



WFGA Abstracts

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Geographic Variation and Paleo Influence

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

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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 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.

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

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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% \pm 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.

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 *Australiae* 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.

Local Population structure

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?

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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.

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

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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.

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.

Current Issues in Conservation

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.

Applied Forest Tree Improvement

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.

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.

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.

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.

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.

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.

Breeding For Resistance

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.

Molecular Forest Genetics

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.

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

