

Western Forest Genetics Association

2001 Annual Conference

Davis, California

Hosted by: **The Institute of Forest Genetics
Pacific Southwest Research Station
USDA Forest Service**

Conference Chair: **David Neale**

Organizing Committee:
Andrew Groover, Valerie Hipkins, Det Vogler

WFGA Sitting Officers:
John Russell, President
Valerie Hipkins, Vice-President
Thimmappa Anekonda, Secretary/Treasurer



Board of Trustees luncheon and reorganization meeting at which the name of the Eddy Tree Breeding Station was changed to the Institute of Forest Genetics – a preamble to donating the Institute to the Forest Service (April 26, 1932).

Left to right: John Diederff, Walter A. Starr, James G. Eddy, Walter Mulford, Donzel Stoney, Lloyd Austin, J.H. Hutchinson, G.O. Tamblyn, James A. Irving, Francis I. Righter, Clyde R. Berriman, William C. Cumming.

Schedule:

Monday, July 30

5:00-7:00pm Opening Reception and Registration
(University Club Dining Room)

Tuesday, July 31

8:15AM-12PM Plenary Session #1
(Alumni Center, AGR Hall)

12:00-1:30PM Lunch
(University Club Dining Room)

1:30-5:00PM Concurrent Workshop Sessions

- Geographic Variation and Paleo-Inference
(University Club, Clubroom 1)
- Local Population Structure
(University Club, Dining Room)
- Current Issues in Conservation
(University Club, Lounge)

5:00-6:30PM Swimming available at Recreation Pool
(\$1.25 charged at gate – **BRING A TOWEL!**)

6:00-9:00PM Banquet
(Recreation Pool Lodge)



Making site determinations (August 1931). (L-R) Jensen, Lloyd Austin, Pete Righter, and Bill Cummings.

Wednesday, August 1

- 8:15AM-12PM Plenary Session #2
(Alumni Center, AGR Hall)
- 12:00-1:30PM Lunch
(University Club Dining Room)
- 1:30-6:00PM Field Trip
Institute of Forest Genetics, Placerville
- 6:00-9:00PM Refreshments and Dinner
Institute of Forest Genetics, Placerville.
- 9:00-10:00PM Return to Davis

Thursday, August 2

- 8:15AM-12PM Plenary Session #3
(RM 198 Young Hall)
- 12-1:30PM Lunch, WFGA Business Meeting
(University Club Dining Room)
- 1:30-5:00PM Concurrent Workshops
- Applied Forest Tree Improvement
(University Club, Clubroom 1)
 - Breeding for Resistance
(University Club, Dining Room)
 - Molecular Forest Genetics
(University Club, Lounge)



Nicholas Mirov, Institute of Forest Genetics, Placerville.

Plenary Session 1. Evolutionary and Population Genetics

Lauren Fins, University of Idaho, Moderator.

ROOM: Alumni Center, AGR Hall

- 8:15 – 8:30 David Neale, Institute of Forest Genetics
Opening Remarks
- 8:30 – 9:15 Connie Millar, Institute of Forest Genetics
From El Nino to Milankovitch: The Influence of Climate on History of Southwestern Forests
- 9:15 – 10:00 Aaron Liston, Oregon State University
Contributions of Molecular Phylogeny to Forest Genetics: Examples from the Pinaceae
- 10:00 – 10:30 Break
- 10:30 – 11:15 Jeff Mitton, University of Colorado
Mt DNA Phylogeny of Limber and Ponderosa Pines
- 11:15 – 12:00 Tom Conkle, Institute of Forest Genetics
Patterns of Population Differentiation in Western North American Conifers.



Plenary Session 2. Adaptation and Response to Stress

Tom Adams, Oregon State University, Moderator.

ROOM: Alumni Center, AGR Hall

- 8:15 – 8:30 David Neale, Institute of Forest Genetics
Opening Remarks
- 8:30 – 9:15 Sally Aitken, University of British Columbia
Some Like it Hot: Adaptation to Temperature and Moisture Regimes
- 9:15 – 10:00 Glenn Howe, Ohio State University
Physiology and Genetics of Dormancy – Related Traits in Populus.
- 10:00 – 10:30 Break
- 10:30 – 11:15 George Newcombe, University of Idaho
Adaptive Responses among Exotic Hosts and Pathogens
- 11:15 – 12:00 Tom Gordon, UC Davis
Resistance to Pitch Canker in Monterey Pine



Plenary Session 3. Tree Improvement and Biotechnology

Andrew Groover, Institute of Forest Genetics, Moderator.

ROOM: RM 198 Young Hall

- 8:15 – 8:30 David Neale, Institute of Forest Genetics
Opening Remarks
- 8:30 – 9:15 Tim White, University of Florida
Roles of Plantations and Tree Improvement in Global Forestry
- 9:15 – 10:00 Jay Kitzmiller, USDA Forest Service
Promoting Healthy California Forests: Overview of the USFS Genetic Resources Program
- 10:00 – 10:30 Break
- 10:30 – 11:15 Ron Sederoff, North Carolina State University
Genomics of Forest Trees; Evaluations and Prospectus
- 11:15 – 12:00 Steve Strauss, Oregon State University
Genetically Modified Trees: Their Place, Their Benefits, Their Concerns and Their Myths



Workshop: Geographic Variation and Paleo-Inference – A Workshop Dedicated to the Memory of Bill Critchfield.

Organized by: Ron Schmidting, Southern Institute of Forest Genetics
University Club, Clubroom 1: Tuesday, July 31, 2001

- 1:30 – 2:15 Steve Jackson, University of Wyoming
Impacts of Quaternary dynamics on conifer genetics: case studies from eastern and western North America
- 2:15 – 3:00 Tom Ledig, Institute of Forest Genetics / UC Davis
Endangered Mexican spruces
- 3:00 – 3:30 Nick Wheeler, Weyerhaeuser Co.
Biogeography of lodgepole pine: genetic evidence
- 3:30 – 3:45 Break
- 3:45 – 4:15 Bill Dvorak, CAMCORE
*Evolutionary history of the Mexican *Oocarpae* and Caribbean Pines*
- 4:15 – 4:35 Ron Schmidting, SIFG
Genetic evidence for refugia and migrations of the southern (Australes) pines after the last glacial maximum
- 4:35 – 4:55 Kristin Mylecraine, Rutgers University
Rangewide allozyme variation in Atlantic White-cedar
- 4:55 – 5:15 Lisa O’Connell, University of British Columbia
*Genetic diversity, mating system, and inbreeding depression of western red-cedar (*Thuja plicata*)*



Workshop: Local Population Structure.

Organized by: Bob Westfall, Institute of Forest Genetics
University Club, Dining Room: Tuesday, July 31, 2001

- 1:30 – 2:15 Peter Smouse, Rutgers University
A 2-generation model of the analysis of gene flow.
- 2:15 – 2:35 Victoria Sork, University of Missouri St. Louis
*Pollen movement in declining populations of California Oak, *Quercus lobata*: Where have all the fathers gone?*
- 2:35 – 2:55 Rodney Dyer, University of Missouri St. Louis
Effects of spatial variation on Two Generation estimates of gene flow.
- 2:55 – 3:15 Jarek Burczyk, Institute of Biology and Env. Protection
Investigations on plant reproductive success through selection and ecological gradients using genetic markers and neighborhood models.
- 3:15 – 3:45 Break
- 3:45 – 4:05 Bob Westfall, Institute of Forest Genetics
*Co-ancestry in Sugar Pine, *Pinus lambertiana*.*
- 4:05 – 4:25 Dilara Ally, University of British Columbia
*Spatial genetic structure in mountain hemlock (*Tsuga mertensiana*), both at the geographic and at the local population level.*
- 4:25 – 4:45 John Church, UC Davis
*Respiration based growth rate predictions match *Pinus ponderosa* elongation season, ambient temperature and show differences between genotypes.*



Workshop: Current Issues in Conservation

Organized by: Yan Linhart, University of Colorado, and
Valerie Hipkins, US Forest Service – NFGEL
University Club, Lounge: Tuesday, July 31, 2001

- 1:30 – 2:00 Yan Linhart, University of Colorado
The application of genetic and evolutionary perspectives to conservation and management of native gene pools
- 2:00 - 2:30 Bill Libby, Zobel Forestry Associates
Some strategies for production forestry and forest conservation in the face of climate change.
- 2:30 - 2:50 Valerie Hipkins, USDA Forest Service
Gene conservation and genetic markers in the US Forest Service: From conifers to grasses
- 2:50 - 3:15 Sara Lipow, Oregon State University
Results of the gene conservation effort for conifers in Oregon and Washington
- 3:15 - 3:45 Break
- 3:45 - 4:10 Alexander Alexandrov, Forest Research Institute
Forest genetic resources of southeastern Europe and their conservation
- 4:10 - 4:30 Kristin Kolanoski, Northern Arizona University
Effects of thinning on genetic variation of ponderosa pine in northern Arizona
- 4:30 - 4:55 Cuauhtemoc Saenz-Romero, Instituto de Investigaciones sobre los Recursos Naturales
Altitudinal genetic variation among Pinus oocarpa natural populations in Michoacan, Mexico. Preliminary results on 6-month-old seedlings



Workshop: Applied Forest Tree Improvement – Genetics, Physiology, and Breeding of Forest Trees.

Organized by: Thimmappa Anekonda, Oregon State University
University Club, Clubroom 1: Thursday, August 2, 2001

- 1:30 – 2:05 John Major, Canadian Forest Service
Significant ecophysiological adaptations of early and late successional spruce species from Eastern Canada.
- 2:05 – 2:40 Thimmappa Anekonda, Oregon State University
Genetics of cold and drought hardiness in coastal Douglas-fir
- 2:40 – 3:15 Chris Hawkins, University of Northern British Columbia
*Testing interior spruce (*Picea glauca* (Moench) Voss X *P. engelmanni* Parry ex Engelm.), somatic embryogenesis clones in the central British Columbia interior*
- 3:15 – 3:45 Break
- 3:45 – 4:05 Charlie Cartwright, BC Ministry of Forests
Genotype-by-Shade Interactions in Western Hemlock.
- 4:05 – 4:25 Sue Nykoluk, University of Northern British Columbia
*Resistance of interior spruce somatic embryogenesis clones to white pine weevil, *Pissodes strobi* (Peck), attack.*
- 4:25 – 4:45 Nicole Wilder, University of Northern British Columbia
Genecology of 20 Paper Birch Sources from British Columbia and Northern Idaho.
- 4:45 – 5:05 Tongli Wang, University of British Columbia
Impacts of coancestry on growth and yield in commercial plantations.



Workshop: Breeding for Resistance – Developments and Issues for the Future.

Organized by: Richard Sniezko, USDA Forest Service, Dorena Tree Improvement Center

University Club, Dining Room: Thursday, August 2, 2001

- 1:30 – 1:55 Fatih Temel, Oregon State University
Early Testing of Douglas-fir for Swiss Needle Cast Tolerance
- 1:55 – 2:20 Bohun B. Kinloch, Jr., Institute of Forest Genetics
Genetic Interactions in the White Pine/Blister Rust Pathosystem
- 2:20 – 2:45 Richard A. Sniezko, USDA Forest Service
*Breeding for resistance to *Phytophthora lateralis* in Port-Orford-cedar : Current Status and Considerations for Developing Durable Resistance*
- 2:45– 3:10 John Russell, Ministry of Forest
*Linking deer browsing, monoterpene production, and genetic variation: Case study in Cupressaceae (*Chamaecyparis nootkatensis* and *Thuja plicata*)*
- 3:10 – 3:45 Break
- 3:45 – 4:10 Timothy L. White, University of Florida
Synergistic Interactions Among Programs in Tree Improvement, Forest Pathology and Molecular Biology
- 4:10 – 4:35 John King, BC Forest Service
*Genetic resistance of Sitka spruce (*Picea sitchensis*) populations to terminal weevil (*Pissodes strobi*): possible major gene involvement*



Workshop: Molecular Forest Genetics

Organized by: Garth Brown, UC Davis

University Club, Lounge: Thursday, August 2, 2001

- 1:30 - 1:50 Kermit Ritland, University of British Columbia
Forestry Genomics: a major new Canadian initiative in structural and functional genomics
- 1:50 - 2:10 Xin-Sheng Hu, University of British Columbia
Joint Full- or Half-Sibs Linkage Mapping in a Natural Population
- 2:10 - 2:30 Kathie Jermstad, Institute of Forest Genetics
Quantitative trait analysis for adaptive traits and their environmental elicitors in a three-generation pedigree of Douglas-fir
- 2:30 - 2:50 Konstantin Krutovskii, Institute of Forest Genetics
Comparative mapping across three conifer genera
- 2:50 - 3:10 Cherdsak Liewlaksaneeyanawin, University of British Columbia
Investigating mating system of white pine weevil, (Coleoptera: Curculionidae) using microsatellite DNA markers
- 3:10 - 3:50 Break
- 3:50 - 4:10 Karen Thamarus, CSIRO Forestry and Forest Products
A genetic linkage map for Eucalyptus obulus with candidate loci for wood and fibre traits
- 4:10 - 4:30 Carol Loopstra, Texas A&M University
Regulation and function of genes involved in wood development
- 4:30 - 4:50 Andrew Groover, Institute of Forest Genetics
Identification of genes controlling vascular development and wood formation using gene traps



Field Trip
75th Anniversary Celebration at the
Institute of Forest Genetics
Organized by Tom Ledig and Chris Nelson

August 1, 2001, 1:30 – 9:00pm

- 1:30 Buses depart Davis for Placerville
- 2:30 - 2:40 Welcome to the Institute of Forest Genetics, Pacific Southwest
Research Station, USDA Forest Service - Garland Mason,
Acting Director
- 2:40 - 3:00 Dedication of the Eddy Arboretum interpretive displays - Chris
Nelson
- 3:00 - 3:40 History, accomplishments, and current program of the Institute
of Forest Genetics - F. Thomas Ledig, Senior Scientist
- 3:40 - 4:00 Memories of James G. Eddy - John W. Warjone, President,
Timber Division, Port Blakely Tree Farms, and grandson of
James G. Eddy
- 4:00 - 6:00 Poster Session and self-guided tour of the Institute of Forest
Genetics
- 6:00 - 9:00 Barbecue dinner
- 9:00 Buses depart for Davis



Workshop Abstracts

Geographic Variation and Paleo- Inference – A Workshop Dedicated to the Memory of Bill Critchfield.

University Club, Clubroom 1: Tuesday, July 31, 2001



Biogeography of Lodgepole pine: genetic evidence

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Lodgepole pine (*Pinus contortae*) has been a component of the western North America flora throughout recent geological history although its present widespread distribution may be a relatively recent phenomenon (Late Pleistocene). The differentiation of populations within and between subspecies, and the distribution of rare alleles across the natural range, both suggest that at the time of maximal Wisconsin glacial advance lodgepole pine was restricted to a number of refugia in the western United States, the islands along the north Pacific coast, and in an unglaciated region of the west-central Yukon. Subsequent to glacial retreat, lodgepole pine migrated to its present range with northern and southern interior populations meeting in northern British Columbia, possibly within the last 4000 years. In north central Alberta, lodgepole pine populations met and hybridized with the closely related Jack pine (*Pinus banksiana*), presumably migrating from the east.

Evolutionary History of the Mesoamerican Oocarpae and Caribbean Pines

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The Mesoamerican Oocarpae include *Pinus greggii*, *P. jaliscana*, *P. oocarpa*, *P. patula*, *P. pringlei*, *P. tecunumanii* and several varieties. *Pinus caribaea* from the Australes subsection has been known to naturally hybridize with both *P. oocarpa* and *P. tecunumanii* in Central America for years. The lack of reproductive barriers between certain species in the Oocarpae and Australes raises interesting questions about their evolution.

Phylogeny assessment of both taxonomic groups was conducted by CAMCORE, North Carolina State University. A total of 110 primers were tested that yielded 76 intense scorable RAPD bands. From these 127 markers were assessed for band presence or absence in the pooled DNA sample.

The results suggest that ancestral oocarpa was progenitor of the Mesoamerican closed-cone pines. *Pinus patula* evolved from ancestral oocarpa and served as the progenitor of *P. greggii*. *Pinus tecunumanii* is closely related to *P. oocarpa* but appears to be of much more recent origin than *P. patula* or *P. greggii*. *Pinus pringlei* originated from *P. oocarpa* and apparently served as one of the ancestors of *P. jaliscana*.

Pinus caribaea var. *hondurensis* occupies an intermediate position on the phylogeny tree between the Mesoamerican Oocarpae and the Australes pines. Essentially, it is both a "closed-cone" and a "southern pine". It appears to have evolved from an Oocarpae ancestor and migrated from Central America into the Caribbean as variety "caribaea".

The importance of these evolutionary relationships are discussed in terms of gene conservation and disease resistance.

Genetic Evidence for Refugia and Migrations of the Southern (AUSTRALES) Pines After the Last Glacial Maximum

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The current natural ranges of the southern pines (SUBSECT. AUSTRALES Loud.) includes the warm-temperate to sub-tropical climate of the southeastern United States. Little is known about the location of the southern pines during the Pleistocene glaciation. Macrofossils of spruce (mostly the extinct *Picea critchfieldii*) dating from the late Pleistocene have been found within the current range of the southern pines indicating that the climate was considerably colder at that time. It is reasonable to assume that the southern pines were situated south of their present range during the Pleistocene. Variation in adaptive and non-adaptive genetic traits suggests that loblolly pine (*P. taeda* L.) existed in two refugia, one in south Texas/north Mexico, and one in south Florida. Longleaf pine (*P. palustris* Mill.) probably existed only in the western refugium. Slash pine (*P. elliottii* Engelm.), on the other hand, presumably resided only in the Florida refugium, whereas shortleaf pine (*P. echinata* Mill.) is cold-hardy enough to have existed in a continuous distribution across the Gulf Coast.

Rangewide allozyme variation in Atlantic white-cedar (*Chamaecyparis thyoides* (L.)BSP)

Kristin A. Mylecraine, John E. Kuser, George L. Zimmermann, Peter E. Smouse, Thomas R. Meagher

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Atlantic white-cedar is a wetland tree species, found along the Atlantic and Gulf coasts of the United States, from southern Maine to central Florida and west to Mississippi. It is primarily a lowland species, being largely confined to a narrow coastal belt, 50 to 100 miles wide. The distribution within this belt is very patchy, depending on the occurrence of suitable sites. The populations in the Florida panhandle, Alabama and Mississippi have been considered a separate variety, *C. thyoides* var. *henryae*. There has been a significant decline in the area occupied by this species over the past 200 years. As a result, there has been growing interest in this species, its management and restoration, because of its economic value as a timber species, as well as its ecological value. The distribution of genetic variation within and among Atlantic white-cedar populations should be an important element in management and restoration plans for the species. We present the first rangewide genetic study of this species. We examine the distribution of genetic variation within and among a set of 40 populations, including both *C. thyoides* var. *thyoides* and *C. thyoides* var. *henryae*, for allozyme variation at eleven loci. We used Analysis of Molecular Variance (AMOVA) to delineate the extent of geographic variation within and among varieties, regions, and populations. We also calculated time-linearized genetic distances between pairs of populations, performed a UPGMA cluster analysis, and examined the relationship between genetic and geographic distances between population pairs. We found significant regional differentiation among populations. There also appear to be slight differences between varieties, although the degree of differentiation is not large, compared to the degree of regional differentiation. We found a significant relationship between genetic and geographic distances between population pairs. This information should be taken into account when developing management plans, in order to prevent gene depletion and ensure conservation. It is also important for choosing stock materials for breeding purposes and restoration efforts.

Genetic diversity, mating system, and inbreeding depression in western redcedar (*Thuja plicata*)

Lisa O'Connell and Kermit Ritland

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In general, conifers have high genetic diversity, high inbreeding depression and low self-fertilization rates compared to other plant groups. A conspicuous exception in conifers is the genus *Thuja* (Cupressaceae) which has shown low genetic diversity, low inbreeding depression and high selfing rates. Even though *Thuja plicata* occupies a large geographical range along the coast of western North America, measures of genetic diversity at both neutral molecular markers and phenotypic traits are among the lowest in conifers. Using one polymorphic isozyme marker we estimated outcrossing rates in six natural populations of *T. plicata* in southwestern British Columbia. Population outcrossing rates ranged from 17 to 100% (mean = 71% +/-4.5 SD), indicating a labile mating system with marked among-population differences. Early inbreeding depression in redcedar is lower than in most other conifers and can allow for ecological differences among populations and trees to influence levels of outcrossing. We used highly polymorphic microsatellite markers to look at variation in mating system within populations. Polyembryony has often been suggested as a mechanism that could mitigate the effects of self-pollination in conifers. Several embryos pollinated by different parents co-occur within an ovule but only one ultimately survives. We conducted controlled pollinations in a seed orchard and found that polyembryony did not decrease rates of selfing in western redcedar. One possible scenario for the low genetic variation and inbreeding depression found in *T. plicata* is that the species experience a bottleneck during the last glaciation which led to high levels of inbreeding and purging of deleterious mutations and a reduction in genetic variation at linked loci through background selection. We use nine microsatellite loci to study patterns of range wide genetic structure. Populations were separated into a northern and southern clade suggesting two glacial refugia. By combining microsatellite mutation rates with the distribution of alleles presently found in western redcedar we can date the species' genetic bottleneck and test whether it coincides with the last glaciation.

Workshop Abstracts

Local Population Structure.

University Club, Dining Room: Tuesday, July 31, 2001



Two-Generation Analysis of Pollen Flow in Forest Tree Species

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Current studies of gene flow are driven by questions concerning contemporary patterns of gene movement that are not well served by treatments based on evolutionary equilibrium. We propose a novel two-generation approach to the study of genetic structure, labeled TWOGENER, a hybrid approach that utilizes some of the better features of 'genetic structure' and 'paternity assessment' methods to quantify heterogeneity among the male pollen pools sampled by maternal trees scattered across the landscape. The method provides estimates of mean pollination distance and effective neighborhood size, allowing us to address a number of questions about the factors that influence the movement of pollen across contemporary landscapes.

First, we describe the model's elements: a genetic distance matrix to estimate inter-gametic distances, and molecular analysis of variance to determine whether pollen profiles differ among mothers. We illustrate the procedure for species where the male gamete is categorically (e.g., conifers) or ambiguously (e.g., angiosperms) determined, because both cases are feasible. Spatial heterogeneity in pollen pools is gauged by the intraclass correlation, F_{ft} , the among-female portion of paternal gametic variation, an analogue of Wright's F_{st} , but with the females as strata.

Second, we relate the expected value of F_{ft} to the expected mean distance of pollen flow, d , using theoretical arguments that parallel 'isolation by distance' models of Wright and Malécot, showing that F_{ft} is an inverse function of mean pollination distance. With TWOGENER, we have now examined pollen flow in empirical studies of several species, and have shown that while forest tree pollen can disperse for kilometers, effective pollination is generally quite localized.

Third, we indicate the direction of ongoing work concerning the impact of adult 'genetic structure', selectively-maintained gradients, and temporal variation on our inference about 'pollen structure'. We also indicate how the method can be used for comparative study of pollen flow, designed to examine the relative impacts of such factors as population density and canopy structure, the differences between continuous forest and anthropogenically created forest fragments, and the impact of pollen flow on the genetic cohesion of our increasingly fragmented populations of forest tree species.

Pollen movement in declining populations of California Valley Oak, *Quercus lobata*: Where have all the fathers gone?

Victoria L. Sork, Frank W. Davis, Peter E. Smouse, Rodney J. Dyer, and Juan F. Fernandez

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The fragmented populations and reduced population densities that result from human disturbance are issues of growing importance in evolutionary and conservation biology. A key issue is whether the extant individuals are becoming reproductively and genetically isolated. Valley oak is a widely distributed California oak species that is increasingly jeopardized by anthropogenic changes in the biota and land use. We studied pollen movement in a savanna population of Valley oak at Sedgwick Reserve, Santa Barbara County to estimate effective number of pollen donors (N_{ep}), average distance of effective pollen movement (d), and effective pollination neighborhood (A_{ep}). Using our recently developed TWOGENER model, a hybrid of paternity analysis and genetic structure statistics, we analyzed maternal and progeny multilocus genotypes derived from one microsatellite primers and eight allozyme loci. We found that the $N_{ep} = 3.68$ individuals and average pollen dispersal distance of $d = 62.4$ m, based on average adult stem density of $d = 1.18$ ha⁻¹. Our results also show that $A_{ep} = 3.09$ ha, which means that a given mother is sampling pollen these ~ 4 males from a circle with a radius of approximately 100 meters. We then deployed our parameter estimates in spatially explicit models of the Sedgwick population to ask whether N_{ep} has changed due to progressive stand thinning between 1944 and 1993. Using information from aerial photographs mapped onto a GIS database, we simulated the consequences of pollen movement for the changed landscape. We discovered that under denser stand conditions in 1944, 16 individuals would have contributed 99.9% of effective pollination within 113m, and the 3 nearest neighbors would have contributed 50% of the pollen. By 1993, density was reduced to the point that 13 individuals supplied 99.9% of the effective pollen within 128m; the nearest neighbor (36m from the index tree) would account for 50% of the effective pollen. Effective pollen movement may be much more restricted than has previously been realized, and it may become even further restricted with continuing demographic attrition.

Effects of spatial variation on Two-Generation estimates of gene flow.

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The analysis of spatially separated pollen pools provides insights into the patterns of contemporary pollen movement through estimates of mean pollen dispersal distance and the genetic effective pollination neighborhood size. However, in many cases, factors external to the intrinsic pollen dispersal distance can influence the distribution of genetic variation both within and among spatially separated pollen pools. For example, allele frequency gradients associated with elevation changes as well as variation in both the number of local pollen donor densities and the physical architecture of the sampled stands have been shown to influence pollen pool composition and differentiation. Here I present an extension of the Two-Generation model which allows the investigator to identify the influence of external factors on the distribution of genetic variation observed in spatially separated pollen pools. By re-parameterizing the Two-Generation analysis as a multivariate step-wise regression analysis (dubbed the Two-Generation Stepwise model), the effects of external variables are removed prior to the estimations of both mean pollen dispersal distance and effective pollination neighborhood size. The model is checked for validity using an extensive battery of simulations to illustrate how bias effects the differentiation of spatially separated pollen pools as well as how the Two-Generation Stepwise model can make the appropriate corrections. Finally, the Two-Generation Stepwise model is applied to a *Quercus alba* data set, of which we know a priori that adult genetic structure is non-uniform across the sampling landscape.

Investigations on plant reproductive success through selection and ecological gradients using genetic markers and neighborhood models.

J. Burczyk and W.T. Adams

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One of the greatest challenges in plant mating system studies is to obtain reliable estimates of male and female reproductive success. Recent advances in estimation methodology greatly increase the utility of genetic markers for evaluating reproductive patterns. Although some researchers attempt to estimate reproductive success of each individual in a population, others attempt to quantify various factors influencing reproductive success. These factors are frequently referred to as 'selection and ecological gradients'.

A family of neighborhood models has been successfully applied to a variety of wind and insect pollinated forest trees, both in natural and seed orchard populations, allowing for precise estimation of several selection and ecological gradients. For example, one model is used to compare the genotypes of naturally regenerated seedlings to genotypes of putative parents within a local population. Applying the model to observed data using maximum likelihood methods makes possible estimates of parameters influencing selection and ecological gradients of both male and female functions and the evaluation of their influence on effective reproductive success realized at the seedling stage. Examples of recent investigations demonstrate the flexibility and usefulness of the neighborhood model approach.

Mating systems and coancestry in sugar pine (*Pinus lambertiana*)

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We extend previously reported results on mating systems analyses of four stands (clusters) of sugar pine (*Pinus lambertiana*) in the Mountain Home State Forest, in the southern Sierra Nevada, California. Mating system parameters were estimated by Ritland's MLTR. In all clusters except one, outcrossing rates were not significantly different from one (at the 5% rate). Differences between the single and multilocus outcrossing rates were not significantly different from zero, except in one spatially-discontiguous cluster. In that same cluster, the parental F was -0.47 and significantly less than zero, suggesting that males and females were siblings. The effective numbers of males per family ranged between 1.1 and 7.2 over the four clusters. To further examine coancestry, we regressed the outcross pollen frequencies on the maternal genotype, using SAS' PROC GLM. Regression slopes generally increased with the spatial size of the clusters. However, slopes were significantly different from zero in only one cluster, the spatially smallest one, and that slope was negative. The partial correlation between maternal and paternal gametes in this cluster was -0.45 , suggesting a trend towards negative assortative mating. We conclude then, that positive coancestries in the stands of larger area is due primarily to spatial heterogeneity in parental allelic frequencies. To examine the degree of migration of pollen into a genetic neighborhood, we used the multivariate equivalent of a variant of the t-test, whereby each candidate individual is tested for membership in a population. We used two alternatives of the methods: one basing means and variances on maternal allelic frequencies and the other on pollen allelic frequencies. With the 5% probability criterion, 4% were different from the neighborhood using maternal frequencies and 6% were different using pollen frequencies. However, in Wright's bivariate neighborhood, assuming normally-distributed dispersal, 91% of the parents are assumed to contribute to the central individual. Under this probability distribution, about 6% of the pollen genotypes were migrants under both data distributions. Thus gene flow into the general mating neighborhood is small.

Space, time, and a montane species: genetic structure in mountain hemlock (*Tsuga mertensiana*).

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The mating system, dispersal mechanism, and relative contribution of pollen or seed to total gene flow can modify the scale at which patterns of genetic diversity are observed. We contrast the regional distribution of genetic variation in populations of mountain hemlock (*Tsuga mertensiana*) across British Columbia to the genetic structure found at the local population scale. At the regional level, using 19 isozyme loci, we found 32% of the loci were polymorphic and expected heterozygosity was 0.087, half the heterozygosity found in other conifers. Outcrossing rates did not significantly differ from unity and there was no significant level of inbreeding. Overall, populations of mountain hemlock across British Columbia had low levels of differentiation ($G_{st} = 0.077$) consistent with a high level of pollen flow. By comparison at the local population level, spatial genetic structure within a single old growth stand was assessed using microsatellites. Measures of genetic diversity, inbreeding, and relatedness were computed for different diameter classes. Expected heterozygosity was high (0.919) across all classes and inbreeding was significantly different from zero increasing exponentially to a maximum (0.182 ± 0.0001) as diameter class increased. High levels of inbreeding observed may have indirectly resulted from family clustering, the presence of null alleles, and a temporal Wahlund effect. Adults and seedlings differed significantly in both allelic and genotypic composition suggesting that these seedlings were not simply a subset of the neighboring adult gene pool. The 1976 clearcut of 43.3 ha surrounding the old growth may have substantially altered the density of trees enhancing seed flow. Significant relatedness was detected only in the adult class between trees 5m apart. Genetic structuring within a single stand of mountain hemlock can be attributed to local seed dispersal, seedling recruitment over a long period of time, and long distance founding events.

Respiration based growth rate predictions match *Pinus ponderosa* elongation season, ambient temperature and show differences between genotypes

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The interaction of metabolism with environmental factors is central to defining growth and distribution of plants. Respiratory and growth measurements of estimated net metabolic energy (predicted growth rate, RSGDHB) were made on three *Pinus ponderosa* (PP) planted in Davis, CA and 12 PP clones grown in Malin, OR. Prior to bud elongation RSGDHB measurements were low. One to four weeks before bud elongation became visible RSGDHB began to increase. RSGDHB continued to increase during rapid bud elongation and decreased as bud elongation slowed in early summer. Temperature responses of RSGDHB were consistent with the temperature response of PP seedling growth rates measured in numerous studies. RSGDHB measurements indicated that PP are able to respond rapidly to temperature variation between 15 and 25C, which corresponds well with the reported maximum seedling growth rates at approximately 23C. Genotypic variation in RSGDHB was measured. RSGDHB fluctuated with ambient temperature, increasing when daily high temperatures (DHT) began to exceed 15C and declining when DHT began to regularly exceed 25C. The timing and temperature responses of RSGDHB appropriately matched ambient temperature data collected during elongation on both exotic and native PP sites. The data indicates that PP is adapted to climates where 20 to 25C are some of the warmest temperatures present while soil moisture remains abundant. Should site climates change, PP will not grow at warmer temperatures, but will grow at different times of the year. An important question then becomes how would climate change affect the duration and characteristics of the bud elongation season.

Workshop

Current Issues in Conservation

University Club, Lounge: Tuesday, July 31, 2001



The application of genetic and evolutionary perspectives to conservation and management of native gene pools

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First, I will discuss briefly some examples of the remarkable diversity of empirical and conceptual issues for which genetic perspectives are important in the conservation biology of forest trees. The emphasis will be on getting some audience participation to add to the list.

I will go on and present concrete examples of the role played by herbivores and parasites as agents of natural selection in forest stands. The principal conclusion of this body of empirical evidence is that such selection is often diversifying, and therefore it is imperative to maintain large amounts of genetic variability within native and planted stands.

The second topic I will develop deals with the potential problems associated with gene flow from non-local seed sources into native forest stands and the resulting potential for genetic pollution and maladapted regeneration in such stands.

Some strategies for production forestry and forest conservation in the face of climate change.

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Historically, climate changes have presented challenges to populations of forest dwellers of all sorts, both for populations that stay in place, and for those that need to migrate. This issue is worthy of interest and concern. It is the opposite of maintaining local populations. Rather, it employs using some immigration or mixed plantings to create a broader genetic base when the population needs to adapt or migrate, particularly if humans have placed impediments in previous migration routes, or if human needs for forest resources require faster adaptation than might otherwise occur. These strategies by no means imply that we should not protect the genetic integrity of most native populations, but they are contingency strategies for some of them.

Gene conservation and genetic markers in the US Forest Service: From conifers to grasses

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In 1992, National Forest System geneticists in the USDA Forest Service adopted a “Genetic Resource Program Strategic Action Plan” to focus genetic activities in support of the agency’s agenda for ecosystem management. The Strategic Plan concentrates on five primary areas, one of which is genetic conservation. The genetic conservation imperative calls for developing and implementing genetic conservation and genetic resource management programs for plant and animal species. A partial list of goals includes measuring genetic diversity of plant and animal species, developing seed transfer guidelines for native and/or introduced woody and herbaceous plant species, inventorying patterns of genetic variation within and among populations of species, determining appropriate long-term management methods for *in situ* reserves, maintaining threatened, endangered, sensitive, and special emphasis species (TES), and developing propagation and/or breeding programs.

The National Forest Genetic Electrophoresis Laboratory (NFGEL) is a national USDA Forest Service facility. Our role is to assess and monitor genetic variation in plant species by analyzing molecular genetic markers (protein and DNA) in material submitted by Forest Service employees and those from other cooperating entities. Genetic data are used to determine genetic structure, measure genetic diversity, clarify taxonomic relationships, study mating systems and genetic relatedness, and address other issues of biodiversity and conservation. NFGEL provides baseline genetic information, determines the effect of management on the genetic resource, supports genetic improvement programs, and contributes information which supports conservation and restoration programs, especially those involving native and TES species. Our services include project proposal development, sample design and collection strategy formulation, protein and DNA marker electrophoresis, data interpretation and analysis, and reports of results and management implications.

The conservation concerns of Forest Service managers are broad. Recently, we have completed studies which have identified the source of off-site plantations of ponderosa pine (*Pinus ponderosa*), determined the origin of an isolated stand of limber pine (*Pinus flexilis*), determined the clonal distribution in quaking aspen (*Populus tremuloides*) in Washington, clarified taxonomic relationships in several TES plants, and offered guidelines for the collection and management of the native grass, *Elymus glaucus*.

Results of the gene conservation effort for conifers in Oregon and Washington

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Concerns for biological diversity and the genetic aspects of sustainable forest management recently prompted a group of forest geneticists representing government, university and private interests to form the Pacific Northwest Forest Tree Gene Conservation Group. One objective of this group is to identify whether there are areas in Oregon and Washington where additional conservation measures are necessary to ensure that the adaptation and evolutionary potential of important conifer species is maintained. As part of this effort, we developed a method of gap analysis to examine genetic resources conserved in situ. The method involved displaying three types of data as GIS layers. The first layer showed the location of various types of protected areas. The second layer displayed the distribution of each tree species across the landscape as inferred from available GIS coverages and grids showing vegetation type. The third layer presented a scheme for stratifying the distribution of each species into genetically meaningful units for analysis, in this case seed zones and ecoregions. GIS allowed us to intersect these layers to determine for each species in each seed zone or ecoregion the minimum expected population size in protected areas. The results show that in much of the study area the genetic resources of most species are well protected in in situ reserves. Additional gene conservation, however, is warranted for western white and sugar pine in many of their ranges and for noble fir in the Willapa Hills of southwest Washington. We also compiled data on genetic resources conserved ex situ. We summarized all resources present in seed stores, provenance and progeny tests, seed orchards, and clone banks both in western Oregon and Washington and in other countries when the germplasm originated in western Oregon and Washington. These combined approaches provide more detailed information on the gene resource status of tree species than any other approach taken to date.

Forest Genetic Resources of Southeastern Europe and Their Conservation

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As a result of the climatic and the geo-morphological peculiarities of Southeastern Europe and of the historical development of its vegetation, especially during the Tertiary and Quaternary, at the present this part of the Old Continent possesses the largest number of forest tree species presenting valuable genetic resources. The paleontological and palinological surveys in the investigated region show that the forests here are of an autochthonous origin and had been not submitted to catastrophic impacts during the Pleistocene. Southeastern Europe represents an enormous refugium, in which forest tree vegetation of an ancient origin has been preserved. Today this part of Europe includes three phytogeographic regions: European Deciduous Forest Zone, European Steppe-and-Forest-Steppe Zone and Mediterranean Sclerophilic Forest Zone, which possess significant vegetation diversity. The horizontal zonal differentiation from 35° to 48° southern latitude and the vertical zonal differentiation from 0 up to 9600 feet in the big mountain massifs as Rila-Rhodopes, Balkan Mts., Carpathians, Dinar Chain, Olymp, Pind and other mountains determine to a great extent the wide phyto-geographic range of species - from boreal to Mediterranean ones. The natural phyto-geographic links of Southeastern Europe with Asia Minor and Caucasus enrich the flora of this region. The composition of the deciduous forests is dominated mainly by representatives of Fagaceae, Aceraceae, Tiliaceae, Betulaceae, Rosaceae, Oleaceae, Salicaceae, Ulmaceae, Juglandaceae, Platanaceae and other families. The conifers are from Pinaceae, Cupressaceae and Taxaceae families, of greater economic importance being the species from genera *Pinus*, *Picea*, *Abies* and *Larix*. Some of the tree species as Macedonian pine (*Pinus peuce* Griseb.), Heldreich pine (*Pinus heldreichii* Christ.), Serbian spruce [*Picea omorika* (Panc) Purk.] and horse chestnut (*Aesculus hippocastanum* L.), which are endemics of relict origin, i.e. paleoendemics, need additional measures for their protection. The conservation of the forest genetic resources in this region is performed by the in situ and ex situ methods, predominantly by the first one. This activity in Europe is performed in accordance with the Resolution 2 "Conservation of Forest Genetic Resources" of the Ministerial Conference for the Protection of Forests in Europe, held in Strasbourg in 1990 and under the auspices of the International Plant Genetic Resources Institute (IPGRI), together with FAO. The established European Forest Genetic Resources Programme (EUFORGEN), including five networks: 1. Conifers; 2. Social Broadleaves; 3. Noble Hardwoods; 4. Mediterranean Oaks and 5. Poplars, is in process of execution. The development of conservation strategies and guidelines and the establishment of national gene reserve forests, which is one of the main activities of the Programme, is being successfully implemented in Southeastern Europe.

Effects of thinning on genetic variation of ponderosa pine in northern Arizona

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Structure and function of the ponderosa pine ecosystem in northern Arizona has changed considerably since pre-Euro-American settlement in the late 1800's due to the introduction of grazing, fire suppression, and logging. Extensive thinning and prescribed burning treatments are being used to return historic conditions to the ponderosa pine forests of Flagstaff's urban-wildland interface (FUWI). The purpose for this genetic study is to assess the impact of thinning treatments on the genetic diversity of ponderosa pine in FUWI. The study compared allozyme variation for 23 loci of pre- and post-settlement ponderosa pine trees on five sites subjected to two different thinning treatments to address the following research questions: 1) Do pre- and unthinned post-settlement populations differ genetically from each other? 2) Do thinned post-settlement populations differ genetically from unthinned post-settlement, and pre-settlement populations?

We found no significant differences among pre- and post-settlement trees for HE, HO, %P and the number of alleles per locus (A). Also, the number of loci fixed for one allele and the number of loci with genotypes deviating from H-W equilibrium did not significantly differ among pre- and post-settlement trees. A lack of significant differences in these parameters was also found when post-settlement trees randomly selected as "leave trees" were compared to post-settlement trees spatially selected to re-create specific historic, clumpy patterns. However, two loci showed allele frequency differences among randomly and spatially selected post-settlement trees, while allele frequencies for eight loci were different among pre- and unthinned, randomly selected post-settlement trees. These results suggest genetic differences occur at the individual loci level among pre- and post-settlement populations, but that there is little difference at individual loci between randomly and spatially selected post-settlement trees.

Results from thinning simulations showed that reduction in post-settlement populations by 50% and 75% of their original size did not significantly change HE, HO, %P, and allele frequencies. However, significant differences in A were found when pre-settlement populations were compared to 75% thinned post-settlement populations, and when unthinned post-settlement populations were compared to 50% thinned and 75% thinned post-settlement populations. In addition, significant allele frequency differences were found at nine loci in comparisons between pre-settlement and 50% thinned post-settlement populations, and at three loci in comparisons between pre-settlement and 75% thinned post-settlement populations. Although changes due to thinning were not large, selection guidelines for restoration thinning should include leaving more post-settlement trees on site to conserve genetic diversity and to minimize changes in allele frequencies in post-settlement populations.

Altitudinal genetic variation among *Pinus oocarpa* natural populations in Michoacan, Mexico. Preliminary results on 6-month-old seedlings.

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We are investigating if there is genetic differentiation among natural populations of *Pinus oocarpa* along altitudinal gradients in the southern slope of the Neovolcanic axis, western Mexico. Open pollinated seeds from about eleven individual trees were collected from each of five *Pinus oocarpa* natural populations distributed along an altitudinal gradient from 1100 to 1500 m, near to Uruapan city, Michoacan state, Mexico. A provenance/progeny test was established in a nursery in Morelia, Michoacan, Mexico and evaluated from 1 to 6 months of age. Preliminary analyses suggest the existence of an altitudinal gradient for the number of cotyledons: populations at lower altitudes had a larger number of cotyledons than populations at higher altitudes (except for the population at the highest altitude, which had an unexpected large number of cotyledons). Basal diameter at 5 months of age was almost significantly different ($p = 0.0777$) among populations, where the population from 1300 m of altitude had the largest diameter. The length of the longest cotyledon and the height at 2 and 6 months of age were not significantly different among populations. However, all the variables were significantly different among half-sib families within populations. The provenance/progeny test was transplanted to two field locations at one year of age. It remains to be seen if at older ages populations would present significant differences among them.

Workshop Abstracts

Applied Forest Tree Improvement – Genetics, Physiology, and Breeding of Forest Trees.

University Club, Clubroom 1: Thursday, August 2, 2001



Significant ecophysiological adaptations of early and late successional spruce species from Eastern Canada

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Red spruce (*Picea rubens* Sarg.) is a valuable timber species that is an important component of late successional forests of eastern Canada. However, red spruce has experienced substantial decline over the majority of its geographic range related to past forestry practices, atmospheric pollution and climate change. Except in the southern part of its Appalachian range, red spruce is largely sympatric with early successional black spruce (*Picea mariana* (Mill.) B.S.P.). The two species appear closely related morphologically, in their isozyme and molecular marker profiles and are capable of hybridization. Hybridization with black spruce has resulted in identification and management problems for these otherwise ecologically distinct species. Benchmark information on growth, development and physiological traits that might be useful in differentiating these species and particularly their hybrids are generally lacking. Current research on adaptive traits differentiating these species and their hybrids will be presented. We examined, compared, and analysed a number of morphometric developmental and physiological adaptive traits including, early development, and allometric relationships. Ecophysiological responses were also examined such as light response curves, freezing tolerance and response to atmospheric moisture stress. Summary results from growth and adaptive physiological response will be reported.

Genetics of cold and drought hardiness in coastal Douglas-fir

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The Pacific Northwest Tree Improvement Research Cooperative (PNWTIRC) conducted artificial freeze tests (1991-2001) on more than 290 coastal Douglas-fir families in five breeding populations in the Pacific Northwest and assessed injury to needle, stem and bud tissues in both seedlings and saplings. Results of these studies demonstrated (1) a high degree of within-population (Coast and Cascade sources) genetic variation in both spring and fall cold injury traits; (2) greater genetic variation among clones of a single full-sib family relative to families within a breeding population; (3) high genetic correlations between cold injury traits within seasons, between sites within breeding zones, between seedlings and saplings within breeding zones, and between artificial and natural cold injury; and (4) low (or inconsistent) genetic correlation between cold injury and stem growth.

The Coop also investigated genetic variation in drought hardiness traits in 39 full-sib families (1996-2001) of coastal Douglas-fir from British Columbia. Seedlings of these families were grown in raised nursery beds under control and drought treatments, applied in second and third growing seasons, and assessed for stem growth, foliage damage, and stem hydraulic traits, with fall cold injury traits assessed only in the second season. Growth ring traits (wood density and ring width) of these families were also characterized for their response to drought in a field environment. These investigations showed that (1) families varied considerably in their response to summer drought in both nursery and field environments; (2) relative to cold injury, genetic control of drought hardiness is weak but of a similar magnitude to that observed for height and diameter traits under well-watered treatments; (3) genetic correlations between drought hardiness traits in the same year were high, low to moderate between different years, and low between the hardiness of seedlings and saplings; and (4) drought hardiness was uncorrelated with cold injury traits in seedlings and showed low to moderate genetic correlation with growth traits.

Because cold hardiness has shown consistent and reliable results, the Northwest Tree Improvement Cooperative (NWTIC), the applied breeding Coop, is considering cold hardiness screening in its second-generation tree-breeding program. Drought hardiness, however, appears to be under less genetic control than cold hardiness. Comparable methods in nursery and field should be developed prior to breeding trees for drought hardiness.

Testing interior spruce (*Picea glauca* (Moench) Voss X *P. engelmanni* Parry ex Engelm.), somatic embryogenesis clones in the central British Columbia interior

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The BC Forest Service (BCFS) initiated an interior spruce somatic embryogenesis (SE) testing program in the central BC interior in 1994. Between 1994 and 1998, 22 candidacy test sites (>54,000 single tree plots) and 25 clonal block sites (>100,000 emblings) were established. The parents used in the crosses were from the BCFS breeding - seed orchard program. This paper reports on six candidacy test sites established in 1995 in four different Biogeoclimatic sub-zones. Full sib seedlings were planted along with SE clones from tested crosses on each of the six sites. In addition, seedlings from a seed orchard and a natural stand seedlot were planted on all sites. Of the reported clones, parental ranks out of 172 parents ranged from 4 to 165 for 15 year height growth and 3 to 146 for spruce leader weevil (white pine weevil, *Pissodes strobi* (Peck)) tolerance. At two years, full sib seedling growth was better than that of SE clonal material. Given the harsh site conditions, survival was good: 78 to 96 percent at year 5. There was significant spruce leader weevil attack on one site. Seed orchard and natural stand seedlot height growth rankings were variable across sites. Generally within a cross, full sib seedlings still have better height growth than clones. None of the clones were suitable for deployment in the Engelmann Spruce - Sub Alpine Fir Subzone. Mean family breeding values did not correlate well with family/clonal performance across sites. Six and seven year post planting examples will be used to highlight these observations. At this time, operational deployment of SE clones is not economically justified in the central BC interior.

Genotype-by-Shade Interactions in Western Hemlock

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Two separate studies examined whether family rankings differed under different light environments. If significant family-by-light interaction is present, then one would have to question whether families tested in clearcut situations (full sunlight) would be optimal for use in silvicultural systems that maintain an overstory (shade) during artificial regeneration. In British Columbia, 53 families from BC and Washington were grown for 2 years under four different light regimes (100%, 72%, 45% and 17% transmission). In Oregon, 39 families from the Tillamook State Forest were grown for 3 years under three light regimes (100%, 70% and 30% transmission).

Shade had a significant impact on growth. The shortest trees were those in the full sun at the end of the second growing season. However, during the third growing season in Oregon, shade began to obviously reduce growth, the greater the shade the greater the reduction in diameter and biomass. More shade also resulted in more biomass allocated to the shoots.

Significant differences among families were found for all traits when analyzed over all light environments. However, not all individual light environments had significant family differences in all traits. Family-mean heritabilities were usually maximized when there was a moderate level of shade.

Family-by-light interactions were non-significant for all traits at both locations. At the BC location the f values for the interaction terms were all less than 1.0. At the Oregon location the interaction variance components ranged from 0 to 42% of the family variance component, but in all cases the interaction was not statistically significant.

The lack of any family-by-light interaction suggests that families selected in one light environment will still be optimal for other light environments. Therefore, current selections tested in clearcut situations may be used in regeneration systems that provide some level of shade.

Resistance of interior spruce somatic embryogenesis clones to white pine weevil, *Pissodes strobi* (Peck), attack.

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Four somatic embryogenesis interior spruce *Picea glauca* (Moench) Voss x *Picea engelmannii* Parry ex Engelm. clones, planted in 1995, on a mesic site in the Sub Boreal Spruce Zone, wet, cool subvariant (SBSwk1 01) and 1996 in the SBSmk1 07, (moist cool, subhygric) were observed for resistance to white pine weevil, *Pissodes strobi* (Peck) attack. The two clones planted in 1996 were subjected to three light intensity treatments and seeded with five weevils per tree in a 2 (clone) x 3 (light) x 2 (weevil density) factorial design in the spring of 2001. Clone 107-1930 originated from parents of high growth (1 female x 5 male) and weevil resistance (10 x 2) rankings while clone I-1026 originated from parents of moderate growth (4 x 78) and weevil resistance (20 x 97) rankings. There are 172 parents in the breeding population. The clones planted in 1995 were also weevil seeded in 2001, but without shade treatments in a 2 (clone) x 2 (weevil density) factorial design. Clone U-284 originated from parents of moderate growth (60 x 36) and weevil resistance (83 x 15) ranking and clone J974 originated from parents of moderate (4 x 102) growth and low weevil resistance (20 x 130) ranking. At this location another clone, U185, with the same growth and weevil ranking as U284, was planted in four blocks respectively with seed orchard seed lot 6863 (Central Plateau) in the proportion 100-0, 67-, 33-67, and 0-100 percent clonal material replicated twice. All blocks were surveyed for growth and weevil attack in 2000 and 2001. From this study tolerance or resistance to weevil attack was quantified by recording feeding and oviposition densities for each clone and by the success (leader kill) of the attack. Success of weevil larvae development is associated with aggregation of oviposition. Reduced light levels have been shown to cause weevils to deposit their eggs dispersed along the stem rather than in an aggregated pattern. The use of clonal material in determining the effects of weevil attack reduces variation for genetic resistance within a spruce genotype. Weevil attack varied significantly by clone. Relationships among clone, light and weevil density, as well as growth will be discussed.

Genecology of 20 Paper Birch Sources from British Columbia and Northern Idaho

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Forty-eight sources of paper birch (*Betula papyrifera* Marsh.) collected along latitudinal, longitudinal and elevational transects from across British Columbia and northern Idaho are being compared in three separate common garden trials. These trials were established in the spring of 2000. A subset of twenty sources was assessed for bud flush in 2001. Bud flush data indicate that the same sources at Skimikin (elevation: 550 m, latitude: 50° 45') and Sandpoint (elevation: 640 m, latitude: 48° 13') flushed at similar times. Trees at these two sites, however, flushed significantly earlier than those at Red Rock (elevation: 725 m, latitude: 53° 45'). The period of time required for each population to flush also differed among sites. The most southerly population from Wrenco, Idaho completed flushing by April 22 at Sandpoint and by April 24 at Skimikin, whereas the same population grown at Red Rock suffered from severe winter injury, resulting in very few trees completing bud flush (buds formed but did not flush); a condition that affected many of the trees at Red Rock. Those buds that did flush, however, did so much later (between May 12 and June 4). The most northerly population from Beaver Lake (latitude: 59° 01') flushed on April 16-20 at Sandpoint, April 15-26 at Skimikin and April 22-May 12 at Red Rock, a period of 4, 11 and 20 days respectively. Seedlings from all forty-eight sources were also grown at 3 different nurseries to assess nursery effects. Seedlings grown in Idaho flushed earlier than those grown in Vernon or Prince George. At Sandpoint, Wrenco seedlings grown in an Idaho nursery completed bud flush on April 9-21, whereas seedlings from the same population grown in Vernon and Prince George nurseries did not complete bud flush until April 17-23 and April 19-25 respectively. Generally, on an elevational transect, high elevation sources flushed before low elevation sources. These preliminary results indicate that there are differences among sites, nurseries and seed sources with respect to bud flush for paper birch.

Impacts of coancestry on growth and yield in commercial plantations

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Inbreeding depression is strong in most species of forest trees, particularly in conifers, and has implications for realized gains from tree improvement programs. In first-generation clonal seed orchards, self-pollination is the only form of related mating and self-fertility is low in many species including Douglas-fir, thus inbred individuals will be at low frequency in improved stands. In advanced generation breeding programs and seed orchards, however, the problem may become more serious. As breeding programs advance, relatedness of individuals in breeding populations will increase in each generation, as some top-ranking individuals are from the same family or share a common ancestor. Thus, in advanced generation seed orchards, crossing between relatives, such as full-sibs, half-sibs, parents-offspring, etc. may be inevitable. These lower levels of inbreeding have a much greater chance of producing viable seed than self-pollination. Therefore, decisions need to be made on tradeoffs between genetic gain obtained by selecting the best, but related individuals, and potential loss due to inbreeding between these individuals.

Studies reported for investigating inbreeding depression are all based on individual-tree performance. The effects of a small proportion of inbred individuals in seed lots on final stand yield may be less important, as these individuals may be culled from nursery stock or be lost in the process of self-thinning once crown closure occurs in stands. However, the effects of inbreeding on wood production of mature stands cannot be assessed empirically in the short term. Through using results from 10-year-old inbreeding field trials in simulations using the growth and yield model TASS (Tree and Stand Simulator) developed by BC Ministry of Forests, the long-term implications of different levels of inbreeding on final stand yields were evaluated in this study.

Results indicate that the probability of related matings is small in seed orchards, and the chance of producing acceptable seedlings from related matings is also small. Competition reduces inbreeding depression at harvest. More productive sites, higher planting densities and longer rotations increase the chance of eliminating inbreds from the final stand.

Slightly higher breeding values of related selections would be able to offset and exceed the effect of inbreeding resulted from related mating, especially when multiple groups of related clones are included. Genetic diversity concerns are more likely to limit use of related clones than inbreeding depression.

Workshop Abstracts

Breeding for Resistance – Developments and Issues for the Future.

University Club, Dining Room: Thursday, August 2, 2001



Early Testing of Douglas-fir for Swiss Needle Cast Tolerance

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Swiss needle cast (SNC), a foliage disease of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) caused by an ascomycete (*Phaeocryptopus gaeumannii* (Rohde) Petrak), has become problematic in recent years along coastal Oregon and Washington. Infected needles lose their ability to control water loss and turn yellow. Premature casting of these needles results in significant growth losses. Early testing is a tool in forest tree breeding that allows evaluation of families at seedling stage, thus resulting in more efficient progeny testing and shortened breeding cycle given sufficient genetic correlation exists between seedlings and older trees. This study was undertaken in order to investigate genetics of SNC tolerance in Douglas-fir and to develop an early testing procedure for breeding purposes. Fifty-five open-pollinated Douglas-fir families from Siuslaw National Forest in the Oregon Coast Range were included in this study. Trees at two juvenile (2-year old) and two older (10- and 12-year old) progeny test plantations were naturally inoculated by spores released from surrounding infected Douglas-fir stands and visually assessed for foliage color, needle retention and foliage density in the field. In an attempt to find other possible SNC tolerance indicator traits needle color (using a color chart), needle retention (by actually counting number of needles), needle dry weight, needle length, percent stomata occluded with fungal fruiting bodies and needle specific area were assessed in the laboratory only on needle samples collected from the seedlings. Although the traits assessed in the laboratory were under some genetic control (narrow sense heritability estimates ranged from 0.06 to 0.21) they either had very strong genetic correlations with the traits assessed in the field or did not correlate well with the traits assessed on older trees. SNC traits assessed in the field appear to be under moderate genetic control and controlled by same sets of genes. Individual and family mean heritability estimates were similar at both ages and ranged from 0.14 to 0.37 and from 0.33 to 0.73, respectively. Genetic correlations among traits at different ages were significant and ranged from 0.30 to 0.75. Early selection for color was found to be 50% as efficient as selection at age-10, and for needle retention early selection was as efficient as later selection. Relative efficiency of early selection for foliage density was lower (28%) than that of the other two traits. Our results indicate that natural inoculation is effective and visual assessment of SNC symptoms in the field is sufficient for early testing purposes. Genetic gains realized from the juvenile selections combined with the potential to be able to screen large number of families at the seedling stage make early selection feasible in Douglas-fir breeding for SNC tolerance.

Genetic Interactions in the White Pine/Blister Rust Pathosystem

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White pines exhibit three main kinds of heritable resistance to blister rust:

Major gene resistance (MGR), is controlled by single genes that condition classic hypersensitive necrosis in needles, the primary infection courts. These genes are usually dominant, but can be modified by suppressor or enhancer genes that affect penetrance and even dominance relationships in some genetic backgrounds.

Slow rusting resistance (SRR), is a single phenotypic expression that integrates different underlying resistance mechanisms. SRR is a rate reducing, partial resistance expressed by lower infection frequencies in different families, and by different kinds of bark reactions that abort infections after they establish in stem tissues. More complexly inherited than MGR, its strongest effects are observed in specific combinations of parents.

Ontogenetic resistance (OGR) is a kind of resistance that develops with age in some adult trees. It is recognized by parent trees that are phenotypically highly resistant compared to cohorts in natural stands or seed orchards, but which produce highly susceptible offspring. It is genotype specific, but appears to be very strong and stable. OGR would be useful in stabilizing a crop in later parts of a rotation, but is the least understood and probably most difficult of all the mechanisms to develop. On the negative side, it masks juvenile susceptibility.

MGR is present in at least three species: sugar pine, western white pine, and southwestern white pine; probably also limber pine, and perhaps others. Although their phenotypic expression is similar, the genes are not the same, because they interact differentially with different races of rust. The designated loci, so far, are Cr1, Cr2, and Cr3. Two virulence alleles in the rust have been identified corresponding to Cr1 and Cr2, designated vcr1 and vcr2. These loci interact in a way typical of gene-for-gene systems, such that vcr1 neutralizes Cr1 but not Cr2, and vice versa for vcr2. Cr3 conditions hypersensitivity to both vcr1 and vcr2 (as well as the wild type race), but no race with vcr3 has been found yet. This specificity among major genes for resistance and virulence is remarkable, considering that this pathosystem has not coevolved. Frequencies of Cr alleles are low to rare in natural white pine populations, yet are much higher than mutation rates. The origin and persistence of these alleles in natural populations, in the absence of any obvious selective agent (prior to blister rust) is mysterious, and suggests genetic memory of similar encounters in past geological epochs, perhaps with native pathogens, persists in these species.

The epidemiological unity that characterized the introduction and spread of blister rust from population bottlenecks from Asia to Europe and then Europe to North America strongly suggests a corresponding genetic unity. Overall variability of *C. ribicola* in western North America is low (only 8% polymorphic loci, 2.5% expected heterozygosity), with a genetically fragmented, metapopulation structure (G_{st} 0.21). Gene flow is low, in spite of high outcrossing. Variation in virulence also appears limited; none has been found on different highly resistant *Ribes* cultivars in extensive trials in Europe and North America, and only the two races that neutralize major resistance genes in sugar pine and western white pine are confirmed. Virulence is inherited cytoplasmically, an unusual phenomenon among pathogen virulence genes. Although other races may exist in North American rust populations, a greater threat to genetically improved white pines may lie in new introductions from Asia, the ancestral gene center of the pathogen.

The challenge is to develop durable resistance. The key is to prevent sudden and drastic increases in frequency of pathogen races of wider virulence. This might be effected by concentrating and deploying different kinds of resistance genes that are mutually buffered against different virulences into synthetic populations. Basic strategies include building gene pyramids and developing multilines, or a combination of the two.

Breeding for resistance to *Phytophthora lateralis* in Port-Orford-cedar : Current Status and Considerations for Developing Durable Resistance

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Port-Orford-cedar (*Chamaecyparis lawsoniana*) is an important component of the forest ecosystems of southwestern Oregon and northwestern California. The presence of a non-native root disease caused by *Phytophthora lateralis*, is causing widespread mortality throughout the range of Port-Orford-cedar. In 1997, the Forest Service and BLM in collaboration with Oregon State University initiated a operational breeding program for resistance. Including a few selections made prior to 1997, a branch dip test has been use to evaluate over 9700 field selections through 2000. Over 1000 candidates ranked high in the branch lesion test are being evaluated further using rooted cuttings in a root dip test or in field tests. Although the branch dip test appears only weakly correlated with other tests many of the highest surviving parents also are highly ranked in all tests. The frequency of resistant candidates is low, and depends on the criteria used to define a candidate as resistant. Large differences in family survival occur, sometimes varying from 0% to 100%. The oldest field tests indicate good survival through 12 years for rooted cuttings and seedlings of top parents. Some results from crossing suggest a major gene for resistance, but some minor conflict exists among the different types of tests. The number and types of resistance mechanisms are unknown, and may be difficult to discern without diagnostic races of the pathogen. Current evidence suggests there is little genetic variability in this introduced pathogen. Breeding in Port-Orford-cedar can be done at a very early age which favors the development of increasing levels of resistance. Management activities that reduce the spread of the pathogen, the size of the pathogen population size, or the introduction or new strains will aid in developing effective resistance.

**Linking deer browsing, monoterpene production, and genetic variation:
Case study in Cupressaceae (*Chamaecyparis nootkatensis* and *Thuja plicata*)**

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In order to investigate whether differential herbivore browsing could reflect genetic variation in the expression of defense mechanisms, we analyzed variation in needle monoterpenes and damage caused by black-tailed deer (*Odocoileus hemionus*) browsing on yellow-cedar (*Chamaecyparis nootkatensis*) and western redcedar (*Thuja plicata*) between trees planted in the same environment. In yellow-cedar, multiple copies of three clones that were heavily browsed amongst over a hundred, had extremely low levels of monoterpene, with one clone producing none. Similarly, in western redcedar, total monoterpenes were more than twice as low in heavily-browsed trees than in non-browsed trees, with one seedling expressing none. This variation in monoterpene production wasn't induced by browsing because the same variation was found for individuals grown in the absence of deer. Therefore only genetic variation in defense mechanisms as expressed through terpene production can explain the observed differences. Genetic correlations among individual monoterpenes were all positive and high, indicating that perhaps a single enzyme controls multiple monoterpene products or else a pleiotropic relationship among genes controlling different monoterpenes. In each of the species, the extreme low monoterpene genotypes were from the same open-pollinated families, which could be indicative of a parental rare recessive mutation at a gene controlling monoterpene production, which has been expressed through selfing. In addition, heritability estimates for individual and total monoterpene production in western redcedar were high (0.64 to 0.90), indicative of significant additive genetic variation. This suggests that western redcedar is far from depauperate in genetic variation as previously thought, allowing the occurrence of the evolution of defense mechanisms imposed by natural selection from herbivores.

Synergistic Interactions Among Programs in Tree Improvement, Forest Pathology and Molecular Biology

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Tree improvement programs have generated valuable research materials over the years by identifying trees that vary in their resistance to diseases. These efforts have been successful in large part due to collaborations between forest geneticists and forest pathologists. More recently, we have used half-sibling seedlots of southern pine species (and in the near future we will be using clones) that vary dramatically in their resistance to fusiform rust disease and to pitch canker disease, as raw materials for discovering genes that are involved in conditioning disease phenotypes. We expect molecular biology results to inform tree improvement efforts, and ultimately lead to ‘smart breeding’ applications.

Genetic resistance of Sitka spruce (*Picea sitchensis*) populations to terminal weevil (*Pissodes strobi*): possible major gene involvement.

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Sitka spruce (*Picea sitchensis*) is highly susceptible to terminal leader damage by the terminal or white pine weevil (*Pissodes strobi*; Coleoptera: Curculionidae) which severely detracts from its growth potential and value. Individual and population differences for susceptibility were earlier noted from older IUFRO provenance trials. Strong weevil resistance was noted from hybrid sources (the natural hybrids of Sitka and white spruce found in Northwestern British Columbia (BC)), but also in populations of pure Sitka in the dry variants of the biogeoclimatic (BEC) zones. Such ecological zones are concomitant with high weevil hazard. It was earlier postulated that this resistance might be broadly based within this high hazard dry zone because of selection pressures from high weevil populations. Results from a population transect of this zone established as an open-pollinated progeny - provenance trial, however, show that this resistance is not broadly based in this zone but is centered about one of the original IUFRO resistant sources. This strong geographic demarcation of resistance and high heritability estimate led us to investigate whether major genes may be part of this observed resistance. We used two techniques from human population genetics for indicating major genes: 1) admixture analysis - which looks for a better fit for a model of a bimodal distribution for resistance within the high hazard zone. 2) regression of within family variance on family mean - segregation of a major gene will provide higher variance within families and a polynomial regression fit. Both these techniques supported evidence indicating the possibility of major gene effects in this resistance.

Workshop Abstracts

Molecular Forest Genetics

University Club, Lounge: Thursday, August 2, 2001



Forestry genomics: a major new Canadian initiative in structural and functional genomics

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In 2000, the Canadian Federal Government established a not-for-profit corporation, Genome Canada, charged with creating Genome Centres in five Canadian regions, including British Columbia (Genome BC). These will carry out large-scale genomics projects in various biological sectors. The goal of the 4-year Genome BC Forestry Genomics project is to maximize the value of Canadian forests by unlocking their genetic potential. It will focus upon two important forest species, spruce and poplar, and on two areas critical to the future of Canadian forestry, forest health and wood quality. The overall strategy is to develop comprehensive genomic resources for these two species, and to integrate these both with each other and with the powerful genetic resources already available around the model plant *Arabidopsis*. This comparative genomics approach will generate a unique, vertically integrated model of the genetic controls underlying woody perennial plant growth, stress resistance and defense. Comprehensive EST collections from both spruce and poplar will be generated using multiple cDNA libraries. DNA microarray-based expression profiling of spruce, poplar, and *Arabidopsis* ESTs will focus on xylem development and responses to stress, and will be complemented by large-scale analysis of *Arabidopsis*, poplar, and spruce protein expression profiles and by a pilot reverse genetic project in *Arabidopsis*. EST collections will be used to acquire a large number of polymorphic genetic markers in both spruce and poplar, providing powerful tools for marker-assisted selection in long-term tree breeding programs. Using fingerprinted BAC libraries, we will generate a framework physical map of the *P. tremuloides* (aspen) genome and will integrate this with the poplar genetic map. The physical map will provide an ideal platform for large-scale gene mapping by BAC end-sequencing and hybridization, and potentially for eventual full genome sequencing. Genome BC Forestry Genomics data will publicly released, and collaborations with other forest genomics initiatives and forest scientists will be actively pursued.

Joint Full- or Half-Sibs Linkage Mapping in a Natural Population

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A new strategy for constructing a global linkage map with dense markers is proposed on the basis of jointly using multiple full- or half-sib families randomly sampled from a panmictic natural population and assessed using either codominant or dominant markers, or both. Expectation-maximization (EM) algorithm combined with Newton-Raphson (NR) is applied to simultaneously attain maximum likelihood estimates (MLEs) of both recombination fraction and allele frequencies of pairwise markers. Simulation results show that adequate estimates of three unknown parameters can be obtained through the combined algorithm. There is a trade-off in allocation of samples between and within families, and an optional sampling scheme is expected under certain conditions. A better linkage map can be constructed using multiple full-sib families than using multiple half-sib families, and using codominant or codominant-dominant markers than using dominant markers. Because of the advantage in providing flexible choices in types of offspring families and markers, the strategy has great implications in the study of genome organization in a natural population, and to improve the linkage maps currently available by analyzing additional families.

Quantitative trait analysis for adaptive traits and their environmental elicitors in a three-generation pedigree of Douglas-fir

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The environmental factors affecting bud phenology differ across latitudinal, altitudinal and geographical clines, making it important to understand the interaction among them and the traits they induce. Environmental factors include winter chill, spring heat sum, photoperiod and moisture deficit. Quantitative trait locus (QTL) analysis was used to identify factors responsive to environmental cues that influence the timing of adaptive traits in Douglas-fir (*Pseudotsuga menziesii* [Mirb] Franco). Clonally replicated progeny of a large three generation out-crossed family (n = 435) were subjected to different chilling and heat sums in a 2 x 3 factorial experiment before measuring the timing of spring bud flush. In a second experiment, growth cessation traits were evaluated under different photoperiod and moisture treatments in a 2 x 2 factorial design. In order to increase precision of phenotypic values, treatments were replicated and clonal means were used in the analysis. ANOVA was performed on the phenotypic data and showed that winter chill and heat sum both played a significant role in determining the length of dormancy, while growth cessation was influenced more strongly by moisture availability than by photoperiodicity. QTL estimations for several traits will be discussed.

Comparative mapping across three conifer genera

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Conifer genomes are very large compared to most other plant species and unlikely to be completely sequenced in the near future. Therefore comparative genomics and comparative mapping will play a central role in many areas of conifer genome research. Comparative mapping requires orthologous polymorphic markers that can be studied across different species. Expressed sequence tag polymorphisms (ESTPs) are the good candidates for such markers. They represent functional genes and can be developed relatively easily from already available cDNA/EST libraries and large gene discovery projects. Comparative mapping helps to (1) produce consensus genetic maps within and between species, (2) verify quantitative trait loci (QTLs), (3) identify candidate genes for quantitative and complex traits and (4) understand the evolution of the conifer genome. We tested about 200 ESTP markers previously developed in several conifer species and used shared markers for comparative genome mapping in loblolly pine, Norway spruce and Douglas-fir. We also used homology search among DNA sequences deposited in the Genbank databases to find orthologous genes suitable for comparative mapping. This study is part of the Conifer Comparative Genomics Project (CCGP) formed as an international collaboration at the Institute of Forest Genetics (USDA Forest Service) to develop orthologous genetic markers, publicly available reference mapping populations that can be shared among different laboratories, and the bioinformatic tools to facilitate comparative mapping.

Investigating mating system of white pine weevil, *Pissodes strobi* (COLEOPTERA: CURCULIONIDAE) using microsatellite DNA markers

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White pine weevil is the most destructive forest pest in British Columbia, severely restricting the growth and range of Sitka spruce. Knowledge of the mating system is important for designing sterile insect release programs, and generally, for understanding the evolution and genetic diversity of this weevil. In this study, aspects of the weevil mating system were inferred via experiments that varied the number of available mates, involving the four combinations: (a) groups 2 or 4 males, competing for single females, and (b) groups of 2 or 4 females, competing for single males. Each treatment was replicated 7 to 11 times, and 1869 progeny in total were assayed for four microsatellite loci. All microsatellite loci segregated in a Mendelian fashion. The results of 1 Female: 2 Males mating showed that sperm precedence occurs in 82% of the studied replications. The 1 Female: 4 Males mating revealed not only mixed paternity, but supported the occurrences of sperm precedence as well. In this experiment, female weevils mated to four different males had a mean paternity of 2.8. The existences of sperm mixing were observed in both 1 Female: 2 Males and 1 Female: 4 Males matings. In addition, the possibility of sperm depletion was also observed in both of the 2 Females: 1 Male and 4 Females: 1 Male matings. The evidences of sperm precedence and multiple paternity will influence the style of *the Integrated Pest Management*;. Sperm precedence has important implications on the ability of sterile insect techniques. Also, the incidence of multiple paternity seems likely to affect the long-term outcome of tree breeding program via the adaptation of white pine weevils to overcome resistant trees.

A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood and fibre traits.

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A genetic linkage map containing potential candidate loci for wood and fiber traits has been constructed for *Eucalyptus globulus* (Labill.) based on segregation of 249 codominant loci in an outbred F1 population of 148 individuals. The map contains 204 RFLP loci, including 31 cambium-specific expressed sequence tags (ESTs) and 14 known function genes. There are also 40 microsatellite and 5 isozyme loci located on the map. The 249 loci mapped to 11 major linkage groups (n = 11 in eucalypts) and a 12th small linkage group containing 3 loci that segregated in the male parent only. Total map distance is 1375 cM with an average interval of 6 cM. Forty-one of the mapped loci identify known proteins (5 isozymes) or sequences with known function (14 genes and 22 ESTs). The mapped genes include enzymes involved in lignin and cell wall biosynthesis. The map is being used to locate quantitative trait loci for wood and fiber traits in *Eucalyptus*.

Regulation and function of genes involved in wood development

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We have a long-standing interest in the identification, function, and regulation of genes involved in wood development. Much of our work has involved two xylem-specific genes from loblolly pine, PtX3H6 and PtX14A9. By transforming tobacco with c-myc tagged versions of the genes, we were able to demonstrate that both encode protein cores of arabinogalactan-proteins (AGPs), a group of glycoproteins thought to play important roles in plant development. Additional xylem-specific AGP-like genes are found in developing xylem of loblolly pine. 5' and 3' flanking sequences of both genes have been examined to determine their roles in the high levels of xylem-specific expression observed (No et al. 2000). PtX3H6 has a negative element at the 3' end, reducing expression in non-vascular tissues while PtX14A9 has an enhancer at the 3' end, which increases expression in vascular tissues. The plant growth regulators auxin, gibberellin, and ethylene were shown to be involved in the regulation of these genes (No and Loopstra 2000). We are now expanding the gene expression studies to a genomic scale with the use of microarrays. Loblolly pine xylem ESTs isolated at North Carolina State University as part of a NSF Plant Genome project are being analyzed for tissue-specificity, regulation by plant growth regulators, and differences between populations.

Identification of genes controlling vascular development and wood formation using gene traps.

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We have taken two approaches towards identifying genes regulating vascular development and wood formation in forest trees. The first approach utilizes a gene trap-based gene tagging system in hybrid Poplar. A gene trap vector carrying the GUS reporter was inserted at random as T-DNA into the poplar genome. When the gene trap vector inserts into an expressed gene, the GUS reporter is expressed in a fashion that mirrors the expression pattern of the tagged gene. Results from the screening of several hundred gene trap lines will be presented, including the tagging of genes expressed during vascular development and wood formation.

The second approach utilizes Arabidopsis, and assumes that the major genes and pathways regulating vascular tissue patterning and cell differentiation events will be similar in diverse plants. A collection of gene trap-tagged Arabidopsis were used to identify genes expressed in specific vascular cell types and at different stages of vascular development. The genes identified include genes expressed at the earliest stages of vascular development during embryogenesis and encode potential regulatory proteins. The gene trap insertion lines indicate significant similarities between primary and secondary vascular growth at the gene expression level. The function of the cloned Arabidopsis vascular genes are currently being assayed in Poplar, and will represent a functional comparison of vascular development in herbaceous and woody plants at the molecular genetic level.

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1931-1932 Nursery Measurement Crew, Eddy Tree Breeding Station