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THE ROLE OF TREE IMPROVEMENT PROGRAMS FOR *EX SITU* GENE CONSERVATION OF COASTAL DOUGLAS-FIR IN THE PACIFIC NORTHWEST

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ABSTRACT

We enumerate the genetic resources for coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) in tree improvement programs in the Pacific Northwest USA and evaluate how they contribute to gene conservation of the species. The first-generation programs include over four million progeny from 33,928 selections planted on 999 test sites. Nearly 2,000 of the selections tested in the first-generation have been incorporated into second-generation programs. The first-generation tests serve as repositories for low-frequency alleles of potential future import and for variation in quantitative traits not presently under selection. Much of this genetic variation also resides in the many large *in situ* populations of Douglas-fir, but it can be more easily detected in genetic tests. Additionally, useful genetic variation can be more rapidly integrated into tree improvement programs with the least loss of genetic gain if it is identified in genetic tests rather than in *in situ* populations. Because first-generation genetic tests are of limited life, we develop a method for creating gene resource outplantings to retain for a longer period the valuable genetic resources in them.

Key words: genetic tests, conservation genetics, *Pseudotsuga menziesii*, tree breeding

INTRODUCTION

Genetic diversity is important for sustainable forest management, and this has been recognized in international agreements (*e.g.*, the Montreal Process, http://www.mpci.org/). The size and breadth of a tree species' gene pool directly affects its ability to evolve and thrive under changing environmental conditions. Without sufficient genetic diversity, species cannot adapt to new stresses such as disease and climate change, nor can tree breeders continue to achieve genetic improvement objectives.

Gene conservation of forest trees can be accomplished through both *in situ* and *ex situ* methods. Most gene conservation plans stress *in situ* protection of natural populations in protected areas. Protected areas are cost effective since they simultaneously serve a variety of conservation objectives, including safeguarding the genetic resources of numerous species. Furthermore, populations in protected areas are subject to normal evolutionary processes. *Ex situ* methods, which include holding germplasm in cold storage or growing trees in off-site plantations, are emphasized for threatened populations or species that cannot easily be conserved *in situ*. Species undergoing domestication also have genetic resources conserved *ex situ* in the form of the hierarchy of populations that comprise the breeding and testing programs.

Large, natural populations of coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) can be found in protected areas across the Pacific Northwest, resulting in strong *in situ* gene conservation (LIPOW *et al.* 2003; YANCHUK & LESTER 1996). In this paper, we explore the many ways that *ex situ* genetic resources also contribute to gene conservation in the region, with emphasis on western Oregon and Washington. There are several reasons why this is an interesting and relevant issue. First, Douglas-fir is the region's most important timber tree and a keystone species in many ecosystems, with the survival of many other plants and animals depending on it. Second, the sheer magnitude of the *ex situ* genetic resources, mainly open-pollinated first-generation progeny tests, is unparalleled for any commercial forest tree species. Third, the Pacific Northwest, more than any region in the United States, attracts scrutiny for evidence of responsible forest management, including management of its genetic resources. We therefore review the size and structure of the region's first- and second-generation breeding programs and show how they achieve specific gene conservation objectives. We also describe the advantages associated with maintaining genetic material previously tested in the breeding programs for the purpose of gene conservation. As genetic tests are of limited life, we outline a practical method that allows these valuable resources to be retained over the long term.

EX SITU GENETIC RESOURCES OF COASTAL DOUGLAS-FIR

Oregon and Washington: First-generation breeding programs

Throughout western Oregon and Washington, forest land management organizations are engaged in coastal Douglas-fir tree improvement programs, most of which were founded on the "progressive" system first introduced by Roy Silen of the USDA Forest Service and Joe Wheat of the Industrial Forestry Association (SILEN 1966; SILEN & WHEAT 1979). In this system, first-generation parent trees were selected from wild stands, and a series of six to 12 open-pollinated half-sib progeny tests were used to generate information for roguing the corresponding first-generation seed orchards and for making second-generation selections. The parent trees came from small breeding zones that varied in size from about 12,000 to 80,000 ha, with an average elevation change of less than 305 m (QUAM, 1988). The breeding zones spanned most of the species' regional range (Figures 1). They displayed little overlap since most organizations engaged in tree improvement participated in one or more of the 20 local Douglas-fir cooperatives comprising the Northwest Tree Improvement Cooperative (NWTIC). Seventy-two of the region's 124 separately managed first-generation programs belonged to the NWTIC (Table 1, Figure 1). The partitioning of selections into breeding zones effectively created multiple breeding populations (*sensu* NAMKOONG, 1976).

The size and composition of most first-generation breeding populations were designed conservatively with the goal of maintaining genetic diversity. The breeding zones were kept small, so that selections would retain adaptations to local conditions and preserve coadapted gene complexes (SILEN & WHEAT 1979). Moreover, large numbers of parent trees were selected from each breeding zone to allow for continued selection without appreciably eroding genetic diversity in the breeding population (SILEN & WHEAT 1979). These factors led to the testing of about 31,178 selections in western Oregon and Washington alone, or an average of about 7.5 selected trees per 1000 ha (Table 1).

The methods used to select parent trees for the breeding population also assured the capture of much of the species' regional genetic diversity. Roughly 80 % of the selections, at least those in NWTIC programs, were not intensively selected "plus trees". Rather, a "roadside selection" approach emphasized sampling trees from throughout the breeding zone that appeared healthy, vigorous, and well formed. The remaining NWTIC selections (approximately 20 %), as well as many selections in non-NWTIC programs (*e.g.*, Weyerhaeuser Company's), were identified using recommendations that considered diameter growth per zone space (STONECYPHER *et al.* 1996). Even with this refinement, the gain achieved was typically only a few percentage points, and significant bias in the genetic composition of the selections is therefore unlikely (STONECYPHER *et al.* 1996). In summary, the selection process, enormous number of parents trees, and stratification into many small breeding zones have assured an extremely broad genetic base both within and among breeding populations.

The most extensive and significant of the *ex situ* genetic resources of coastal Douglas-fir in Oregon and Washington are the progeny tests of offspring from the ~31,178 parent trees. The tests are distributed on more than 861 test sites that sample a wide range of environmental conditions (Table 1). Because breeding zones are small, however, they remain in native environments and are subject to "normal" forces of natural selection. Number of offspring per test varies among programs. NWTIC tests typically include at least 12 trees per family grown at each of six to 12 test plantations; in other words, at least 72 offspring per parent tree. Many tests, especially older ones begun in the 1960s and 1970s, incorporate more individuals, and the total number of progeny in NWTIC programs surpasses a mind-boggling 2,700,000 trees, an average of roughly 130 progeny per parent tree (Table 1). Similar family sizes were used in non-NWTIC programs, with the total number of progeny under test in western Oregon and Washington equaling about four million!

Oregon and Washington: Advanced generation breeding programs

In the past decade, cooperative second-generation breeding has gotten underway in most of western Oregon and Washington. Note, however, that despite testing of almost 11,000 first-generation parents in southwest Oregon (in the Oakridge, McKenzie, Roseburg, Medford, Powers and Gold Beach areas), there has been no move to proceed to cooperative second-generation breeding in this area.

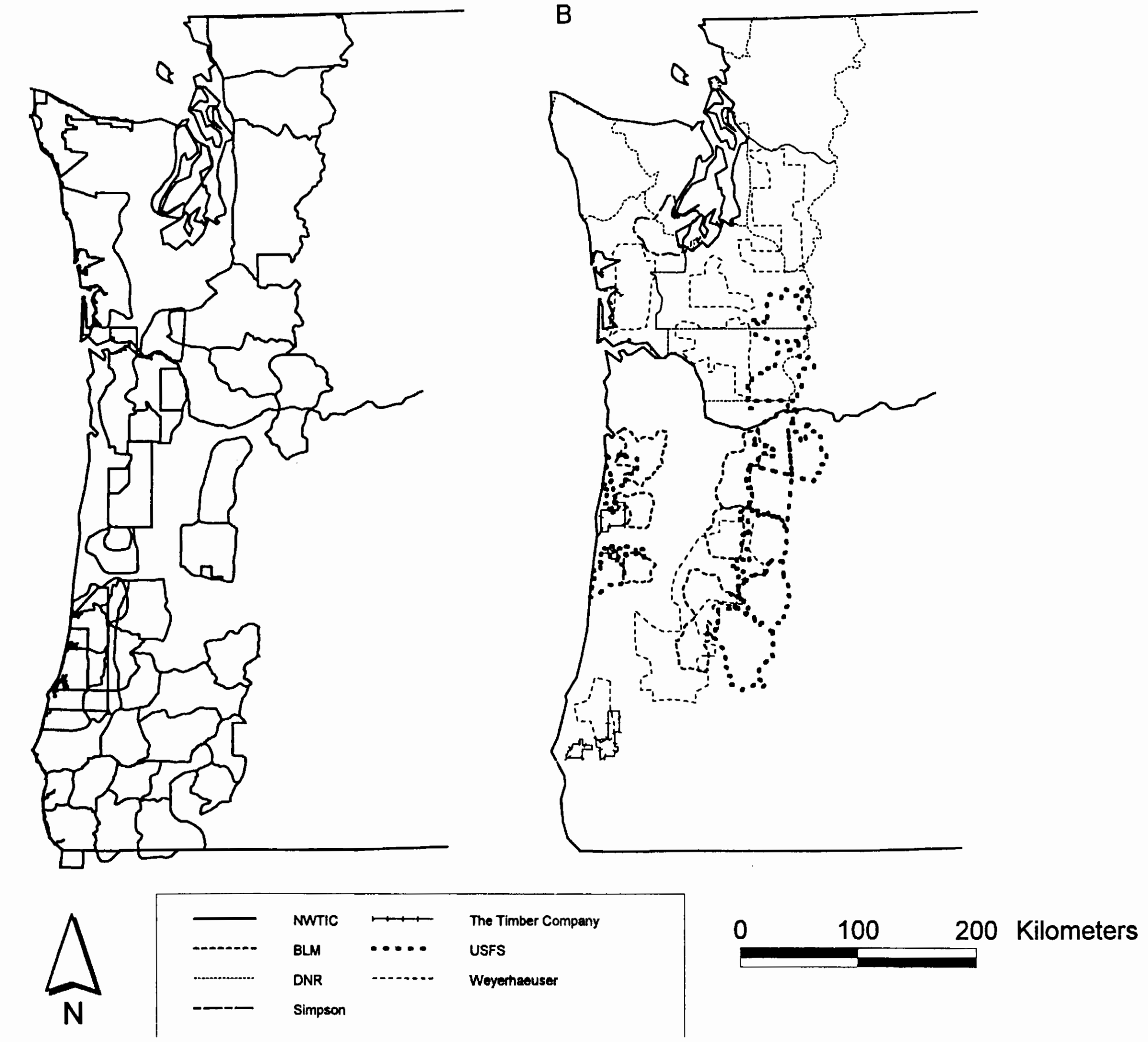

Figure 1. In western Oregon and Washington (USA), first-generation breeding zones for programs (a) in the Northwest Tree Improvement Cooperative and (b) of the USDA Forest Service, USDI Bureau of Land Management, Washington Department of Natural Resources, and industry (The Timber Company, Weyerhaeuser Company, and Simpson).

The ongoing, cooperative second-generation programs include only eight breeding zones, which encompass 72 first-generation programs (34 of which were NWTIC) (Figure 2). Each of the eight programs plans to test 51 to 379 second-generation families (1,947 total) created by crossing selections from within the breeding zone. These represent about 13 % of the first-generation families from the same area (Table 2). Weyerhaeuser Company, acting independently, is using only one low-elevation second-generation breeding zone for Washington, and it is incorporating 150–300 selections from each of four corresponding first-generation breeding zones (WOODS 1993). The intense selection in the second-generation programs is expected to yield substantial genetic gains.

Organizations restructured and enlarged breeding zones for the second generation because, although Douglas-fir populations possess unusually high levels of genetic variation and display considerable local adaptation (SILEN & MANDEL 1983; SORENSEN 1983; CAMPBELL 1986, 1991; CAMPBELL & SUGANO 1993), several lines of evidence suggested that the original breeding zones need not have been so small. Results from Weyerhaeuser Company's low elevation, first-generations tests, for example, showed that the same good families performed well across four first-generation breeding zones (STONECYPHER *et al.* 1996). Likewise, the performance of progeny in field tests in

Table 1. Estimates of the numbers of parental selections included in first-generation tree improvement programs throughout western Oregon and Washington, British Columbia, and northern California, as well as the number of progeny tested. Most data are from a database of the NWTIC that underestimates slightly the real values.

Program	Number of breeding zones	Number of first-generation selections	Number of progenies	Number of test plantations
Oregon and Washington		19421	2,701,004	620
NWTIC	72	3152	221,080	110
USDA Forest Service[a]	14	2219	297,528	60
USDI Bureau of Land Management[a]	9			
Oregon Department of Forestry[a]	1	150	13,500	3
Washington DNR[a]	6	720	Not available	21
Weyerhaeuser Company	6	3707	639,239	Not available
Other industry	16	1809	119,296	47
California USDA Forest Service	9	1800	Not available	36
British Columbia	2	650	203,500	102
Total	135	33628	>4,195,147	>999

[a] These organizations have independent programs in some regions, but in other regions they participate in NWTIC programs. Only the independent programs are listed here.

Table 2. Summary of the organization of the ongoing second-generation NWTIC tree improvement programs for coastal Douglas-fir. Local families are tested within a breeding zone, whereas non-local families are tested across breeding zones

Program	Number of first-generation programs	Number of first-generation selections	Target-number of second generation local families	Target-number of second generation non-local families
Washington Coast	5	1,046	140	152
Puget Sound	4	670	51	209
Washington Cascades	10	2,515	210	249
Vernonia	3	1,372	379	207
Trask Coast	12	2,590	289	104
Trask Inland	9	1,625	272	171
South Central Coast	6	2,048	278	109
North Oregon Cascades	14	3,418	367	142
Total	**72**	**15,386**	**1,947**	**1,343**

four NWTIC breeding zones and in a seed orchard correlated reasonably well (Silen 1985). Additionally, families tended to rank similarly across sites within six breeding zones in Oregon; differences that did occur were generally not correlated with environmental differences among sites, suggesting that the first-generation breeding zones were not overly large (Johnson 1997).

Despite evidence indicating that breeding zones could be expanded for the second-generation, few data address exactly how far families can be safely deployed. For this reason, NWTIC programs developed a breeding strategy emphasizing local crossing and wide testing. Second-generation crossing is concentrated within first-generation breeding zones, creating multiple populations within each second-generation breeding zone. Wide testing refers to the fact that each of these "local" crosses is planted on test sites throughout the new breeding zone. If future test data indicate that breeding zones were expanded too far, second-generation families can be subdivided into smaller breeding zones in the next generation. Because most crosses for the second-generation involved selections from the same first-generation breeding zone, subdividing could be done without losing any second-generation families because the parents involved in the cross were too far separated to be considered appropriate for any single breeding zone. With subdividing, however, future

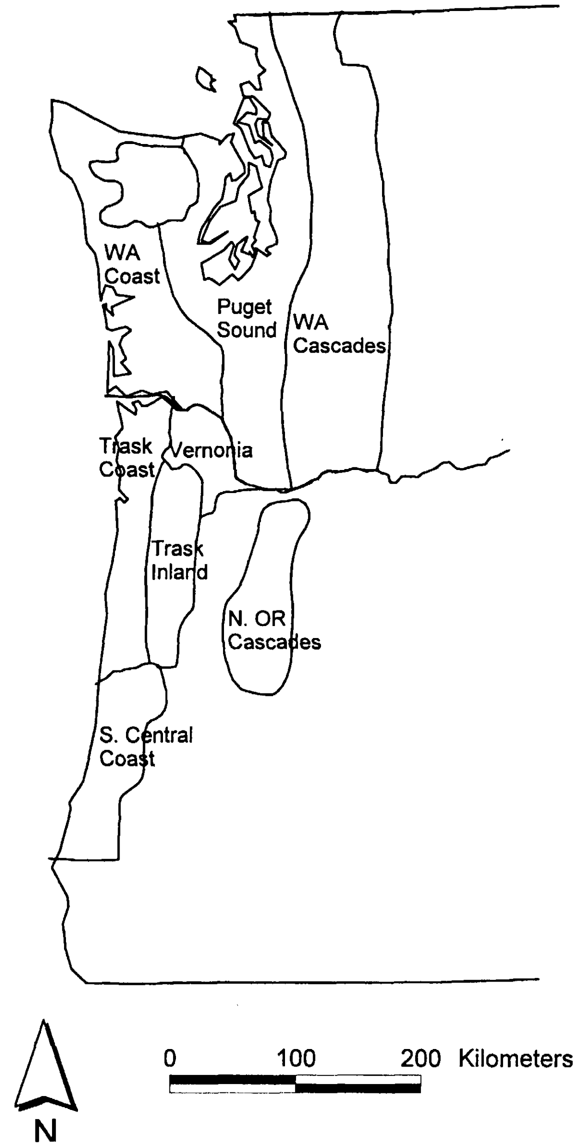

Figure 2. In western Oregon and Washington (USA), second-generation breeding zones for programs in the Northwest Tree Improvement Cooperative.

programs might require infusion of additional selections from the first-generation programs. The multiple populations in the second-generation breeding zones also serve as sublines that should facilitate avoidance of inbreeding in seed orchards and in subsequent generations of breeding. In addition to the testing of "local" families over a wider breeding zone, elite crosses are being made across first-generation programs within a second-generation cooperative and the higher-ranked families from second-generation breeding programs are being tested in other second-generation cooperatives (non-local families) to provide the data necessary to determine if second-generation zones can be safely expanded even further.

Oregon and Washington: Seed stores

In Oregon and Washington, extensive seed stores exist for coastal Douglas-fir. Databases obtained from the USDA Forest Service and Bureau of Land Management show that these agencies presently hold approximately 19,000 family seed lots in addition to bulked collections. This seed is stored for a variety of reasons, including for use in reforestation following logging or natural disturbance, research needs, and gene conservation. Most of the seed was collected from wild stands on federal lands, although some was obtained from seed orchards or trees on private lands.

California

Tree improvement programs for coastal Douglas-fir in California also contain valuable genetic resources, although they are less extensive than programs in Oregon and Washington. For first-generation testing, the USDA Forest Service set up nine California breeding zones (KITZMILLER 1976), one of which was managed as a cooperative with industrial partners. These breeding zones are larger than the first-generation ones in Oregon and Washington. There are also several small industrial programs. The Forest Service selected 200 plus trees per breeding zone (1,800 total) and planted open-pollinated progeny from each at four test sites per breeding zone. The first progeny tests were initiated in 1976, and 12- to 20-year results are generally available. All parental selections have been archived in a clone bank that the Forest Service intends to maintain indefinitely to permit inclusion of all first-generation selections into second-generation programs. Second generation breeding has not begun, but, should it go forward, plans call for crossing the top 50 % of first-generation families in small partial diallels to test for general and specific combining ability and subjecting the remaining families to single pair matings followed by within-family selection (J. KITZMILLER, USDA Forest Service Regional Geneticist, pers. comm.). This strategy should maintain a broad genetic base in the second generation.

British Columbia

A coastal Douglas-fir tree improvement program run by the British Columbia Ministry of Forests comprises another substantial *ex situ* genetic resource. The program was designed with a single, large breeding zone for elevations below 700 m, because early provenance tests (CHING & HINZ 1978; ILLINGWORTH 1978) and a study of wide crossing (ORR-EWING *et al.* 1972) indicated relatively weak genetic differentiation among British Columbia populations. The first-generation program included plus trees identified in natural populations. Despite an intense selection process, low heritability of growth and form traits resulted in little genetic gain, and the selections are therefore expected to sample well genetic diversity in the breeding zone. A half-diallel design was used to progeny test 372 parent trees, with each family planted on 11 of the 88 test sites. Open-pollinated progeny from additional British Columbia trees and from 161 parent trees from western Washington were tested across five sites. The test results further affirmed that seed could be safely moved within the large breeding zone (WOODS 1993). The first-generation tests remain in place, and many of the original selections have been archived in clone banks (WOODS 1993). For the second generation breeding population, 450 parents were chosen based on forward selection of progeny from the half-diallels. The parents were divided into sublines of 12 to 16 clones to enable control of inbreeding in the associated seed orchards, although the sublining may be reevaluated as the tests generate data.

Ex situ genetic resources for coastal Douglas-fir in other countries

Coastal Douglas-fir has been widely exported to other parts of the world for use in plantation forestry, and genetic tests can be found in Europe, New Zealand, and Chile. An International Union of Forestry Research Organizations provenance trial set up from 1967–1978 in 36 European countries, for example, tested 134 provenances of coastal Douglas-fir (KLEINSCHMIT & BASTIEN 1992). Many of these provenances still exist on at least one test site. Additional provenance trials and a breeding program that includes 1170 half-sib families from 35 provenances from coastal Oregon and Washington have been established in the United Kingdom, Belgium, France, Germany, Spain, and Italy, countries where Douglas-fir is widely grown commercially (J. C. BASTIEN, National Institute for Research (INRA), pers. comm.; J. KLEINSCHMIT, Lower Saxony Forest Research Institute, pers. comm.). In New Zealand, where Douglas-fir ranks as the second most important softwood, two provenance trials containing 82 sources were planted in 1957 and 1959 at 14 test sites, with at least 31 additional provenances from coastal Oregon and California included in more recently initiated tests (MILLER & KNOWLES 1994; C. B. LOW, New Zealand Forest Research Institute, pers. comm.). Many of the better provenances from these trials have been incorporated into a breeding program and archived in clone banks. In Chile, four recently established provenance tests include more than 700 Douglas-fir families from 25 provenances from the Pacific Northwest (P. RAMIREZ DE ARELLANO and C. BALOCCHI, Arauco, pers. comm.). Interestingly, in these non-native environments, individual families are being deployed over a much wider geographical and elevational range than in North America.

TREE IMPROVEMENT PROGRAMS AND GENE CONSERVATION OBJECTIVES

The *ex situ* genetic resources of coastal Douglas-fir in breeding populations and genetic tests in the Pacific Northwest are more extensive and complete than for any other forest tree species. The huge number of selections included go a long way towards meeting the three objectives most frequently associated with gene conservation (KANG 1979; KANG & NIENSTAEDT 1987): (1) to retain sufficient genetic variation in a breeding program to permit continued gain in subsequent generations; (2) to capture most rare (low-frequency) alleles; and (3) to preserve the normal adaptive potential of a population by maintaining variation in traits under quantitative genetic control (and not under strong directional selection).

In forest trees, high levels of genetic gain can be realized for multiple generations in even relatively small breeding populations (e.g., initial sizes of 50 –100) (reviewed by WHITE 1992; JOHNSON *et al.* 2001). First-generation Douglas-fir test populations in the PNW average about 250 selections, with the smallest containing 70 selections; they should therefore permit continued genetic gain. Similarly, second-generation breeding populations average about 215 local families, with the smallest program (Puget Sound) including 90 local families. These population sizes should also be adequate unless new data indicate that some second-generation breeding zones require subdivision, in which case infusion of additional families from the first generations of testing may be necessary.

Since relatively large population sizes are required to capture rare alleles, this gene conservation objective is usually accomplished by conserving trees *in situ* in protected areas (NATIONAL RESEARCH COUNCIL 1991). Yet, for Douglas-fir, the breeding and test populations are so extensive that they are expected to incorporate most alleles present in the natural populations from which they were drawn. While estimates vary, most suggest that a sample size of 50–160, which is smaller than that found in most Douglas-fir breeding programs, is sufficient to include, with 95 % probability, at least one copy of every neutral allele of frequency 0.05 or more (KANG 1979; NAMKOONG *et al.* 1988; FRANKEL *et al.* 1995). We should keep in mind, however, that for a rare allele to be useful for breeding - for example because it confers disease resistance - multiple copies are needed to avoid problems associated with monocultures and inbreeding (YANCHUK 2001). Furthermore, a single copy of a recessive allele could not be detected, as identification would require homozygous expression. Nevertheless, according to YANCHUK'S (2001) calculations, a breeding population of 250 selections (the average size of first-generation programs) is expected to include about twenty individuals expressing in either homozygous or heterozygous form 95 % of the dominant alleles of frequency 0.05 in the natural population. Fewer individuals expressing rare, recessive alleles would be found in the same breeding population, however, since only for allele frequencies greater than 0.30 can we expect 20 homozygous individuals. Very large sample sizes (approximately 10,000) are required to sample, with 95 % probability, twenty individuals with homozygous expression of a recessive allele of frequency 0.05. In short, the very large first-generation testing populations are expected to include multiple individuals expressing all but the very rarest alleles, while the second-generation programs likely miss the expression of some rare, recessive alleles.

A relatively large population size is also needed to capture and maintain over the long-term the "normal" genetic variance for quantitative traits present in a population and is not experiencing strong, directional selection. Again, estimates of the effective population size required vary. LYNCH (1996) proposed a target effective population of 1,000 based on an evaluation of how the forces of natural selection, random genetic drift, and mutation influence the genetic variance in a population. He showed that the genetic variance in populations of this size or larger is controlled mostly by selection-mutation balance, with genetic drift having a negligible effect. LANDE (1995), considering the population size at which genetic variation created by mutation is balanced with that lost by genetic drift, argued for an effective population size of 5000. These values apply only to populations subject to many generations of evolution. Thus, although Douglas-fir second generation breeding population sizes, which range from 287–586 when non-local crosses are included, fall short of these targets, they nevertheless contain considerable genetic variation for quantitative traits. Moreover, since an average of 1,798 first-generation families were tested per second-generation breeding populations, we can be confident that these tests capture *ex situ* much of the quantitative trait variation present in natural populations.

ADVANTAGES OF *EX SITU* GENE CONSERVATION IN TREE IMPROVEMENT PROGRAMS

The hundreds of millions of coastal Douglas-fir trees growing in protected areas distributed throughout the Pacific Northwest provide for excellent *in situ* gene conservation (LIPOW *et al.* 2003; YANCHUK & LESTER 1996) and are of great value to the region's ecology and social systems. Nevertheless, *ex situ* genetic resources in tree improvement programs have the potential to offer some advantages with respect to utilizing valuable genetic variation. If, for example, a new disease were to strike Douglas-fir, or the impact of an existing disease increased (*e.g.*, Swiss needle cast), screening existing genetic tests, rather than in *in situ* populations, could speed up the process of identifying genetic resistance and incorporating it into a breeding population. Once a resistance allele was detected in a tree in a genetic test, a breeder could turn to other family members for additional copies, since they would have high probability of also harboring the allele (*e.g.*, for open-pollinated tests, an allele inherited from a parent tree is expected in half its progeny). This would be especially helpful if the resistance allele was at low frequency or recessive. Moreover, by discovering resistance in an already tested population, breeders could minimize the gain lost when developing new breeding and production populations. Genetic tests also permit evaluation of the effectiveness of resistance or other traits, since repeatability can be examined over several sites. Additionally, as mature trees, saplings, and seedlings can differ in traits expression, assessment of certain traits might be most successful when screening mature trees. The advantages of decreasing the time needed for breeding, and the gains lost, apply to traits under quantitative genetic control as well as to those controlled by low-frequency alleles.

The prospect of global climate change creates another incentive for valuing *ex situ* genetic resources in genetic tests in addition to those protected *in situ.* Mounting evidence suggests that rapid global climate change will alter the distribution of many forest trees, including Douglas-fir (*e.g.*, THOMPSON *et al.* 1998). Should this occur, the survival of any given population might be determined by whether it possesses the genetic variation necessary to respond to new selection pressures. Armed with information about adaptive tolerances from genetic tests, however, forest geneticists may be able to actively move germplasm and facilitate the establishment (or reestablishment) of well-adapted populations. Indeed, if left in place, genetic tests will continue to provide worthwhile and relevant data for decades.

The provenance trials and breeding programs for coastal Douglas-fir in Europe, New Zealand, and Chile also serve to conserve genetic resources and may someday be beneficial to the forest industry or to ecosystem preservation in the Pacific Northwest. Given its worldwide use as a plantation species, the first encounter Douglas-fir has with a future pest or pathogen, and the first uncovering of tolerance or resistance, could be in a non-native environment, as could the detection of variants for a variety of valuable traits. It is therefore conceivable that improved Douglas-fir germplasm now in another country may someday be returned to North America for restoration or commercial purposes. Indeed this process is underway for noble fir (*Abies procera* Rehd.); stock native to Oregon and Washington and subjected to several cycles of breeding for bough traits in Denmark is being returned to the United States, where interest in the bough industry is expanding and breeding is less advanced (LIPOW *et al.* 2001).

Genetic variation cannot be easily detected in seed stores, since they first require outplanting. For this reason, and because they are not subject to the normal evolutionary processes experienced by trees in the ground, the seed stores for Douglas-fir are less useful for gene conservation than trees in progeny tests or provenance trials. All the same, they contain considerable genetic variation that could be tapped into if needed.

LONG-TERM CONSERVATION OF GENETIC RESOURCES REPRESENTED IN FIRST-GENERATION GENETIC TESTS

The gene conservation value of the *ex situ* genetic resources for coastal Douglas-fir hinges, in part, on maintenance of the first-generation progeny tests, since the second-generation programs alone are too small to accomplish the objectives of conserving most rare alleles and providing the full benefits associated with breeding for new traits or responding to climate change. The first-generation progeny tests, however, were not designed to last indefinitely. As they are often measured only through age 15, many have fulfilled or will soon fulfill their primary objectives of contributing data to rank the performance of parental selections. Left alone, self-thinning will naturally occur in the maturing tests – typically starting at age 15–30 years, depending on site conditions, elevation, and plantation spacing – resulting in a decline in stand health, loss of test balance, and possibly loss of some slower growing families. In any case, measures are in place in all regions engaged in Douglas-fir tree improvement that aim to hold onto first-generation tests and selections, at least for a few decades.

The NWTIC established guidelines designed to preserve enough first-generation progeny and test sites to allow for future assessment of new traits or reassessment of current traits and to maintain selections so that additional first-generation selections are available for inclusion into advanced generations should subdivision of second generation breeding zones be necessary (J. DANIELS, previous NWTIC Director, pers. comm.). The guidelines called for systematic thinning of three to four test sites per breeding zone to retain a more or less balanced test with about six trees per site for most families. Removing alternate diagonals is usually sufficient for this purpose. The remaining three to nine plantations per breeding zone are to be left standing, although they may be thinned with less concern about family balance. In all test sites, the goal is to hold onto all advanced generation selections. To date, these guidelines have been fairly well implemented (D. CRESS, Oregon State University, pers. comm.). Most tests were established between 1969 and 1993 and, because thinned tests are expected to remain viable until stands are 40 to 60 years old, we expect that the thinning guidelines will preserve 30–40 % of the progeny, representing >95 % of the first-generation parents, until at least 2020. Importantly, successful maintenance of tests sites will require continued coordination, planning and monitoring so that stands are not inadvertently harvested or otherwise harmed.

In California, first-generation progeny tests were established at tight spacing (6 × 6 ft) and usually required two thinnings before age 15. An interlocking block design allowed two out of three rows to be removed without loss of families or balance. Many tests now need additional thinning, which is expected to remove entirely about half of the most poorly performing FAMILIES (J. KITZMILLER, USDA Forest Service regional geneticist, pers. comm.). For use in a potential second-generation, the agency aims to preserve 20–22 families per test site and 12–16 trees per family, with at least half of the families common to all four test sites in the breeding zone. No family will be wholly lost, however, as all parental selections have been archived in a clone bank. Likewise, in British Columbia, most selections have been placed in clone banks.

ESTABLISHING GENE RESOURCE OUTPLANTINGS

For application when first-generation progeny tests mature to the point that they cannot be maintained, companies and agencies could consider setting up gene resource outplantings. These outplantings would perpetuate genes from previously tested families not included in the second-generation as an "insurance policy" against future changes in breeding objectives (such as selecting for resistance to a new disease). To minimize the costs associated with setting up outplantings and their size, an appropriate strategy might be to sample a fraction (say 10 or 20 %) of the next best performing families not included in the second-generation.

The progeny in a first-generation test represent the F1 generation of the original parental selections (P). Open-pollinated seed could be collected from one F_1 individual per first-generation family and outplanted in rows, thereby creating an F_2 generation. Planting in rows would facilitate site establishment, make for easy demarcation of family identity, and keep costs down. Within an outplanting, replication of family rows would be unnecessary, because the primary objective is to preserve families, not to test them, although replication at multiple sites (two or three) is necessary to guard against catastrophes.

A family size of 20 would ensure the inclusion of nearly all allelic variation in the F_1 selection, as it leads to a very high probability probably (0.998) of sampling both alleles at a heterozygous locus. Therefore, assuming no inbreeding, the F_2 family, like the F_1 individual, would include, identically by descent, about half of the alleles present in the parental selection (P). Even more genes from the parental selections would be represented in the gene resource outplantings because many F_1 individuals were likely pollinated by other F_1 individuals in the progeny test.

With respect to choosing specific F_1 trees for seed collection, preference would be given to superior genotypes within a family. Because within-family heritabilities tend to be low (<0.20) in Douglas-fir (JOHNSON *et al.* 1997), any effects of within-family selection on allelic frequencies are likely to be small. Nevertheless, if growth were an adaptive trait, mild selection on growth would mimic natural selection and potentially increase slightly the frequency of important alleles needed in the future. Alternatively, the frequency of favorable alleles would be reduced if the favorable alleles were associated with slower growth or poorer form, but only by a small amount. Furthermore, the gain lost when establishing a new breeding population could be minimized somewhat by starting with an "improved" gene resource population produced by within-family selection.

Gene resource outplantings extend some of the benefits associated with progeny tests for gene conservation. Screening for discernable differences among families would be possible and could be done easily in outplantings where families are planted in rows. While family differences are unlikely to be evident for many characteristics, the outplantings might provide important observations on major effects such as disease resistance or adaptation to a significant change in climate. They would also make possible within-family selection for future breeding or gene resource populations, and, because family rows allow for outcrossing, open-pollinated seed could be used following within-family selection without too much concern for inbreeding.

Nevertheless, even though this method is designed to minimize costs, collecting and processing seed and establishing outplantings would be expensive. Therefore, long-term maintenance of first-generations tests remains the most cost efficient and valuable form of *ex situ* gene conservation of coastal Douglas-fir.

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