanding of the population genetic structure of the species that are at risk and detection of potential variation in susceptibility to the disease. We have begun a series of studies to investigate whether variations in field observations of disease symptoms may be associated with genetic structure of coast live oak. An in vitro system was developed to assay response of excised shoots to inoculation of *Phytophthora ramorum*. Inoculum was placed under the bark and the excised shoots were grown on the greenhouse bench for 3 weeks. Response to inoculation was assessed by measuring the size of the resulting lesion. Populations of coast live oak from high infection areas in northern California were contrasted with those from southern California and from an east-west transect in central California in 4 trials during the season. In an early season trial, populations from southern California produced small lesions compared with those from northern California, but this differential response was not evident in a repeat late-season trial. No significant differences were observed among populations from the east-west transect. In all trials and for all populations, variation among individuals in response to inoculation was highly significant. AFLP molecular markers were used to test whether genetic similarity among individuals was associated with similarity in the response to inoculation. For the early season trial, the 5% most genetically similar individuals had significantly shorter pairwise distances in lesion size than the entire pool of inoculated samples for 6 of the 7 AFLP primer pairs that were tested. For the remaining trials, associations between genetic similarity and similarity in response were fewer. Since our rangewide molecular study of coast live oak revealed a differentiation of northern and southern populations, partial Mantel tests were carried out, in which the effect of population differentiation was removed. These still revealed a significant association between genetic similarity and response to inoculation, particularly in the early-season trial. Hybridization is common in oak species and close phylogenetic relationships among hybridizing species and several generations of backcrossing may lead to cryptic hybrid products that go undetected in many ecological studies. We have detected cryptic hybrid structure between interior live oak, coast live oak and Shreve oak through a broad region in northern California. All four species were simultaneously detected in the genetic background of individuals morphologically assigned to interior live oak. In some cases, introgression was detected well outside the region of sympatry with one of the parental species. We are beginning studies to see whether the higher frequency of hybrid genotypes in northern California may play a role in response to inoculation of northern California coast live oak.

Genetic diversity and spatial population structure of Sitka spruce (*Picea sitchensis* (Bong) Carr): Implications for sampling strategies and conservation of rare alleles

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Ex situ conservation maintains genetic diversity in seed banks, clone and tissue banks, breeding populations, and arboreta. It typically provides a back-up to *in situ* conservation, but often has smaller sample sizes. *Ex situ*, unlike *in situ*, conservation strategies can be designed to maximize the capture of genetic diversity across a landscape, particularly rare alleles. While many rare alleles may be selectively neutral or deleterious, some may be vital for meeting future genetic needs, such as those conferring insect or disease resistance. Rare alleles are those most likely to be missed when sampling for gene conservation. To assess the effects of sampling strategy on capture of allelic diversity in widespread species, we studied Sitka spruce (*Picea sitchensis* (Bong.) Carr), populations as a model. Sitka spruce is a conifer that occupies wide geographic and ecological niches from 33^o N to 60^o N latitude along the Pacific coast of North America. Large numbers of individual trees were sampled in eight populations classified as core or peripheral based on ecological niche, and continuous and disjunct based on distribution. In each population, 200 trees were sampled, spatially mapped and genotyped for eight DNA-based sequence-tagged-site (STS) co-dominant markers. One important finding of this study is striking differences in genetic structure but not average levels of genetic diversity, between core and peripheral populations. Another remarkable result found by this study is strong spatial structure as evidenced by coancestry in peripheral populations, both continuous and disjunct. Differences in population structure were attributed to an aggregation of similar multi-locus genotypes, in a structured, isolation by distance manner in peripheral and continuous, and peripheral and disjunct populations but not so in core and continuous populations. Alleles, whether locally common or rare, tended to be distributed throughout the range of the species. Rare and localized alleles were only detected in peripheral and disjunct populations and averaged 5% of all alleles. To capture localized alleles (both common and rare), sampling should cover more populations over the geographic and ecological range of species at a cost of fewer individuals per population. Implications for capture of allelic diversity are discussed.

Species-transferable microsatellite markers developed from loblolly pine ESTs

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Microsatellites are usually regarded as the markers of choice in population genetics research because they exhibit high variability. The development cost of these markers is usually high. In addition, microsatellite primers developed for one species often do not cross-amplify in related species, requiring separate development for each species. However, microsatellites found in ESTs (expressed sequence tags) might better cross-amplify, as they reside in, or near, conserved coding DNA. In this study, we identified 14 *Pinus taeda* (loblolly pine) EST-SSRs from public EST databases and tested for their cross-species transferability to *P. contorta* spp. *latifolia*, *P. ponderosa* and *P. sylvestris*. As part of our development of a *P. contorta* microsatellite set, we also compare their transferability to that of 100 traditional microsatellite markers developed in *P. taeda* and tested on *P. contorta* spp. *latifolia*. Compared to traditional microsatellites, EST-SSRs had higher transfer rates across pine species; however, the level of polymorphism of microsatellites derived from EST was lower. Sequence analyses revealed that the frequencies of insertions/deletions and base substitutions were lower in EST-SSRs than in other types of microsatellites, confirming that EST-SSRs are more conserved than traditional SSRs. Our results also provide a battery of 23 polymorphic, robust microsatellite primer pairs for lodgepole pine.



PRESENTATION ABSTRACTS

Wednesday July 30

Adaptation in forest trees



Genetic dissection of adaptive traits in forest trees

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There may be no topic of greater interest to forest geneticists than the genetic basis of adaptation. For decades, the common garden experimental approach has been used to estimate the genetic control of adaptive traits and to describe patterns of genetic variation in relation to variation in the environment. More recently, the quantitative trait locus (QTL) mapping approach has been used to identify regions in the genomes of forest trees that harbor loci that determine complex adaptive traits. For example, QTLs have been mapped for bud phenology, frost tolerance and drought resistance. QTL studies provide reasonable estimates of the (1) number, (2) size and direction of effect on the phenotype and (3) genomic location of genes effecting complex traits. However, QTL studies do not reveal the specific genes. We are using an approach taken from human genetics, called association mapping, towards identifying the individual genes controlling complex adaptive traits in forest trees, including Douglas-fir. Association mapping is similar to QTL mapping in that its goal is to identify correlations between DNA polymorphisms and whole-plant phenotypes. The major difference between the approaches is that association mapping is based on historical recombination in populations whereas QTL mapping is based on recombination in experimental crosses. The resolution of mapping is therefore much greater with association mapping, making it possible to identify the specific gene controlling the phenotype and ultimately the polymorphisms responsible for allelic effects. We present preliminary results from an association mapping experiment in Douglas-fir to discover alleles responsible for tolerance to frost.

Wednesday July 30, 9:15 am

Ecophysiological implications of paternal inheritance for adaptive traits in red spruce, black spruce, and their hybrids.

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Hybridization between red spruce (*Picea rubens* Sarg.) and black spruce (*Picea mariana* (Mill.) B.S.P.) has resulted in identification and management problems for these otherwise ecologically distinct species. Except in the very southern part of its range, red spruce is largely sympatric with the transcontinental black spruce. Our goal was to establish and compare light-energy processing traits in controlled crosses of red and black spruce, and their interspecific hybrids.

There were a number of light-energy processing traits that were found to be species specific. Generally the dark-adapted traits showed a non-additive or paternal species effect, where hybrid index 25 (25% red spruce) was similar to black spruce and hybrid indices 50 and 75 (50 and 75% red spruce) were similar to red spruce. Parental (paternal and maternal) type (red, black or hybrid) analysis of photochemical efficiency showed significant paternal ($p = 0.000$), maternal ($p = 0.000$), and paternal x maternal interaction effects ($p = 0.005$) ($r^2 = 0.604$), which upon further analysis appear to be largely species-specific, uniparental and paternally based. At face value, light-adapted traits by hybrid index revealed a more additive genetic effect between species. Photosynthesis (f (quantum yield)) analysis, by parental types revealed a significant paternal effect ($p = 0.003$), and non-significant maternal and interactive effects ($p = 0.143$). Any cross having black spruce as the male parent had significantly higher photosynthesis ($p = 0.000$) than with those having either red spruce or hybrids (25, 50, 75) as the male parent. In addition, thermal dissipation efficiency was strongly paternally inherited; consequently; any cross with a red spruce male had higher thermal dissipation efficiency. This suggests that the above traits are strongly controlled by the chloroplast genome. We know of no studies in trees demonstrating the importance of paternal inheritance (e.g., via chloroplasts) in terms of physiological and ecophysiological processes that affect adaptation and fitness.

Adaptation of red and white fir to elevation across an ecotone

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In the Sierra Nevada mountains of California red fir (RF) dominates the upper montane forest, and white fir (WF) is important in the midmontane mixed conifer forest. In the southern-most Sierra Nevada the ecotone between these communities occurs between 2400-2700 m. Microcalorimetric measurements of temperature dependence and timing of respiration (RCO_2) and heat rate (q) were made on RF and WF sampled across the ecotone in the southern-most Sierra Nevada. RCO_2 and q increased when bud break and elongation occurred, peaked during elongation and declined as elongation ended, which is consistent with previous studies on ponderosa pine. WF q and RCO_2 accelerated earlier and remained high longer than RF. RF q and RCO_2 were higher than WF for a shorter later period. This is consistent with WF-RF elongation seasons on the study sites. WF bud break occurred first and elongation continued longer than RF. Total elongation is similar for both species. RF elongated faster for a shorter later period. Lower elevation WF sites are expected to lose available moisture earlier therefore WF must elongate earlier. Higher elevation RF sites have moisture available later therefore RF elongate later at warmer temperatures permitting faster growth and greater protection from frost damage.

Wednesday July 30, 10:20 am

Blister rust resistance on western white pine in Oregon/Washington: evolving perspectives

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Throughout much of western North America, populations of five-needle pines have been dramatically reduced in natural ecosystems by white pine blister rust, caused by an introduced pathogen, *Cronartium ribicola*. In Oregon and Washington, the resistance program has evaluated progenies of over 4900 western white pine (WWP) and 4500 sugar pine (SP) phenotypic selections since its inception in the late 1950's. Rust screening has shown that while progenies of most selected parents are susceptible, major gene resistance and forms of partial resistance are present. Resistance mechanisms occur at low frequencies, and at least one type, a hypersensitive reaction in the needles of WWP and SP, appears to be geographically restricted. Another newly delineated type of complete resistance, Mechanism 'X' (40-60% of half-sib seedlings from selected parents free of stem symptoms following artificial inoculation), may be present in WWP in very low frequency (<1%) among phenotypic selections. Another form of resistance (Mechanism 'Q') includes both a high proportion of stem-symptom-free and bark reaction individuals within a half-sib family. The underlying bases of these resistances (Mechanisms X and Q) and their relations to other observed resistance responses are unknown but may represent a continuum of resistance response that may include latent development of stem symptoms, delayed mortality, or tolerance. Field trials have been established in Oregon, Washington, and California to validate performance of selected WWP and SP families. Early results from these field trials are encouraging.



Screening coastal Douglas-fir for resistance to *Phellinus weirii*

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Phellinus weirii (Murr.) Gilb., causing laminated root rot, is the most serious pathogen affecting coastal Douglas-fir (Fdc) in western North America. A screening trial with 97 full-sib Fdc families conducted by the Canadian Forest Service, in cooperation with the BC Ministry of Forests, provided evidence of genetically-based resistance by Fdc to the fungus. Fifteen of the original 97 Fdc families were subsequently selected for closer scrutiny to both confirm proposed resistance ratings and to investigate potential resistance factors. To date, mortality data for trees from the 15 families that were outplanted and inoculated with *P. weirii* in 1999 confirm original resistance ratings for some but not all families. The original Fdc screening trial also demonstrated that isolates of *P. weirii* vary in their virulence (a quantitative measure of the amount of disease caused by the pathogen) and aggressiveness (a measure of the rate at which virulence is expressed). To better understand the genetic variation present in populations of *P. weirii* from across British Columbia, several isolates are being analyzed using molecular techniques such as PCR (polymerase chain reaction). Results to date suggest that while many isolates have unique banding profiles, isolates with identical banding patterns come from the same or similar geographic locations/biogeoclimatic zones. Proposed future research directions also will be presented.

Provenance variation in susceptibility of western hemlock (*Tsuga heterophylla*) to hemlock dwarf mistletoe (*Arceuthobium tsugense*)

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Hemlock dwarf mistletoe (*Arceuthobium tsugense*) is a parasitic plant that adversely affects the productivity of hemlock stands. In the past, clear-cutting as a harvest method acted as a sanitation cut eliminating the risk of spread of mistletoe other than as an edge effect. Current harvesting more often features partial cuts, resulting in risk of contagion from residual stems. This study compares the susceptibility of hemlock from a broad range with the aim of identifying geographic trends, and observing partitioning of variation between provenance and family within provenance. This is intended as a preliminary trial to guide screening of hemlock seed orchard parents from Oregon, Washington and British Columbia with the purpose of allowing the creation of mistletoe-resistant seedlots. Six individuals from each of 5 open-pollinated families from 10 sources were potted at age 1 year from seed, inoculated with *A. tsugense* seeds collected near Woss, BC in the second year, and assessed for susceptibility at age 4 years. Growth of mistletoe was not correlated with the growth of the host plant. Analysis of variance revealed significant variation in height at age 4 by provenance, but no trends in susceptibility to infection were detected. Duncan's multiple range test identified one provenance as different for this trait. Implications for orchard screening are discussed.



PRESENTATION ABSTRACTS

Thursday July 31

Genomic approaches for understanding adaptation

Population genetics of adaptation - lessons from pines and *Arabidopsis*

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Conifers and many other plants have high population differentiation with respect to traits related to adaptation to local climatic (or other) conditions. Especially traits related to timing of growth and flowering have been extensively studied, but the genes responsible for the variation are mostly not known. The sequencing of the *Arabidopsis thaliana* genome and developmental genetics work has provided improved possibilities for searching for the responsible genes by combining the *Arabidopsis* background with the population genetics of outcrossing species. We have studied Scots pine and an outcrossing relative of *Arabidopsis thaliana*, *A. lyrata*. Comparative mapping, QTL mapping, studies of association and sequence variation are used in search of the genes.



Conserving neutral and adaptive variation in British native trees

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Natural populations of native trees in Britain are generally small and fragmented due to a very long history of anthropogenic forest clearance. Non-native provenances of native tree species have also been widely planted over a long period. There is currently much interest in expanding the area of native species woodland in Britain using planting stock of genuinely local provenance. This position raises a number of complex issues regarding genetic history, conservation of neutral and adaptive variation within tree species and tree breeding work. This paper will highlight some of these dilemmas and discuss possible avenues of supporting research.

An effective, efficient, and economical method of mass propagation for aspen (*Populus tremuloides*)

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This paper describes a new method of mass vegetative propagation for trembling aspen (*Populus tremuloides* Michx.) being developed at the Woodmere Nursery in Fairview, in northwestern Alberta, as part of an operational research program for Daishowa-Marubeni International Ltd. and the Western Boreal Aspen Corporation (WBAC).

Development of this method was undertaken because of difficulties in producing sufficient numbers of uniform clonal test stock from wild root collections. Cost and timing of these collections have also been problematic.

This method of propagation involves suckering from small root segments derived from greenhouse-grown potted root donor (stock) plants. In its present form, it takes two growing seasons. Stock plants are grown in one-gallon pots for one season. Stems are removed, and the root mass is cold stored over the winter. In the spring, roots are washed, cut into short segments and placed into styroblocks where they develop into rootlings, which can be used operationally.

The propagation technique is simple, economical and efficient, in that large numbers of uniform plants can be derived easily from limited supplies of donor material. It is being refined further to serve as a means of mass clonal multiplication of aspen test stock; WBAC members also plan to use this method for operational clonal aspen planting stock production. The method also shows promise for clonal propagation of balsam poplar (*Populus balsamifera*) and may also prove effective with other suckering broad-leaved species.

Spatial patterns of genetic variation for neutral genetic markers and adaptive traits in the *Picea sitchensis* x *glauca* introgression zone

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A genetic analysis of the introgression zone between Sitka spruce (*Picea sitchensis*) and white spruce (*P. glauca*) in northwestern British Columbia was conducted to 1) quantify the contributions of parental species to introgressed populations; 2) characterize genetic patterns of variation across the introgression zone; and 3) address practical issues of gene resource management in this region. Seeds were sampled and seedlings grown from sixty putative hybrid parent trees from sixteen locations across the introgression zone. Foliage samples were also obtained for allopatric Sitka and white spruce reference populations. Based on allele frequencies for Sequence-Tagged-Site (STS) molecular markers, a hybrid index was developed that reflects the relative contribution of Sitka spruce to hybrid populations (0=white spruce, 1=Sitka spruce).

Mean STS hybrid index estimates ranged from 0.46 to 0.95 for locations within the zone of introgression. Significant differences in hybrid index were observed among sampling locations. Hybrid indices decreased from the coast to the interior within the introgression zone in spite of large among-family variation within locations. Variation among locations in hybrid index was better explained by geographic variables than by climatic or ecological variables. Physical drainage distance along river valleys to the nearest marine inlet explained 78% of the variation in STS hybrid index. Concordant clines were obtained when the same analytical techniques were applied to published allozyme frequencies for seedlots originating from a wider geographic area across the introgression zone. Allozyme hybrid index estimates ranged from 0.01 to 0.98. The relatively steep cline observed in hybrid index across the maritime/continental climate ecotone is best managed by limiting longitudinal seed transfer for reforestation in this region.

Thursday July 31, 11:05 am

The potential of using genomics in the resistance breeding programs in British Columbia: a breeders perspective

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Resistance breeding is becoming increasingly important to the tree improvement programs and Forest Genetics research in British Columbia. This includes: resistance to *Cronartium* blister rust for the white pines; *Armillaria* and *Phellinus* root-rot resistance in Douglas-fir; *Comandra* blister rust and gall rust resistance in lodgepole pine; mistletoe resistance in western hemlock; *Keithii* resistance in cedar; and for pests, resistance to the white pine or tip weevil in interior and Sitka spruce. Some level of population and family resistance based on phenotypic screening has been noticed in all of these investigations and in some cases it has been very marked. Selections based on this screening have been the major part of both the white pine and the Sitka spruce programs and are also a very important part of the interior spruce program in British Columbia. Except for hypersensitive response (HR) controlled by the Cr2 gene in white pine, we have little knowledge of what is behind the observed resistance or how it is inherited for blister rust. We are starting to have some knowledge of the mechanisms behind weevil resistance in our spruce populations but although we believe major genes might be behind some of this resistance we are some time away from confirming such gene effects and their inheritance.

The genome project at the University of BC is using material from our phenotypic screening programs for weevil resistance in both Sitka and interior spruce. I would like to talk here, from a breeders perspective, on how genomics research can be useful in these efforts: where it is likely to help and where not. Firstly our phenotypic screening appears to be very effective and with the high heritabilities we are observing (for HR it is 1.0) genomics may not have much to offer as a selection tool in our screening. More importantly we see its potential in elucidating our understanding of the genetic basis of resistance. Some ways in which genomics research could help include: 1) Genomic associations with mechanisms of resistance. 2) Identifying gene effects for these mechanisms and their inheritance – especially as we are just constructing our pedigreed populations. 3) Understanding the evolutionary background of our resistant populations – much of this resistance has been found in unique populations – and likewise the background of pest populations. Certainly there needs to be ongoing communication between breeders and genomic scientists.

Genomics and adaptation: Where do we go from here?

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Genomic approaches will greatly expand our understanding of adaptation in forest trees. Physical, comparative, and functional genomic studies have already identified genes that appear to play important functional roles in adaptive traits such as cold hardiness, drought hardiness, bud phenology, and winter dormancy. The next important challenge is to identify those genes that contribute to genetic variation within and among populations of forest trees. Adaptation to local environments involves the coordinated expression of many traits, each of which seems to be controlled by multiple genes with relatively small effects. Analyses of quantitative trait loci (QTL) also suggest that QTL x environment interactions are common. Given this inherent complexity, we suggest that researchers should focus on developing markers for candidate genes, then use multilocus, multiallelic analytical techniques to uncover the relationships between genotype and phenotype in natural populations (e.g., association genetics). We will focus our discussion on adaptation to cold and describe how genomic approaches can be integrated with genealogical studies to bridge the gap between genotype and phenotype at both the individual and population levels.

POSTER ABSTRACTS

Effects of inbreeding on whitebark pine

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Whitebark pine (*Pinus albicaulis*) is the only North American stone pine (genus *Pinus*, section *Strobus*, subsection *Cembrae*), a group that is distinguished by indehiscent cones, and wingless seeds that are dispersed by nutcrackers (family *Corvidae*, genus *Nucifraga*). Whitebark pine seed is dispersed by the Clark's nutcracker (*Nucifraga columbiana*), which harvests ripe seeds from the unopened cones and buries thousands of seeds in the ground for later consumption. Because of the seed foraging behavior of the nutcracker, it is likely that multiple seeds are collected from the same tree and cached together. Dispersal of whitebark pine's seeds by the Clark's nutcracker results in clumps of related individuals randomly distributed across the landscape. This clumpy growth habit leads to higher levels of inbreeding (both selfing and bi-parental inbreeding) in this species compared with other wind-pollinated conifers. The effects of this inbreeding on quantitative traits (inbreeding depression) as well as on genetic diversity are unknown. A previous study of whitebark pine observed an increase fixation index ($1-H_o/H_e$) with increasing latitude and decreasing longitude throughout British Columbia. Levels of white pine blister rust infection decrease from south to north and from east to west. It has been hypothesized that blister rust may be selecting against more homozygous, inbred individuals, resulting in the observed trends in fixation index. Components of this study will 1) quantify inbreeding depression in quantitative traits by comparing parental outcrossing rate with progeny performance, and 2) try to elucidate the causative factors in the trends in fixation index. Isozyme analysis of different age cohorts (6-month-old seedlings, young saplings, and mature trees) from sites throughout British Columbia with varying levels of blister rust infection will be used to separate the effects of inbreeding and disease on levels of genetic diversity.

Comparison of Conifer Elongation Temperature: Response to Climate Adaptation

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This study compared elongation temperature conditions and resulting adaptation of conifer species native to ranges that cross elevation and moisture gradients in California. Timing and length of elongation were measured at 1-2 week intervals spring and summer for three years in a common garden at Davis California. Hourly temperature data were collected. Elongation initiated following this sequence: gray pine, ponderosa pine, Jeffrey pine, incense cedar, Douglas-fir, sugar pine, and finally white fir. This is consistent with literature. There was overlap in timing of elongation between some species and this varied somewhat from year to year. Data indicates elongation begins under the lowest temperature conditions for gray pine and becomes progressively warmer for species in the listed sequence. As species become progressively adapted to higher elevations and more mesic environments elongation is initiated under warmer conditions. Davis and regions these species are adapted too generally have Mediterranean climates. Mild wet winters and warm dry summers result in spring growth seasons due to mild temperatures and residual moisture from the rainy season. Warmer drier sites lose available moisture earlier and adapted species (gray pine, ponderosa pine) must elongate earlier at lower temperatures to use available moisture. Higher elevation and more mesic sites have moisture available later in spring and adapted species (Douglas-fir, sugar pine, white fir) initiate elongation later under warmer temperatures permitting faster growth and providing greater protection from frost damage.

Is natural selection or genetic drift the primary cause of phenotypic diversification?

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The ability of natural selection to produce an exquisite diversity of phenotypes has enthralled us since Darwin's time. However, phenotypic differentiation between species sometimes appears to be non-adaptive. We begin to validate Darwin's claim by using a three species QTL phylogeny. To compare the role of directional selection *versus* genetic drift in species divergence, we use Orr's (1998) sign test of neutral phenotypic evolution. If a trait has a continuous history of directional selection, QTLs along a phylogenetic lineage should mostly enhance or mostly reduce a trait. If phenotypic evolution is neutral, QTLs with opposing or antagonistic effects should be common. Knowledge from the phylogenetic comparison of QTLs would help to reveal the plausibility of selection in phenotypic evolution.

Gene discovery for adaptive traits in Douglas-fir

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Our research objective, past and present, has been for the discovery of genes controlling important adaptive traits in Douglas-fir. Through quantitative trait loci (QTL) mapping techniques we have identified loci controlling several important quantitative traits and estimated their effects on the phenotype. QTL x Environment interactions for several traits have also been identified in field and greenhouse experiments using clonal populations. The task now is to identify the specific genes that underlie the QTLs we have identified through statistical analyses. EST libraries have been constructed and sequenced in several conifer species, including Douglas-fir, and queried for genes known to contribute to specific physiological processes. Many of these EST markers have been mapped in multiple species and a high degree of co-linearity between linkage groups among species has been found. Although, our first goal is to place known candidate genes on existing maps, our ultimate aim is to determine associations between alleles of candidate genes and phenotypes of interest in large natural populations.

Variation in blister rust resistance in the Pacific Northwest: results from screening half-sib progeny of 226 western white pine and 217 sugar pine phenotypic selections

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Western white pine (WWP) and sugar pine (SP) families sown in 1989 and 1994 were inoculated with *Cronartium ribicola* after two growing seasons, and development of rust symptoms and mortality were followed for five years. In general all types of resistance responses were low in frequency. Survival of infected seedlings varied from 1.6% to 13.1% among the four trials, and family means varied from 0 to 54.8%. However, some half-sib families (open-pollinated progeny of phenotypic selections from natural populations) had survival comparable to the Idaho and Oregon/Washington full-sib resistant checklots. In both species the families with the highest levels of survival generally had higher than average levels of several resistance responses. Survival of seedlings with stem symptoms was low but tended to be higher for seedlings with bark reactions than for seedlings with only normal cankers; trial means for survival ranged from 1.2 to 13.5% for seedlings with bark reactions vs. 0.4 to 4.8% for seedlings with only normal cankers. In the two WWP trials more westerly populations tended to have slightly fewer seedlings with stem symptoms ($r=-0.29$, $p=0.0015$; $r=-0.31$, $p=0.0009$). For the WWP trial with a wider North-South sampling, there was a tendency for the progeny of southern WWP parents to have more seedlings without stem symptoms ($r=-0.27$, $p=0.0038$). For SP, which has a much narrower species range in Oregon, these trends were not significant in the two trials examined here. Major gene resistance is rare for both WWP and SP, but 6 SP and 8 WWP parental selections were identified as candidates for further evaluation. Results indicate that rust resistance in natural populations is very low, even in selected trees. Genetic resistance offers land managers the best 'tool' to reduce the decline of WWP and SP in natural populations. Breeding efforts are focused on the development of seedlings with more durable rust resistance for reforestation and restoration.

DNA mapping and marker assisted breeding

BENOIT LANDRY

DNA LandMarks Inc., 84 Rue Richelieu, St-Jean-sur-Richelieu, Quebec J3B 6X3, 450.358.2621 ext. 101

DNA LandMarks Inc. is a Montreal-based Plant Genomics company specializing in the development of DNA marker technologies to accelerate plant breeding activities. In January 2003, DNA LandMarks purchased ParaGen (previously known as Celera AgGen, PE AgGen, Linkage Genetics and NPI). This acquisition vastly increased our company's marker portfolio and expanded our throughput capacity. DNA LandMarks offers high quality research and development services in molecular mapping, trait tagging and map-based cloning to accelerate research projects of plant breeding companies and public institutions. Other DNA Marker services available include:

- Proprietary High Throughput (HT) DNA marker system (IMP markers)
- HT DNA isolation
- HT DNA fingerprinting and genotyping
- HT DNA sequencing and Bioinformatics
- HT Marker-Assisted Breeding
- Construction of detailed genetic maps
- Germplasm Characterization

DNA LandMarks facilities are state-of-the art, housed in a certified new technology centre. Our mandate is to provide timely, high quality results and ensure complete confidentiality to our customers.



Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl. ex. D. Don.)

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Western white pine (*Pinus monticola* Dougl. ex. D. Don., WWP) shows genetic variation in disease resistance to white pine blister rust (*Cronartium ribicola*) at different levels. Most plant disease resistance (R) genes encode proteins that belong to a super family with nucleotide-binding site domains (NBS) and C-terminal leucine-rich repeats (LRR). In this paper a PCR strategy was used to clone R gene analogs (RGAs) in WWP with oligonucleotide primers designed based on the conserved motifs of NBS domain of angiosperm NBS-LRR genes. Sixty-seven NBS sequences were cloned from disease resistant trees. BLAST search of GenBank revealed that they shared significant identities to well-characterized R genes from angiosperms, including flax *L* and *M* genes, tobacco *N* gene and soybean *LM6* gene. Sequence alignment analysis revealed that western white pine RGAs contained the conserved motifs identified in angiosperm NBS domains, especially those motifs specific for TIR-NBS-LRR proteins. Phylogenetic analysis of plant R and RGAs indicated that all cloned WWP RGAs can be grouped into one major branch together with well-known R proteins carrying TIR domain, suggesting they belong to the subfamily of TIR-NBS-LRR genes. In one phylogenetic tree, WWP RGAs were sub-grouped further into fourteen clusters with an identity threshold of amino acid sequence at 75%. cDNA cloning and RT-PCR analysis with gene-specific primers demonstrated that RGA members in 10 of 14 classes were expressed in foliage tissues, indicating that a large and diverse NBS-LRR gene family are apparently functional in conifers. These results provide evidence for the hypothesis that conifer RGAs share a common origin with R genes from angiosperms, some of them may play important role in defense mechanism to confer disease resistance in western white pine. Ratio of non-synonymous to synonymous nucleotide substitutions (Ka/Ks) in the WWP NBS domains was larger than one or close to one significantly, indicating the presence of diversifying selection or neutral selection on the NBS domains of the WWP RGA family.

Stand structure and reproductive fitness traits of eastern white pine (*Pinus strobus* L.)

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Mating behavior and seed traits were determined for eastern white pine (*Pinus strobus* L.) in six small, remnant and marginal populations from the Island of Newfoundland, and in three large populations from the center of the geographic range in Ontario, to examine the effects of small population size and fragmentation on mating system parameters and reproductive fitness. The Ontario populations showed the highest and the Newfoundland West (NFW) populations the lowest reproductive fitness, with Newfoundland East populations ranking higher than NFW but significantly lower than Ontario populations. Actual inbreeding rates, determined by combining allozyme-based estimates of selfing in the filled seed component with estimates of inbreeding from the proportions of empty seeds, ranged from 7.4 to 31.6%, with an average of 22% for all populations, 11.1% for the Ontario, 24.7% for the Newfoundland East, and 30.1% for the Newfoundland West populations. Multilocus outcrossing rates were significantly correlated (i) negatively with the average distance to the five nearest neighboring trees (a surrogate measure for within-stand densities of reproductively mature trees), and (ii) positively with the proportion of filled seeds per cone. Filial seed progeny fixation index was positively correlated with both (i) average nearest neighbor distances, and (ii) proportion empty seeds per cone. Thus, we detected strong interrelationships between the within-stand density of reproductively mature trees and both outcrossing rates and filled seed production. Interestingly, there was no relationship between the fixation index of the mature parent stands and their density. The genetic status or integrity of the extant parental populations may have been largely unaffected by the large-scale population decline experienced by eastern white pine early in the 20th century, a decline that was clearly having an adverse effect on reproductive fitness of these populations. These results indicate that there are certain thresholds of population size and stand density that may be important for maintaining reproductive fitness and genetic diversity, particularly in small, isolated tree populations, and that maintaining adequate population sizes and within-stand densities has important implications for managing residual populations following forest harvesting operations.

Old-growth red spruce forests as reservoirs of genetic diversity and reproductive fitness

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Old-growth forests are assumed to be potential reservoirs of genetic diversity for the dominant tree species, yet there is little empirical evidence for this assumption. Our aim was to characterize the relationship of stand traits, such as age, height, and stem diameter, with the genetic and reproductive status of old-growth and older second-growth stands of red spruce (*Picea rubens* Sarg.) in eastern Canada. We found strong relationships between height growth (a fitness trait) and measures of genetic diversity based on allozyme analyses in red spruce. The negative relationship between height and the proportion of rare alleles suggests that high proportions of these rare alleles may be deleterious to growth performance. Latent genetic potential, however, showed a significant and positive relationship with height. Stand age was not correlated to height, but was correlated to seedling progeny height. In late-successional species such as red spruce, age and size (e.g., height and stem diameter) relationships may be strongly influenced by local stand disturbance dynamics that determine availability of light, growing space, moisture, and nutrients. In larger and older stands, age appeared to provide a good surrogate measure or indicator for genetic diversity and progeny height growth. However, in smaller and more isolated populations, these age and fitness relationships may be strongly influenced by the effects of inbreeding and genetic drift. Therefore, older populations or old-growth forests may represent superior seed sources, but only if they are also of sufficient size and structure (e.g., stem density and spatial family structure) to avoid the effects of inbreeding and genetic drift. Thus, larger and older forests appear to have an important evolutionary role as reservoirs of both genetic diversity and reproductive fitness. Given the rapid environmental changes anticipated (as a result of climate change, increasing population isolation through fragmentation, or following the introduction of exotic pests and diseases) these older populations of trees may have a valuable function in maintaining the adaptive potential of tree species.

Development of SSR markers in *Picea*

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We have utilised a spruce EST database derived from white, sitka and interior spruce species to develop a set of SSR markers for use in spruce. 188 SSR sequences were identified in the EST database, and after analysis of the repeats and surrounding regions, 44 primer pairs were designed. These primers and other, previously reported spruce SSR primers were tested on 20 individuals from white, sitka and black spruce. Amplification across 23 spruce species was also determined. These analyses showed that the use of an EST database to identify SSRs leads to a high success rate of developing highly informative markers that are able to amplify loci from a wide range of spruce species.

Simply inherited resistance to *Phytophthora lateralis* in Port-Orford-cedar: greenhouse testing

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Phytophthora lateralis, a non-native root rot pathogen, is the primary cause of Port-Orford-cedar (*Chamaecyparis lawsoniana*) mortality throughout its native range in southwest Oregon and northwest California. Most trees in natural stands are very susceptible, but greenhouse testing in the late 1980's demonstrated existence of genetic resistance to this pathogen. We used a root dip technique to examine the magnitude and inheritance of this resistance. Susceptible families show 90-100% mortality over the 10 to 11 month evaluation period, but a few full-sib families show little (0-10%) or only moderate (25-60%) mortality. Although some deviations from expected ratios were evident, results from full-sib families from control crosses using the most resistant parents strongly suggest that a major gene for resistance is present, with survival ratios of 3:1 and 1:1 depending on the parent and cross. A putative homozygous dominant parent, three heterozygous parents, and three homozygous recessive parents were identified from the analysis. The data do not preclude other kinds of resistance across the entire range of Port-Orford-cedar.

Genetic distance-heterosis correlation in western hemlock

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The goal of all tree breeding programs is to produce premium seedlings that show good performance when transferred into the field. The hope is to be able to predict the fitness of F1 offspring before mating, to reduce the number of parents that would be tested and promote increased genetic diversity in nursery grown seedlings. The prediction of F1 performance is of particular value to breeders as field testing is expensive, time-consuming and labor-intensive. Western hemlock comprises 40% of the coastal harvest and 60% of British Columbia's export market. Given the regeneration capacity combined with the biological diversity observed in western hemlock stands, this species is a prime candidate for tree improvement. Linkage disequilibrium between neutral marker loci and other selected loci could result in heterozygote advantage at the neutral marker loci. Genetic distance is a measure of heterozygosity and therefore can potentially be correlated with heterosis. The phenotype of a given breeding line of western hemlock can thus be predicted for the next generation if it displays correlation genotypically (microsatellites and AFLP) with the phenotype (height, DBH, etc.) of its F1. Therefore the plan is to characterize each elite line using the F1's that are a product of crosses between the elites.

Development of bioinformatics tools for the analysis of EST databases

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Access to an EST database allows analysis of sequence data, comparison with other genomes and development of molecular markers. Development of a pipeline to process the information is required to allow full and rapid utilization of the resources. We have utilised spruce and poplar EST libraries to develop SSR markers, identify SNPs and compare spruce and poplar expressed sequences with other genomes. Comparison of EST sequences with other genomes allows annotation of genes, and identification of conserved elements between genomes. The identification of molecular markers from expressed regions of the genome allows the development of highly informative markers, which are useful for population studies and breeding programs.