AN ABSTRACT OF THE THESIS OF

Geoffrey David Gooding for the degree of Master of Science in Forest Science presented on June 29 1998. Title: Genetic Variation and Mating System of Ponderosa Pine in the Willamette Valley of Oregon.

Abstract approved: ______________________________

Sally N. Aitken

The population genetic structure of ponderosa pine (Pinus ponderosa Dougl.) in the Willamette Valley of Oregon was investigated. Cones were collected from native stands of ponderosa pine from the Willamette Valley, eastern Oregon, southwest Oregon and the Puget Sound Basin of Washington. Seeds were subjected to isozyme analysis of 12 enzyme systems controlled by 20 different loci. Population genetic parameters for progeny, parent trees and pollen were estimated from isozyme data using BIOSYS-2, a population genetic statistical software package. For progeny data, total gene diversity (H_T) was 0.249; average genic diversity within stands (H_S) was 0.231; average genic diversity among stands (D_{ST}) was 0.017; the proportion of total genic diversity due to differences among stands (G_{ST}) was 0.067. Based on gene diversity statistics and genetic distances, stands of Willamette Valley ponderosa pine do not differ substantially from one another or from other populations of ponderosa pine in Washington and Oregon. These results suggest that protection of the Willamette Valley population can be accomplished by conserving a large number of individuals in a few large stands or many small stands.

Multilocus and single-locus estimates of outcrossing rate were determined for six stands within the Willamette Valley. Outcrossing rates were estimated for seven polymorphic loci using MLTR, a mating system analysis software package. Estimates of multilocus outcrossing for these stands (mean 0.642; range 0.365 to 0.879) were substantially lower than estimates previously reported for other populations of ponderosa pine, other pine species, and other species in the family Pinaceae. No significant difference was detected among stands in outcrossing rate. The high level of inbreeding observed in this population could be attributable to population fragmentation due to human activities, or to reduced levels of pollen production in this population compared to other populations of ponderosa pine. Genetic conservation strategies for this population should include the protection of the large native stands that remain and the use of local, adapted sources for afforestation. Management of pollen in a seed orchard should improve the genetic quality of seed over wild stand seed by reducing inbreeding levels.

Genetic Variation and Mating System of Ponderosa Pine

in the Willamette Valley of Oregon

by

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A Thesis Submitted

to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Master of Science

Presented June 29, 1998

Commencement June 1999

Master of Science thesis of Geoffrey David Gooding presented on June 29, 1998

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Geoffrey David Gooding, Author.

Acknowledgments

This study was initiated by the Willamette Valley Ponderosa Pine Conservation Committee (WVPPCC). Funding for this study was provided by the College of Forestry at Oregon State University (OSU) and the United States Department of the Interior-Bureau of Land Management. Facilities and equipment were provided by the Pacific Northwest Research Station of the United States Department of Agriculture-Forest Service and the Department of Forest Science at O.S.U.

Many individuals provided me with invaluable assistance and information during the course of this study, and I would not have been able to complete it without their patient and thoughtful help. I would like to thank Bart Thielges of the College of Forestry and Logan Norris of the Department of Forest Science for their help in extending my funding and allowing me continued use of Departmental facilities after my assistantship ran out. Tom Adams of the Department of Forest Science provided me with access to the isozyme lab and all necessary equipment and shared his expertise on mating system analyses and population genetics. From the U.S. Forest Service Pacific Northwest Research Station, I thank Allan Doerksen for his training, assistance and expertise in laboratory methods of isozyme analysis; Frank Sorenson for providing me with the seed collection from Southwest Oregon; Brad St. Clair and Al Sugano for their help in making the collection at the Fort Lewis army base in Washington; Richard Miles for his services in making X-ray photographs of seed, and Chris Niwa for her expertise in cone and seed insects of the Willamette Valley. Kim Delaney of the U.S. Forest Service,

Pacific Southwest Forest and Range Experiment Station provided red pine seed for use as standards in the isozyme analysis. Valerie Hipkins of the U.S. Forest Service, National Forest Genetic Electrophoresis Laboratory in Placerville, CA provided information on isozyme laboratory methods. Gerry Barnes of Tree Improvement Enterprises, Cottage Grove, Oregon, provided seed collections from eastern Oregon and the Willamette Valley, and allowed me to accompany him on several collection trips. Rick Fletcher of the O.S.U. Cooperative Extension in Corvallis, Oregon, and other members of the WVPPCC provided maps of ponderosa pine stands in the Willamette Valley and helped me identify which stands were good candidates for collection. Jan Aagard of the Department of Forest Science at O.S.U. assisted me during several collection trips. Jinghua Zuo of the Department of Forest Science and Tanya Cole of the Department of Horticulture provided a great deal of assistance in the isozyme laboratory. Greg O'Neill and Andy Bower of the Department of Forest Science assisted me in a variety of ways related to seed germination and laboratory work. Steve Strauss and Sheila Vollmer of the Department of Forest Science provided the ultra-cold storage facilities in their lab and expertise in genetics and laboratory methods.

I thank my major professor, Sally Aitken of the University of British Columbia for her support, guidance, and invaluable expertise during all stages of my academic career at O.S.U. I would also like to thank my current employer, Bill Lowe of the Western Gulf Forest Tree Improvement Program in College Station, Texas, for his patience and understanding while I have completed this thesis. Finally I would like to thank my wife, Lorene Broemling, for her continued support and encouragement.

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Genetic Variation and Mating System of Ponderosa Pine in the Willamette Valley of Oregon

CHAPTER 1: GENERAL INTRODUCTION

The Species

Ponderosa pine (Pinus ponderosa Dougl.) is the most widely distributed indigenous pine in the western hemisphere (Wang 1977). The range of ponderosa pine, including its taxonomic varieties P. ponderosa var. ponderosa, P. ponderosa var. scopulorum and P. ponderosa var. arizonica, extends from southern British Columbia east to southwestern North Dakota and central Nebraska, and south to southern California, northern Durango, and San Luis Potosi in Mexico (Figure 1.1, from Critchfield and Little 1966). Ponderosa pine is usually associated with arid or mountainous habitats. However, there is a small disjunct population of P. ponderosa var. ponderosa in the relatively mild and moist Willamette Valley of Oregon.

Ponderosa Pine in the Willamette Valley

Prior to European settlement in the mid-19th century, the Willamette Valley consisted of four major vegetation types: riparian gallery forest, prairie, open forest and upland conifer forest (Towle, 1982). The open forest type was the most important habitat for ponderosa pine. This forest type was largely maintained by the burning practices of the Calapooia Indians. Frequent burning on the valley floor prevented natural succession

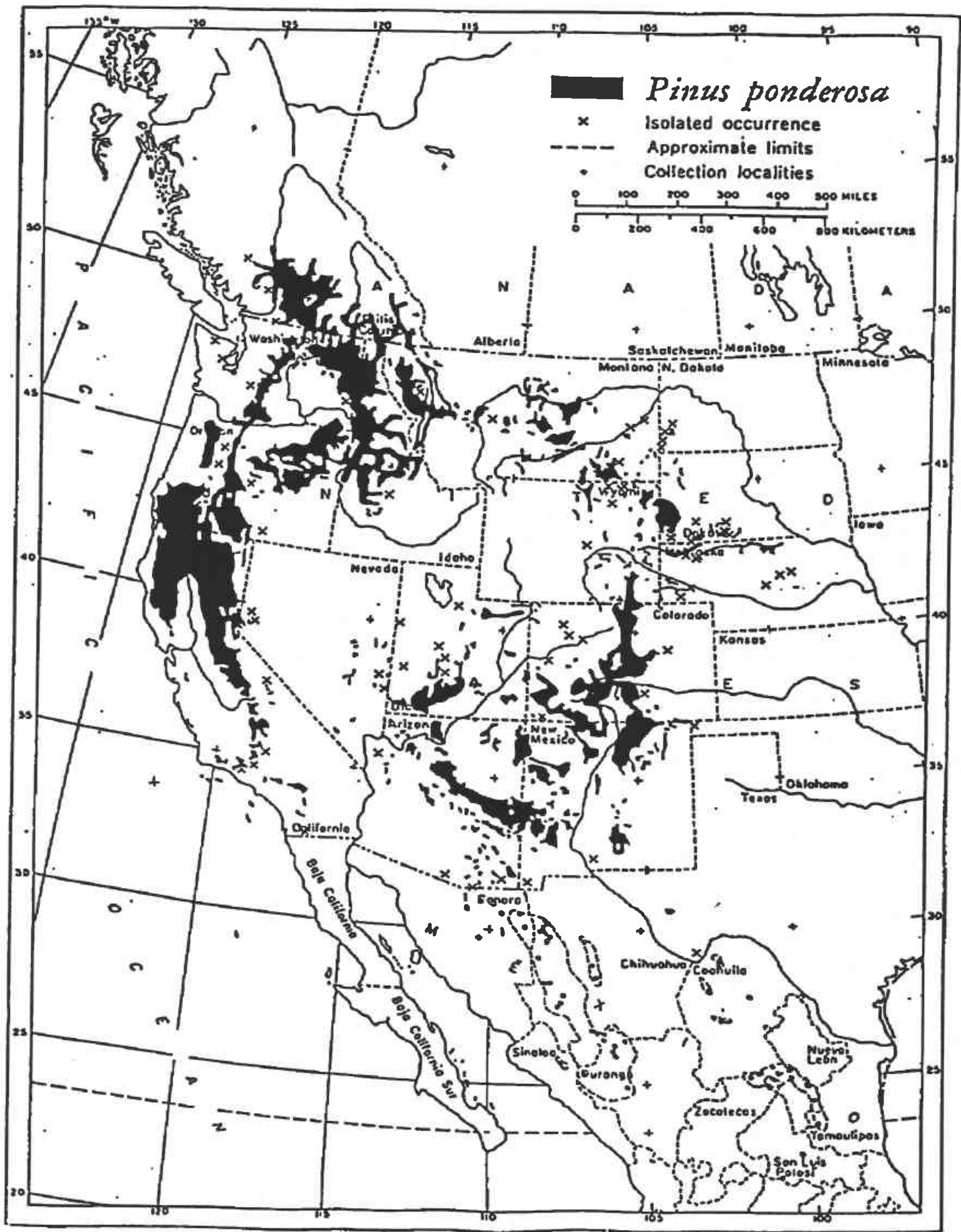


Figure 1.1. Geographic distribution of ponderosa pine (from Critchfield and Little 1966).

to Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and kept the valley mostly open prairie with some isolated groves of Oregon white oak (Quercus garryana Dougl.) and ponderosa pine (Franklin and Dyrness 1973).

Since European settlement, the vegetation of the Willamette Valley has undergone marked change. Urbanization and agricultural development of the prairie and open forest have reduced those vegetation types, and the cessation of uncontrolled burning has allowed the encroachment of Douglas-fir and other conifer species from the surrounding hills into the valley (Towle 1982). As a result of these changes and logging activity, the extent of ponderosa pine in the Willamette Valley has been greatly reduced.

The Willamette Valley population of ponderosa pine (WVPP) has only recently gained some commercial importance, so it has not been included in most studies of genetic and geographic variation. However, one long-term provenance study of ten seed sources planted at five sites in Oregon and Washington included a plantation on McDonald Forest on the western edge of the Willamette Valley near Corvallis, Oregon and a Willamette Valley seed source (Squillace and Silen 1962). Although this study was not designed with statistical rigor in mind and was not replicated, genetic differences in rate of height growth were found among provenances. Significant declines in growth and yield were observed when ponderosa pine seed from sources to the east of the Cascades were planted in the Willamette Valley (Squillace and Silen 1962).

Anecdotal evidence suggests that the incidence of foliage diseases is higher in east side sources than in native sources when planted in the valley, suggesting further adaptive differences between provenances. For a successful plantation program, seedlings must be available that are adapted to the conditions of the Willamette Valley.

The Willamette Valley Ponderosa Pine Conservation Committee

Private, non-industrial woodland owners in the Willamette Valley have given increasing emphasis to ponderosa pine in tree planting programs over the last ten years. The ability of ponderosa pine in this area to grow in conditions either too wet or too dry for Douglas-fir have made it an economically attractive candidate for reforestation on such sites. It is estimated that one million ponderosa pine seedlings would be planted in the Willamette Valley each year if an adequate seed and seedling supply were available (WVPPCC 1994).

Unfortunately, the small number of native stands of trees and the lack of a consistent cone crop over the last few years have caused the supply of seedlings from the Willamette Valley to be far outweighed by the demand. To address this issue, a group of landowners, industrial foresters and agency representatives met in early 1994 and formed the Willamette Valley Ponderosa Pine Conservation Committee (WVPPCC). The group identified five objectives:

1. Conserve Willamette Valley ponderosa pine as a genetic resource.
2. Maintain genetic diversity and adaptability of seed sources used in reforestation programs.
3. Improve the genetic quality of Willamette Valley ponderosa pine through careful tree selection and prescriptive seed deployment.
4. Provide supplies of high quality seed for planting programs over the next ten years.
5. Provide for long-term supplies of high quality seed through development of a seed orchard (WVPPCC 1994).

Previous genetic studies involving WVPP have not included assessments of genetic diversity among stands within the valley, of which about 160 individual stands have been identified, stretching from southern Lane County to Washington County (WVPPCC 1994). More detailed information is needed about the genetics of WVPP, in order to ensure that the appropriate genetic management strategy is undertaken. Before an effective tree improvement program can be established for WVPP, the amount and distribution of genetic variation and knowledge about the mating system of WVPP should be available.

Analyzing Genetic Diversity in Conifers through Isozyme Studies

The objective of this study is to determine the amount and distribution of genetic diversity and to describe the mating system of ponderosa pine in the Willamette Valley through isozyme analysis. The isozyme method has been used extensively as a relatively

quick and inexpensive way to quantify genetic variation by assessing electrophoretic differences in enzymes. Isozymes are variant forms of enzymes that can be differentiated electrophoretically; allozymes are isozymes that differ due to allelic variation. Allozyme variation can be studied within a single individual, between individuals, within populations, and among populations. Allozyme variation can show clear differences in the genotypes of individuals, where a genetic analysis of phenotypic traits through progeny testing might be less clear because of pleiotropy or environmental effects (Pearce 1983).

Conifer seeds offer some advantages for genetic studies because gene products are present in both haploid and diploid tissues. The megagametophyte surrounding the embryo is haploid and has the same genetic makeup (haplotype) as the maternal gamete contributing to the embryo. By analyzing the gametophytes from several seeds of the same tree the maternal genotype can be inferred. The embryo itself is diploid, and the haploid pollen contribution to individual embryos can be deduced by subtracting the maternal haplotype (as assessed from the gametophyte) from the embryo diploid genotype. Isozyme analysis can therefore yield information about both the mating system and the genetic variability of a conifer (Conkle *et al.* 1982). It is also possible to detect allozyme polymorphisms in bud and mature needle tissue of some conifers, including ponderosa pine (Mitton *et al.* 1979). However, to describe the mating system, it would be required to sample vegetative tissue of parent trees and offspring known to come from that parent.

The technique of isozyme analysis has been used for a variety of objectives in studies of Pinus species. One of the most useful applications of the technique is in geographic studies of allozyme diversity and genetic differences among and within populations. Isozyme analyses can be performed with a small amount of material (either seeds or vegetative tissue), so it is relatively easy to sample a large number of trees representing several populations. Geographic population studies using isozymes have been described for several species of pines, including P. banksiana (Dancik and Yeh 1983), P. contorta (Dancik and Yeh 1983; Knowles and Grant 1985, Yeh *et al.* 1985), P. halepensis (Loukas *et al.* 1983), P. jeffreyi (Furnier and Adams 1986), P. leucodermis (Boscherini *et al.* 1994), and P. sylvestris (Rudin *et al.* 1974).

Another common use of isozyme studies in Pinus is the description of mating systems. The mating system of a population is the proportion of self vs. outcrossed matings. The outcrossing rate in a stand can be estimated based on the degree of non-random association of alleles from seed and pollen parents. The loss of genetic diversity in natural or artificial stands can result in inbreeding, which can affect the fitness and viability of a population due to inbreeding depression. An understanding of the mating system allows informed decision making regarding pollen management and genetic conservation techniques. Pine species that have been the subject of mating system studies using isozyme analysis include P. contorta (Perry 1978; Epperson and Allard 1984), P. jeffreyi (Furnier and Adams 1986), P. leucodermis (Boscherini *et al.* 1994), P. monticola (El-Kassaby *et al.* 1987), P. sylvestris (Rudin *et al.* 1974; Shen *et al.* 1981) and P. taeda (Adams and Joly 1980; Roberds and Conkle 1984).

Isozyme analyses can be useful in studies of evolution and phylogeny in pine species and varieties. A study of the Californian closed-cone pines (Pinus subsect. oocarpae) revealed patterns of isozyme differentiation among P. attenuata, P. muricata, and P. radiata that supported their grouping into one subsection of Pinus, but also identified clear species differentiation (Millar *et al.* 1988). Isozyme analysis was also used to describe the phylogenetic relationship of the closely related P. banksiana and P. contorta ssp. latifolia in Alberta, and supported earlier hypotheses that the two species derived from a common ancestor (Dancik and Yeh 1983). A comparison of P. contorta ssp. contorta and P. contorta ssp. bolanderi found that isozyme differentiation between the two taxa was not as high as expected for subspecies. However, ssp. bolanderi did show significantly less variation than ssp. contorta, suggesting that the former was derived from the latter relatively recently (Aitken and Libby 1994).

Another category of studies has related quantitative characters and ecological factors to levels of allozyme heterozygosity. The phenomenon of heterosis, or heterozygote advantage, has been well known for some time and some researchers have attempted to find relationships between allozyme heterozygosity and characteristics related to fitness. However, it has been difficult to determine if heterozygote advantage results from true heterosis, or just a release from inbreeding depression. Some evidence of an association between depressed growth rate and below average levels of allozyme heterozygosity has been found in P. attenuata (Strauss 1986) and P. radiata (Strauss and Libby 1987). In certain environments, there is a positive association between allozyme heterozygosity and germination rate in P. banksiana (Govindaraju and Dancik 1987), but

attempts to relate heterozygosity and biomass production in P. banksiana were less successful, and showed an association in only one of four environments (Govindaraju and Dancik 1986).

Genetic Studies of Ponderosa Pine

Common-garden studies of quantitative traits

Racial studies have shown that there is considerable genetic differentiation among populations of ponderosa pine from different geographical areas. Conkle and Critchfield (1988) reviewed a number of previous studies of ponderosa pine across the species range, and observed that the two northern taxonomic varieties of ponderosa pine consisted of five geographic races. P. ponderosa var. ponderosa, which occurs in the western portion of the species range, can be divided into three races: Southern California, Pacific (which includes the Willamette Valley population), and North Plateau. The eastern variety, P. ponderosa var. scopulorum, can be divided into two races: Rocky Mountain and Southwestern. These five races are well differentiated on the basis of morphological traits, biochemical analyses, and growth responses of provenances in nursery and plantation trials. Genetic differences have also been found among the three taxonomic varieties in cold resistance, growth initiation, growth rate, tree form, needle characteristics, and wood properties (Wang 1977). These taxonomic varieties have been further divided into nine genetically distinct geographic clusters based on seedling characteristics (Read 1980). A study of two ponderosa pine provenances conducted in

northern Idaho and in Oregon and Washington found that approximately 36 percent of the variation in total height among trees within a plantation was due to provenance (Squillace and Silen 1962).

Other common garden studies have repeatedly found significant genetic differences among ponderosa pine provenances and have led to prescriptions for seed movement. A study of southwest Idaho populations found most differentiation was due to elevation of the source, and it was recommended that seed not be transferred more than 450 m in elevation for reforestation purposes (Rehfeldt 1980). A study in central Oregon found significant, but not large, differentiation among populations of ponderosa pine. Based on the results of this study, the authors recommended seed zones consisting of elevation bands 300 m in width below elevations of 1550 m (Sorenson and Weber 1994).

Genecological studies in ponderosa pine have found significant local variation based on habitat type (Madsen and Blake 1977) as well as soil type (Jenkinson 1974). In short, a great deal of genetic differentiation in quantitative traits is present in ponderosa pine at the level of species, geographic variety, and local population.

Isozyme studies in Pinus ponderosa

Several studies of ponderosa pine using isozymes have yielded valuable information about genetic variation in different parts of the species range. These studies

include population studies in Oregon (Rotach 1997), Montana (Woods *et al.* 1983), and Colorado (Mitton *et al.* 1979, Linhart *et al.* 1981).

In the Oregon study, a total of 488 trees were sampled from two separate regions: Southwest Oregon and Central Oregon on the east side of the Cascade Range. In general, both populations had high levels of genetic diversity compared to studies in other areas, with diversity estimates in the upper range of values found for conifers. Levels of diversity were slightly higher in Southwest Oregon than Central Oregon. Differences between regions were hypothesized to be due to environmental differences resulting in differential adaptation, and not to genetic drift (Rotach 1997).

The Montana study sampled 50 trees in each of six small isolated stands and found very low levels of diversity within and among stands (Woods *et al.* 1983). The variation that did exist was almost completely due to variation within rather than among stands. Only 1.5% of the genetic diversity observed could be attributed to among-stand differences. It was suggested that this low level of diversity is due to a frequent fire interval, with considerable gene flow occurring between stands during initial stages of stand development when stocking levels are low and stands are relatively open.

The study in Colorado by Linhart and others (1981) was based on one population of ponderosa pine composed of six related groups of genetically distinct trees. Although these groups were close together, significant differences were found among the family groupings. This is in contrast to the above studies of ponderosa pine, which found less

variation at the local population level. The population was also divided into four distinct age classes, but these did not differ significantly in genetic characteristics. The conclusion offered by the authors was that within this population there is genetic differentiation in space but not in time.

Niebling and Conkle (1990) compared allozyme frequencies for three races of ponderosa pine (Pacific, North Plateau and Rocky Mountain) and Washoe pine (P. washoensis). Although Washoe pine is a narrowly distributed endemic, levels of diversity in the three sampled populations of this species were similar to those for the widespread geographic races of ponderosa pine. The study also found significant differences in heterozygosity among the races of ponderosa pine, and genetic distance values indicated that both Washoe pine and the Pacific and North Plateau races of ponderosa pine were strongly differentiated from the Rocky Mountain race of ponderosa pine.

Both common-garden progeny tests and genetic marker studies have found considerable genetic variation in ponderosa pine both within and among populations. Isozyme studies have confirmed the separation of the species into taxonomic varieties and geographic races based on quantitative traits. While isozyme and other neutral or near-neutral genetic markers cannot provide a complete picture of the population genetics of any species, it appears that for ponderosa pine there is good agreement between the results of studies based on genetic markers and those based on quantitative traits, which have readily recognized adaptive significance. Genetic differences among populations of

ponderosa pine found for isozymes may show at some similarity to differences that might be found using common-garden studies of quantitative traits.

Summary and Justification

The current status of Willamette Valley ponderosa pine can be summarized as follows:

1. There is a large demand for ponderosa pine seedlings to plant on sites in the Willamette Valley that are inappropriate (e.g., too wet or dry) for Douglas-fir.
2. There is little native seed available and other seed sources appear to be maladapted to the conditions of the valley.
3. The remaining native stands are being harvested at a rapid rate.

Little is known about the genetics of ponderosa pine in the Willamette Valley and the relationship of WVPP to ponderosa pine elsewhere. This study investigated genetic diversity among and within stands of ponderosa pine in the Willamette Valley, and the extent of outcrossing versus inbreeding in the stands, using isozyme analysis. The numerous, widely distributed and often isolated stands of ponderosa pine in the Willamette Valley should not be considered a single panmictic unit, but for the purposes of this study they will collectively be referred to as the Willamette Valley population of ponderosa pine (WVPP). An important issue that has been addressed in part by this study is the extent to which WVPP differs from populations of ponderosa pine in the East

Cascades, Southwest Oregon and the Puget Sound Basin of Washington. This issue has implications for seed transfer between and within these areas. While questions of adaptive genetic variation cannot be definitively answered by the current isozyme study, it does provide an indication of genetic diversity among and within populations. Using this information, inferences can be drawn about historic levels of gene flow among populations and relative population sizes.

The information resulting from research described in this thesis will be used to help conserve ponderosa pine as a genetic resource in the Willamette Valley and to help design a seed orchard and breeding strategy so that the stock of ponderosa pine seedlings that are adapted to the Willamette Valley environment can be grown in quantity. These programs will subsequently meet the demand for seedlings by small woodlot owners, and will ensure that the Willamette Valley population of ponderosa pine remains viable and retains the ability to adapt to future environmental conditions.

Objectives

The broad goal of this study is to describe the amount and distribution of genetic variation of ponderosa pine in the Willamette Valley and to determine if it differs significantly from other populations of ponderosa pine in the region. Information from this study will be used to determine the degree to which stands of Willamette Valley ponderosa pine are evolutionarily significant or unique genetic resources that should be conserved.

To achieve the above goal, four specific objectives have been addressed:

1. Determine the amount of genetic variation in WVPP and whether it is different from the amount of genetic variation in other populations of ponderosa pine.
2. Describe the amount and distribution of among-stand genetic variation in WVPP.
3. Analyze the mating system of WVPP, determine the amount of inbreeding in seed collections from native stands, and compare the outcrossing rate of WVPP to other populations of ponderosa pine.
4. Determine if levels of inbreeding are related to current population sizes.

Objectives 1 and 2 are addressed in the second chapter of this thesis. Objectives 3 and 4 are addressed in the third chapter.

CHAPTER 2: AMOUNT AND DISTRIBUTION OF GENETIC VARIATION IN WILLAMETTE VALLEY PONDEROSA PINE

Abstract

Ponderosa pine (Pinus ponderosa Dougl.) in the Willamette Valley of Oregon was investigated to determine if population genetic parameters differed substantially from ponderosa pine in other regions of Oregon and Washington. Cones were collected from native stands of ponderosa pine from the Willamette Valley, eastern Oregon, southwest Oregon and the Puget Sound Basin of Washington. Eleven stands were analyzed, including seven from the Willamette Valley. A total of 1242 germinated seeds from 157 mother trees were subjected to isozyme analysis by starch gel electrophoresis. Results were obtained for 12 enzyme systems controlled by a total of 20 gene loci. Population genetic parameters for parent trees, progeny, and pollen gametes in progeny were estimated. No substantial difference in estimated population genetic parameters, including number of alleles per locus, percent polymorphic loci, observed and expected heterozygosity were found among Willamette Valley stands and between the Willamette Valley region and the other sampled regions of Oregon and Washington. Genetic distance estimates among stands were generally low. Based on allozyme variation, Willamette Valley ponderosa pine does not appear to differ substantially from other populations of ponderosa pine in Washington and Oregon. However, there is anecdotal evidence of important differences in adaptive traits, and future common-garden studies may discover genetic differences between WVPP and ponderosa pine in other regions that were not detected by isozyme analysis. This study found no geographic structure

associated with genetic diversity in WVPP, so protection of the genetic resource represented by this population can be accomplished by conserving a large number of individuals in a few large stands or in many small stands.

Introduction

Although ponderosa pine (Pinus ponderosa Dougl.) is commonly associated with arid, montane environments and continental climates, a disjunct population is present in the mild and moist Willamette Valley of Oregon. Small, isolated stands of ponderosa pine can be found throughout the Willamette Valley from Cottage Grove north to Portland. Although these stands are too widely distributed and isolated to be considered a single panmictic unit, I will refer to these stands collectively as the Willamette Valley population of ponderosa pine. The results of seed source studies (Squillace and Silen 1962) suggest that Willamette Valley ponderosa pine (WVPP) differs from populations on the east side of the Cascade Range in growth characteristics, but there have been no studies of patterns of genetic variation of ponderosa pine within the Willamette Valley.

Agricultural activity, fire suppression and urban development in the Willamette Valley over the last 150 years have resulted in a decline in the numbers of WVPP (Towle 1982, WVPPCC 1994). There is a growing demand for high quality ponderosa pine seedlings in the Willamette Valley by local small woodland owners who wish to cultivate ponderosa pine on sites that are inappropriate for Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) (WVPPCC 1994), yet local seed is becoming increasingly difficult to

obtain. For these reasons it is important to determine if WVPP is genetically distinct from populations elsewhere in Oregon and Washington. Large genetic differences between WVPP and other populations of ponderosa pine, or among WVPP stands would indicate a need for genetic conservation measures to maintain diversity and variability within the native Valley population. Such differences would also indicate a need to establish seed transfer guidelines to ensure that planted ponderosa pine is adapted to the conditions of the Willamette Valley.

Objectives

The purpose of this study is to determine the amount and distribution of genetic variation of ponderosa pine in the Willamette Valley. Isozyme studies have been widely used as a relatively quick and inexpensive method of quantifying levels of genetic variation by assessing electrophoretic differences genetic variants of metabolic enzymes, or allozymes. Through isozyme analysis, estimates of population genetic parameters were obtained and compared and genetic variation in WVPP described. By comparing estimates of population genetic parameters of stands within the Willamette Valley with stands in other areas of Oregon and Washington, I estimated the degree to which WVPP is differentiated from populations elsewhere in the region.

Materials and Methods

Cone Collection

Candidate stands for collection were located by reconnaissance trips around the Willamette Valley and by consultation with members of the Willamette Valley Ponderosa Pine Conservation Committee (WVPPCC). A stand was deemed suitable for collection if it met the following criteria:

A. It appeared to be a native stand. This determination was made by examination of the spacing of trees and estimating the ages of trees. If trees appeared to grow in a straight line, e.g. along a road, or if all the trees in the stand appeared to be evenly spaced and about the same age, the stand was presumed to be planted rather than naturally regenerated. These stands were not collected because of the risk that the seed originated from a source outside of the Willamette Valley.

B. All trees sampled appeared healthy and did not have obvious top dieback. Top dieback is thought to be associated with maladapted seed sources from outside the Valley.

C. The stand included at least ten female cone-bearing trees, each with a minimum of two mature cones. If a stand had fewer than ten cone-bearing

trees, or if two mature cones were not found for each of at least ten trees, it was not sampled. The estimates of genetic variability that were calculated depend on an adequate sample size of seed to accurately reflect the values for the stand.

Cones were collected in the Willamette Valley when they appeared mature, to ensure adequate maturation of seeds, but prior to cone opening and seed dispersal. Prior to collection, several trips were made to monitor cone maturity and to obtain permission of property owners to collect cones. Only large, green or purplish-green cones were collected. Small brown cones that aborted before maturity, quite common in the Willamette Valley due primarily to the Conophthorus spp. cone beetle (C. Niwa, USDA Forest Service PNW Research Station, personal communication 1996), were not collected.

Cones were collected from the top two-thirds of the crown, when possible, to reduce the frequency of selfed seed. Like most pines, ponderosa pine produces most of its male strobili in the bottom one-third of the crown, so female cones in this region of the crown have a higher probability of self-fertilization than cones in the upper crown of the tree. Cones collected from the bottom of the crown would likely give downwardly biased estimates for frequency of outcrossing, because most cones are produced in the upper crown. Cones were collected with a pole pruner when possible, by climbing, or sometimes by using a 12-gauge shotgun. At least two cones from at least ten trees for

each stand were collected. Cones were identified by stand and mother tree and kept in separate mesh or pollen-tec bags throughout the collection process.

I collected cones from as many stands as possible during the 1994 cone collection season. Gerry Barnes of Tree Improvement Enterprises, Inc., (Cottage Grove, Oregon) collected additional stands on contract in conjunction with operational seed collections for the WVPPCC. At the end of the 1994 cone collection season, cones had been collected from seven stands within the Willamette Valley (Figure 2.1). Cones were collected from stands in the north and west sides of the Valley as well, but unfortunately these stands did not include enough cone-bearing trees to be included in the analysis.

Four collections were made from outside the Willamette Valley (Figure 2.2). Two populations from the east side of the Cascades were collected by the contractor in September 1994, one in the Ochoco National Forest (120.8°N, 44.6°W) (Ochoco) and one on the Warm Springs Indian Reservation (121.3°N, 45.0°W) (WSIR). Seed from Southwest Oregon was made available for analysis by Frank Sorenson of the U.S. Forest Service (collection centered around 123.1°N, 42.7°W) (Southwest). Cones were also collected from the small, disjunct population of ponderosa pine located on the Fort Lewis U.S. Army Base near Steilacoom, Washington (122.7°N, 47.1°W) (Fort Lewis). This population was collected twice; in 1994 by Brad St. Clair and Al Sugano of the U.S. Forest Service and me, and again in 1996 by Al Sugano.

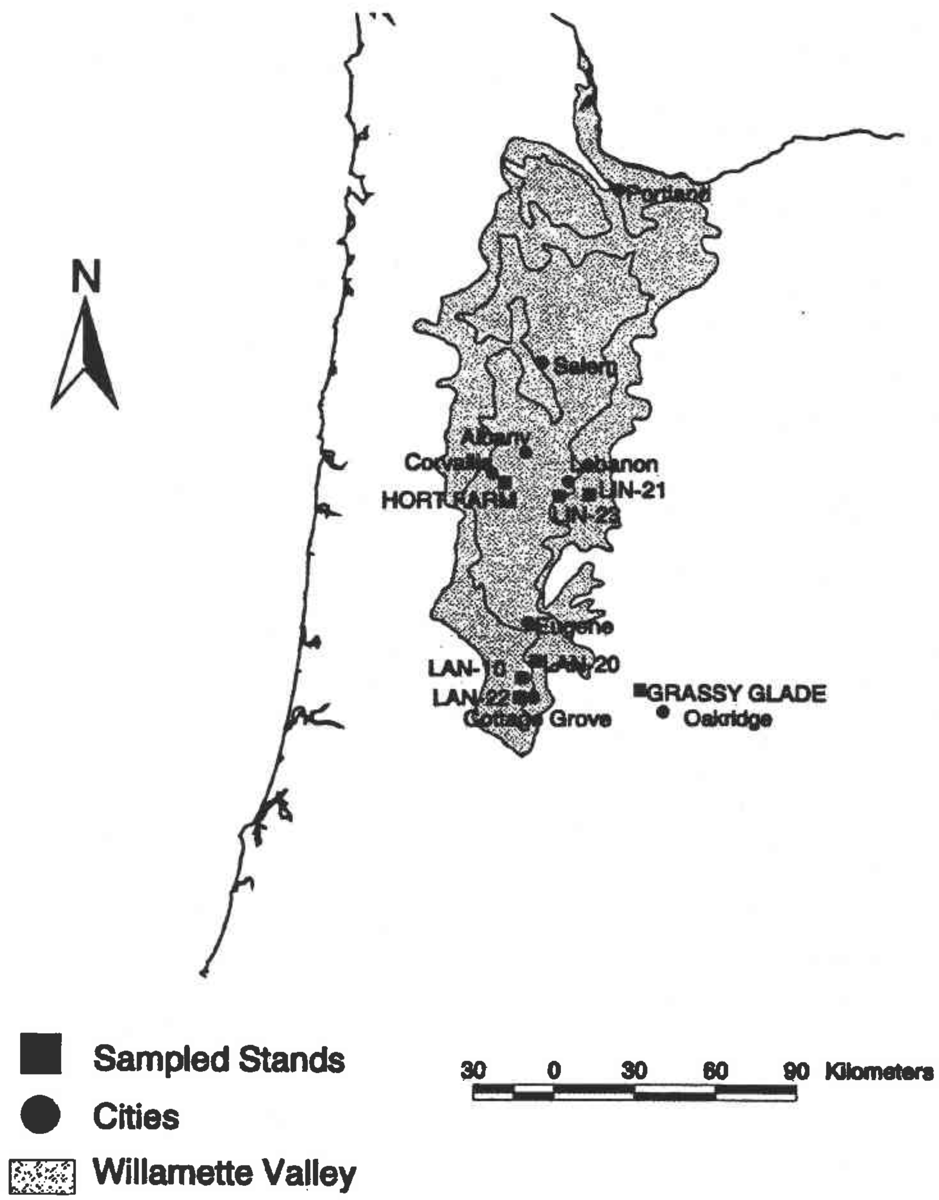


Figure 2.1. Location of sampled stands within the Willamette Valley.

Figure 2.2. Location of sampled stands outside the Willamette Valley.

Procedures for cone collections outside the Willamette Valley generally followed those for collections within the Willamette Valley. With the exception of the 1996 Fort Lewis collection, cones were collected in August or September, 1994. The number of stands and parent trees from which cones were collected are summarized in Table 2.1.

After cones were collected, bags were stored in a cone drying room to allow cones to open, and to minimize the growth of fungi that might decrease seed viability. Seeds were extracted from the cones by hand and stored separately by parent tree at 0°C.

Table 2.1. Location and number of stands, trees and samples used in study.

Region	Stand	Latitude	Longitude	Elevation (m)	Parent Trees	Seeds Analyzed
Eastern Oregon	Ochoco	120.8° W	44.6° N	1520	22	132
	WSIR[1]	121.3° W	45.0° N	910	26	156
Total					**48**	**288**
Fort Lewis	Ft. Lewis	122.7° W	47.1° N	100	13 [2]	130
Southwest Oregon	SW	123.1° W	42.7° N	270-1370	10 [3]	100
Willamette Valley	Hort. Farm	123.1° W	44.4° N	170	11	101
	Lan-10	123.1° W	43.9° N	220	12	113
	Lan-20	123.1° W	43.8° N	210	20	120
	Lan-22	123.1° W	43.8° N	250	10	100
	Lin-21	122.8° W	44.5° N	220	11	110
	Lin-23	122.9° W	44.5° N	200	22	132
	Grassy Glade[4]	122.4° W	43.8° N	980	(Bulked Sample)	48
Total					**86**	**724**
Grand Total	**11 stands**				**157**	**1242**

Average number of seeds analyzed per tree = 7.6 (not counting bulked sample)
Average number of trees analyzed per stand = 15.7 (not counting bulked sample)

[1] Warm Springs Indian Reservation

[2] Two collections made for Ft. Lewis stand: 4 trees in 1994 and 9 trees in 1996.

[3] Several trees collected from several stands over a wide area, considered one stand for the purposes of this study.

[4] The Grassy Glade stand is located on the western slope of the Cascades, and is not in the Willamette Valley proper. I have grouped it with the other WV populations for comparative purposes because of its proximity to the valley, but it is at a higher elevation and farther east than the other WV stands.

X-ray photographs were made of random samples of 25 seeds per tree to estimate filled seed percentages.

All seed were stratified in a 1% solution of hydrogen peroxide at room temperature for two days prior to incubation in order to induce germination. This

procedure was amended after the first two germination attempts to include a two day soak in a solution of Captan™ fungicide (3.31 g/L) to decrease fungal growth during incubation. The peroxide solution was aerated by inserting a hose connected to the laboratory air supply into the beaker containing the seeds and solution.

Approximately four weeks before the isozyme analyses began, random samples of seed were germinated. These test germinations were used to determine how far germination should progress before seed are dissected and the embryonic and gametophytic tissues are removed. It was found that the embryo had absorbed approximately half of the gametophytic tissue after the radicle reached a length of ½ - 1 times the length of the seed. At this stage, there was adequate enzyme activity in both embryonic and gametophytic tissue to perform the isozyme analyses.

Isozyme Analysis

Starch gel electrophoresis was conducted on extracts of both the megagametophyte (1N) and embryo (2N) tissues of seeds sampled from each mother tree. Between six and ten seeds were analyzed for each mother tree, so that the total number of seeds sampled for each stand would be at least 100. The goal of fixing the number of seed per stand at 100, rather than fixing the number of mother trees, was to provide an adequate sampling of pollen gametes so as to derive representative allele frequencies for each stand from pooled male contributions, rather than from the small number of mother trees.

Gametophytes and embryos were initially assayed for a total of 18 enzyme systems under the control of 33 loci, but due to inconsistent staining or poor resolution of bands, six enzyme systems were dropped over the course of the analysis to leave 12 enzyme systems coding by a total of 20 loci (Table 2.2).

Table 2.2. Enzymes, acronyms used in text, enzyme commission reference number (E.C. buffer systems used for electrophoresis, and number of scored loci used.

Enzyme[1]	Acronym	E.C. number	Buffer System[2]	No. of scored loci
Acid phosphatase	ACP	3.1.3.2	B	1
Alcohol dehydrogenase	ADH	1.1.1.1	E	1
Fumarase	FUM	4.2.1.2	E	1
Glutamate dehydrogenase	GDH	1.4.1.3	B	1
Glutamate oxaloacetate transaminase	GOT	2.6.1.1	B	3
Isocitrate dehydrogenase	IDH	1.1.1.42	D	1
Leucine-amino peptidase	LAP	3.4.11.1	A	3
Malate dehydrogenase	MDH	1.1.1.37	E	2
Peptidase	PEP	3.4.13.1	A	3
Phosphoglucomutase	PGM	2.7.5.1	A	1
Phosphoglucose isomerase	PGI	5.3.1.9	E	1
Shikimate dehydrogenase	SDH	1.1.1.25	D	2
Total				**20**

[1]Gel and stain protocols are in Conkle *et al.* (1982) and Strauss and Conkle (1986).

[2]System A. Tray buffer: Tris citrate pH 8.3; gel buffer: Lithium borate pH 8.3.
System B. Tray buffer: Tris citrate pH 8.8; gel buffer: Lithium borate pH 8.0.
System D. Tray and gel buffer: Morpholine citrate pH 6.1.
System E. Tray and gel buffer: Morpholine citrate pH 8.1.

General procedures for starch gel electrophoresis of conifer seeds followed Conkle *et al.* (1982). Methods for resolving peptidase (PEP), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), glutamate oxaloacetate transaminase (GOT), acid

phosphatase (ACP), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), shikimate dehydrogenase (SDH), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), and phosphoglucose isomerase (PGI) were those of Conkle *et al.* (1982), with the exception that morpholine-citrate (pH 8.1) gel and electrode buffers were used for ADH, MDH and PGI (Table 2.2). Fumarase (FUM) was assayed according to the methods of O'Malley *et al.* (1979). Inheritance of all 20 of these loci has been verified in pines by genetic segregation tests (Conkle 1981, Strauss and Conkle 1986).

On the day prior to each electrophoretic run, eight starch gels were poured and sealed in plastic wrap, and the tray buffers prepared and refrigerated at approximately 4°C. Seeds were dissected and gametophytes and embryos separated and placed into separate wells of plastic 72-well microtiter plates. One to two red pine (Pinus resinosa Ait.) seeds were also dissected and placed in the sample containers to be used as standards. Red pine is known to be highly monomorphic for the isozyme loci used in this study (Rotach 1997), making it an excellent standard for a control group. Two drops (approximately 0.1 mL) of extraction buffer (composed of 10 mL 0.2 M pH 7.5 phosphate buffer, 10 mL 5% sucrose solution, 100 mg ascorbic acid, 50 mg D-glucose-6-phosphate, 50 mg Bovine albumin and 10 mg Dithiothreitol; Rotach 1997) were put in each well. The sample containers were frozen in an ultra-cold (-80°C) freezer overnight. The morning of the run, gels were trimmed and sliced. Samples were retrieved from ultra-cold storage, allowed to thaw for 15 to 20 minutes, and then thoroughly crushed with a steel replica plater. Four 3.5 mm by 11 mm wicks cut from Whatman #1 filter paper were put into each well to absorb the extract. Then each gel was loaded with 36 to

44 wicks. Embryo/gametophytic pairs were run side by side to facilitate comparison. Because enzyme activity decreases quickly after tissue has been thawed and crushed, all of these steps were completed as quickly as possible and samples were kept on ice or refrigerated throughout the process.

Two gels for each of the four buffer systems (total of eight gels) were run per day. During the run, trays were in a refrigerator at approximately 4°C. To maintain low temperatures during the run, gels were covered with chilled water bags that were replaced every 60 to 90 minutes. Gels were run at 60 mA for buffer system A, 55 mA for system B, and 50 mA for systems D and E. Amperage was adjusted regularly (at least every 30 minutes), but the voltage was not allowed to exceed 300 V. Wicks were removed from the gels 15 to 20 minutes after the current was switched on. Progress of solvent front migration was monitored by the use of red dye marker wicks placed on both sides of the gels. Electrophoresis continued until the markers migrated 8 cm from the origin for buffer systems A and B and 6 cm for systems D and E. After completion of electrophoresis, gels were sliced horizontally and each slice was stained for one enzyme system and incubated at 37°C. Slices from the top and bottom of the gel were more likely to have undergone partial drying, reducing homogeneity of the gel texture, and were used only if absolutely necessary. Stain recipes followed Conkle *et al.* (1982) with some modifications taken from Rotach (1997). Stained gels were examined and the relative mobility of all allozymes recorded on data collection sheets. After initial scoring, gels were fixed in a 50% alcohol solution and a second assessment was completed at a later

date to verify scores. After the second reading, gels were wrapped in paper towels and plastic film to prevent drying and stored in a refrigerator for future reference.

Statistical Analysis

Estimates of population genetic parameters were calculated using BIOSYS-2, a computer program for the analysis of allelic variation within and among populations (Swofford and Selander 1981). Data were entered into BIOSYS-2 in the form of genotype frequencies. Genotype frequencies for progeny samples were determined by a direct count of genotypes. Genotypes of parent trees in all stands except Grassy Glade were inferred according to the following method. Because the megagametophyte tissue of seeds is haploid in conifers and is genetically identical to the maternal contribution, diploid maternal genotypes can be inferred directly using haploid seed tissue.

The probability of misclassifying a heterozygous mother tree as a homozygote at a particular locus using this method of inference is at most:

$$p = \tfrac{1}{2}^{(k-1)}$$

where k is equal to the number of megagametophytes analyzed per tree. Thus, for six seeds the probability of misclassifying a heterozygote as a homozygote is $\tfrac{1}{2}^5$, or 3.13%. Fewer than six seeds would result in an unacceptably high probability of misclassification (greater than 5%), so no fewer than six seeds were analyzed for each mother tree. Grassy Glade was a bulked sample and seeds were not identified by mother tree, so genotype and

allele frequencies could be estimated for a population sample of seeds, but only allele frequencies and not genotype frequencies could be estimated for the trees in this stand.

Population genetic statistics calculated with BIOSYS-2 to estimate genetic diversity within populations include the mean number of alleles per locus (A), percent polymorphic loci (P), and mean heterozygosity (H). Mean number of alleles per locus is simply the average number of electrophoretic variants detected at each locus for each sampled stand, and is calculated as follows:

$$A = \sum_{j=1}^{n} m_j / r * (100)$$

where m_j is the number of alleles at locus j with $p \geq 0.01$ and r is the total number of loci.

Percent polymorphic loci (P) is the proportion of loci in which the frequency of the most common allele is less than or equal to 0.95, and is calculated as follows:

$$P = n_p / r$$

where n_p is the number of polymorphic loci and r is the total number of loci.

Heterozygosity can be expressed in two ways: expected (H_e) and observed (H_o). Observed heterozygosity is the proportion of individuals heterozygous at a locus, averaged across all loci, according to a direct count of genotypes. Expected heterozygosity is a function of allele frequency, and can be calculated for each locus as:

$$H_e = 1 - \sum_{i=1}^{n} p_i^{\,2}$$

where there are n alleles and p_i is the frequency of allele i.

Unbiased expected heterozygosity is corrected for small sample size (when sample size is small, low frequency alleles will be missed in sampling) and for a single locus is:

$$H_e = (2N / 2N\text{-}1)\,(1 - \sum_{i=1}^{n} p_i^2)$$

where N is the number of samples in the population. Because of the small sample size for some stands, the unbiased measure was used exclusively.

Expected and observed mean heterozygosities are calculated by estimating the appropriate heterozygosity values for each locus, and then averaging over all loci. If a population is under Hardy-Weinberg equilibrium, expected mean heterozygosity and observed mean heterozygosity will be approximately equal. However, differences between H_o and H_e indicate non-random mating. A deficiency of heterozygotes may indicate assortative mating, which is usually due to inbreeding in the population.

In order to estimate genetic differentiation among stands, the eleven sampled stands were divided into four regions. The eastern Oregon (EC) region includes two stands (Ochoco and W.S.I.R.). The Fort Lewis region includes one stand (Fort Lewis). The southwest Oregon (SW) region includes one stand (Southwest), and the Willamette Valley (WV) region includes seven stands (Lan-10, Lan-20, Lan-22, Lin-21, Lin-23, Hort. Farm and Grassy Glade).

Heterogeneity chi-square tests were used to test for differences in allele frequencies among stands within the Willamette Valley and East Cascades regions.

Because the Southwest and Fort Lewis regions were represented by only one stand each, they could not be analyzed in this way. A heterogeneity chi-square test was also used to test for differences among stands in all regions. Genetic distance values, including Nei's unbiased distance (Nei 1978) and Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967), were calculated for all possible pairwise comparisons of stands and pairwise comparisons of regions. Phenetic clustering based on Nei's unbiased genetic distance values was performed using the UPGMA (unweighted pair-group method with arithmetic averaging) procedure (Sneath and Sokal 1973).

Partitioning of variability among and within stands within the Willamette Valley and all stands, regardless of region, was examined using gene diversity statistics (Nei 1987). Total gene diversity, H_T , a measure of total variation in the entire sample of stands, is given as:

$$H_T = 1 - \sum_{i=1}^{n} p_i^2$$

where there are n alleles and p_i is the mean frequency of allele i across all stands.

Total gene diversity is partitioned as:

$$H_T = H_S + D_{ST}$$

where H_S and D_{ST} are average genetic diversities within and among populations, respectively. The proportion of total gene diversity due to genetic differences among populations is:

$$G_{ST} = D_{ST} / H_T$$

(Nei 1987).

All statistical tests in this study were conducted at the p = 0.05 level of significance.

Results

Stands of ponderosa pine in the Willamette Valley and in other areas of Oregon and Washington exhibit generally high levels of genetic diversity. Of the 20 loci assayed, 19 were polymorphic in at least one of the eleven sampled stands. Many loci were highly polymorphic, with up to five alleles for some loci observed in both progeny samples (Appendix A) and parent trees (Appendix B) and up to four alleles for some loci in pollen (Appendix C). Progeny samples had slightly more allelic diversity than either parent trees or pollen. Progeny had from 45% to 80% polymorphic loci in individual stands and from 2.0 to 2.8 alleles per locus (Table 2.3). Parent trees had from 55% to 90% polymorphic loci in individual stands and between 1.8 and 2.3 alleles per locus (Table 2.4). Pollen had from 45% to 75% polymorphic loci and between 1.8 and 2.7 alleles per locus (Table 2.5).

Observed heterozygosity was lower in progeny samples, averaging 0.186 in progeny across all stands (range from 0.124 to 0.236) compared to an average of 0.219 in parent trees (range from 0.182 to 0.334). Expected heterozygosity estimates for stands based on allele frequencies were lowest for pollen data, averaging 0.216 (range from 0.131 to 0.260) compared to 0.219 in progeny samples (range from 0.153 to 0.261) and 0.228 in parent trees (range from 0.189 to 0.287).

Table 2.3. Population genetic statistics for all progeny. Calculations were made using BIOSYS-2, a population genetics software package. Numbers in parentheses are standard errors.

Stand	Mean sample size per locus	Mean no. alleles per locus	Percent polymorphic loci [1]	Mean heterozygosity: Direct-count	Mean heterozygosity: Hardy-Weinberg expected [2]	Mean heterozygosity: Difference (expected-observed)
Hort. Farm	101.0 (0.0)	2.7 (0.2)	70.0	0.215 (0.039)	0.245 (0.039)	0.030
Lan-10	102.1 (3.6)	2.3 (0.2)	75.0	0.192 (0.032)	0.261 (0.038)	0.069
Lan-20	110.1 (2.2)	2.6 (0.2)	75.0	0.175 (0.030)	0.240 (0.038)	0.065
Lan-22	75.3 (6.8)	2.0 (0.2)	45.0	0.149 (0.039)	0.153 (0.036)	0.004
Lin-21	84.4 (5.1)	2.3 (0.2)	75.0	0.204 (0.041)	0.223 (0.039)	0.019
Lin-23	126.5 (1.6)	2.8 (0.2)	80.0	0.190 (0.029)	0.246 (0.041)	0.056
Grassy Glade	47.7 (0.3)	2.4 (0.2)	80.0	0.236 (0.028)	0.237 (0.029)	0.001
Mean	**92.4 (25.8)**	**2.4 (0.3)**	**71.4 (12.1)**	**0.194 (0.028)**	**0.229 (0.036)**	**0.035**
Ochoco	124.7 (3.4)	2.5 (0.2)	65.0	0.165 (0.033)	0.220 (0.041)	0.055
W.S.I.R.	105.7 (7.9)	2.7 (0.2)	60.0	0.124 (0.028)	0.168 (0.035)	0.044
Ft. Lewis	130.0 (0.0)	2.3 (0.2)	70.0	0.191 (0.035)	0.199 (0.036)	0.008
Southwest	97.0 (1.6)	2.2 (0.1)	75.0	0.209 (0.034)	0.226 (0.035)	0.017
Mean	**114.4 (15.6)**	**2.4 (0.2)**	**67.5 (6.5)**	**0.172 (0.037)**	**0.203 (0.026)**	**0.031**

[1] A locus is considered polymorphic if the most common allele frequency does not exceed 0.95.

[2] This estimate is unbiased for sample size.

Table 2.4. Population genetic statistics for all parent trees. Calculations were made using BIOSYS-2, a population genetics software package. Numbers in parentheses are standard errors.

				Mean heterozygosity		
Stand	**Mean sample size per locus**	**Mean no. alleles per locus**	**Percent polymorphic loci [1]**	**Direct-count**	**Hardy-Weinberg expected [2]**	**Difference (expected-observed)**
Hort. Farm	10.3 (0.2)	2.3 (0.1)	90.0	0.334 (0.053)	0.283 (0.039)	-0.051
Lan-10	10.9 (0.4)	2.2 (0.2)	75.0	0.191 (0.035)	0.287 (0.046)	0.096
Lan-20	18.9 (0.4)	2.3 (0.2)	80.0	0.194 (0.033)	0.249 (0.043)	0.055
Lan-22	7.6 (0.7)	1.8 (0.2)	55.0	0.197 (0.050)	0.189 (0.047)	-0.008
Lin-21	8.6 (0.5)	1.8 (0.1)	70.0	0.228 (0.049)	0.222 (0.042)	-0.006
Lin-23	21.0 (0.3)	2.3 (0.2)	55.0	0.223 (0.049)	0.230 (0.048)	0.007
Mean	**12.9 (5.6)**	**2.1 (0.2)**	**70.8 (13.9)**	**0.228 (0.054)**	**0.243 (0.038)**	**0.015**
Ochoco	20.3 (0.7)	2.3 (0.1)	55.0	0.182 (0.041)	0.222 (0.042)	0.040
W.S.I.R.	18.0 (1.3)	2.3 (0.2)	65.0	0.188 (0.040)	0.193 (0.036)	0.005
Ft. Lewis	13.0 (0.0)	2.0 (0.1)	65.0	0.227 (0.045)	0.198 (0.037)	-0.029
Southwest	9.7 (0.2)	2.0 (0.1)	85.0	0.228 (0.049)	0.206 (0.038)	-0.022
Mean	**15.3 (4.8)**	**2.2 (0.2)**	**67.5 (12.6)**	**0.206 (0.025)**	**0.205 (0.013)**	**-0.001**

[1] A locus is considered polymorphic if the most common allele frequency does not exceed 0.95.

[2] This estimate is unbiased for sample size.

Table 2.5. Population genetic statistics for pollen component of samples. Calculations were made using BIOSYS-2, a population genetics software package. Numbers in parentheses are standard errors.

Stand	Mean sample size per locus	Mean no. alleles per locus	Percent polymorphic loci [1]	Hardy-Weinberg expected heterozygosity [2]
Hort. Farm	101.0 (0.0)	2.5 (0.2)	70.0	0.242 (0.041)
Lan-10	103.1 (3.6)	2.3 (0.2)	65.0	0.240 (0.040)
Lan-20	111.5 (2.1)	2.5 (0.2)	70.0	0.242 (0.046)
Lan-22	75.6 (7.1)	1.8 (0.2)	45.0	0.131 (0.036)
Lin-21	84.4 (5.1)	2.2 (0.2)	70.0	0.220 (0.040)
Lin-23	128.1 (1.6)	2.7 (0.2)	75.0	0.260 (0.041)
Grassy Glade	48.0 (0.0)	2.1 (0.2)	75.0	0.219 (0.036)
Mean	**93.1 (26.3)**	**2.3 (0.3)**	**67.1 (10.4)**	**0.222 (0.043)**
Ochoco	126.5 (3.2)	2.2 (0.2)	60.0	0.230 (0.046)
W.S.I.R.	110.2 (8.1)	2.3 (0.2)	55.0	0.163 (0.036)
Ft. Lewis	130.0 (0.0)	2.2 (0.2)	70.0	0.205 (0.039)
Southwest	97.0 (1.6)	2.1 (0.2)	75.0	0.229 (0.040)
Mean	**115.9 (15.3)**	**2.2 (0.1)**	**65 (9.1)**	**0.207 (0.031)**

[1] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[2] This estimate is unbiased for sample size.

Willamette Valley stands had slightly more allelic diversity than stands outside the Willamette Valley for all life history stages sampled (parents, progeny and pollen). The mean number of alleles per locus, percent polymorphic loci, and heterozygosity values were generally lower in stands outside the Willamette Valley (Tables 2.3, 2.4, 2.5). However, the differences observed are small.

Private alleles (alleles that occur in only one stand) were found in all stands but two: Lan-10 and Lan-22. Semi-private alleles (defined as alleles that occur in three or fewer stands) were found in every stand. Table 2.6 shows the number of private and

semi-private alleles found in each stand for each of the three different types of data, progeny, parent trees, and pollen.

Table 2.6. Number and distribution of private and semi-private alleles.

Stand	Progeny		Parent Trees		Pollen		Total	
	Semi-private	Private	Semi-private	Private	Semi-private	Private	Semi-private	Private
Ochoco	2	1	3	0	2	1	**7**	2
W.S.I.R.	5	1	2	0	3	1	**10**	2
Ft. Lewis	0	0	2	0	1	1	**3**	1
Southwest	2	1	4	1	1	0	**7**	2
Hort. Farm	4	2	3	1	5	0	**12**	3
Lan-10	1	0	2	0	3	0	**6**	0
Lan-20	3	1	4	1	4	1	**11**	3
Lan-22	3	0	3	0	1	0	**7**	0
Lin-21	1	1	1	0	1	1	**3**	2
Lin-23	4	2	4	3	5	1	**13**	6
Grassy Glade	2	1	N/A	0	0	0	**2**	1
Total	**27**	10	**28**	6	**26**	6	**81**	22

An allele is "semiprivate" if found in three or fewer stands, and "private" if found in only one stand.

Estimates of genetic distances between individual stands were wide ranging. Values for Nei's unbiased genetic distance between stands ranged from 0.007 to 0.049, while values for Cavalli-Sforza and Edwards chord distance ranged from 0.130 to 0.223 (Table 2.7). Nei's unbiased genetic distance between regions ranged from 0.018 to 0.026 (Table 2.8).

There does not appear to be any geographic pattern to genetic variation in the sampled stands. Genetic distances between regions (Table 2.8) are similar to genetic distances between individual stands (Table 2.7). In addition, stands within the same region are not necessarily more closely related to one another than to stands outside the

Table 2.7. Matrix of genetic distance coefficients based on progeny data.

Stand	1	2	3	4	5	6	7	8	9	10	11
1 Ochoco	****	.171	.164	.163	.202	.196	.164	.196	.164	.140	.190
2 W.S.I.R.	.024	****	.155	.191	.197	.178	.163	.162	.153	.159	.150
3 Ft Lewis	.025	.011	****	.134	.171	.197	.150	.161	.170	.151	.156
4 Southwest	.024	.021	.018	****	.169	.194	.149	.187	.189	.147	.161
5 Hort. Farm	.047	.034	.028	.028	****	.213	.171	.214	.199	.182	.182
6 Lan-10	.049	.026	.031	.029	.042	****	.181	.223	.171	.168	.213
7 Lan-20	.032	.017	.014	.009	.022	.026	****	.184	.159	.130	.157
8 Lan-22	.032	.012	.017	.022	.030	.042	.017	****	.196	.194	.190
9 Lin-21	.032	.015	.018	.025	.037	.024	.020	.029	****	.158	.157
10 Lin-23	.023	.015	.016	.011	.036	.023	.010	.220	.022	****	.151
11 Grassy Glade	.030	.007	.011	.013	.026	.027	.011	.013	.012	.014	****

Below diagonal: Nei (1978) unbiased genetic distance.
Above diagonal: Cavalli-Sforza and Edwards (1967) chord distance.

Table 2.8. Matrix of Nei's genetic distances averaged by region based on progeny data (numbers in parentheses are ranges of estimates).

Region	Number of Stands	1	2	3	4
1 Eastern Oregon	2	.024 (.024-.024)			
2 Fort Lewis	1	.018 (0.11-.025)	*		
3 Southwest Oregon	1	.023 (.021-.024)	.018 (.018- .018)	*	
4 Willamette Valley	7	.026 (.007-.049)	.019 (.011- .031)	.020 (.009-.029)	.024 (.010-.042)

* No comparison, only one stand sampled in these regions.

region. For example, the genetic distance value between Warm Springs Indian Reservation (WSIR) and Ochoco (two stands in the East Cascades region) is estimated at 0.024. This is the same as the estimated distance between the Ochoco stand and the Southwest stand (two stands in different regions) and the same as the average genetic distance between stands in the Willamette Valley.

A cluster diagram of individual stands using Nei's unbiased genetic distance values for progeny data is shown in Figure 2.3. Cluster diagrams based on parent tree and pollen data using Nei's unbiased genetic distance values are shown in Figures 2.4 and 2.5, respectively. These phenograms were constructed using the unweighted pair group method with arithmetic means (UPGMA) and show the simplest possible evolutionary relationship between the stands, assuming all groups evolve at the same rate.

Contingency Chi-square analyses were used to test for differences among stands in allele frequencies. Highly significant differences ($P<0.0001$) in allele frequency were found among stands for all polymorphic loci in Willamette Valley progeny samples (Table 2.9). Allele frequencies in six of 19 loci were not significantly different ($P>0.05$) among stands in the progeny sample from the East Cascades region (Table 2.10).

The progeny sample for the Willamette Valley region shows a relatively large amount of total genic diversity ($H_T = 0.258$), but most of this diversity is due to within stand variation ($H_S = 0.241$) and relatively little is due to differences among stands ($D_{ST} = 0.017$). The proportion of total genic diversity due to among stand variation (G_{ST}) in the Willamette Valley is 0.063 (Table 2.11). Genic diversity measures are similar for the entire progeny sample taken as one population (Table 2.12). Total genic diversity is slightly smaller than in the Willamette Valley region only ($H_T = 0.249$), and within-stand variation is similarly smaller ($H_S = 0.231$), so among-stand variation in the entire sample is the same as for the Willamette Valley region ($D_{ST} = 0.017$). The proportion of total

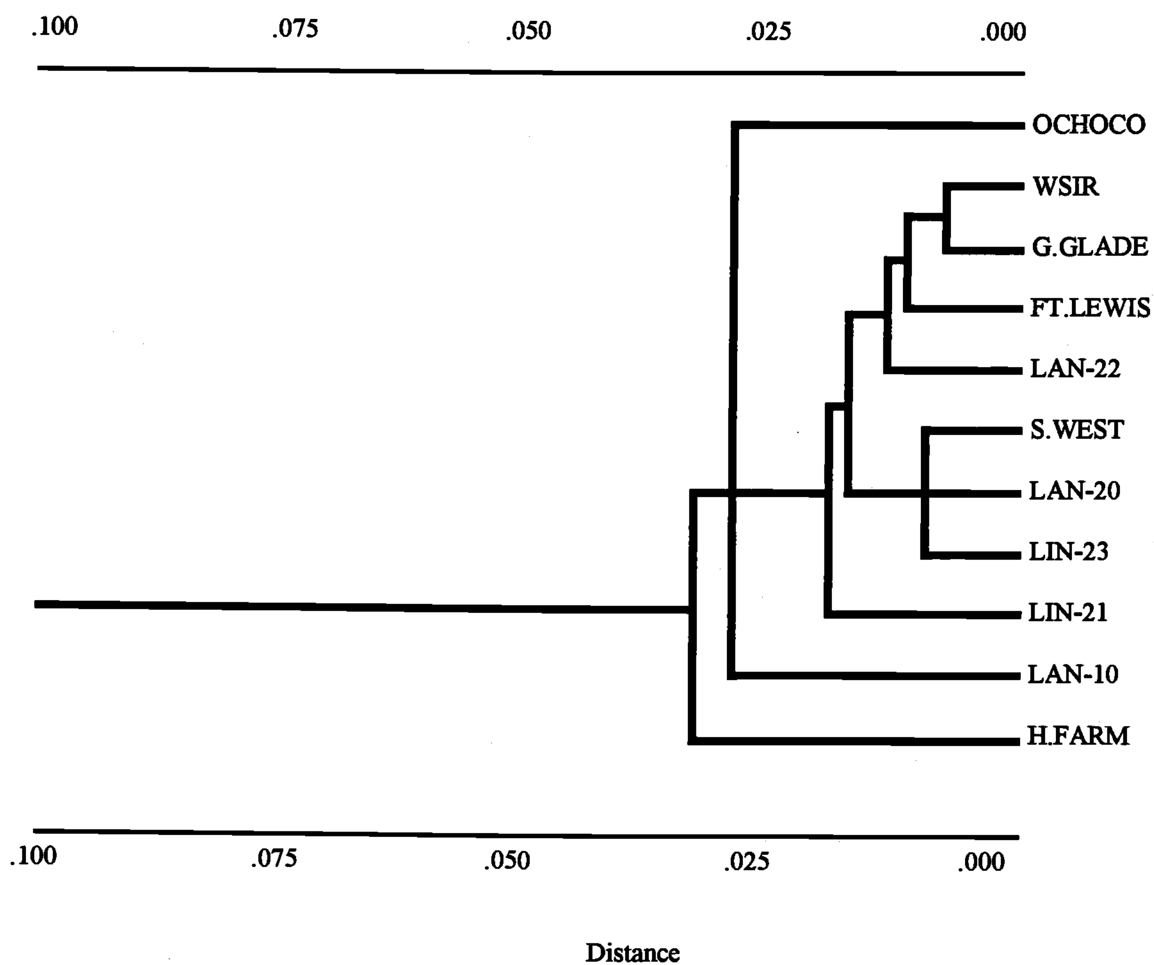


Figure 2.3. Population clustering based on Nei's unbiased genetic distance using the UPGMA method (progeny data)

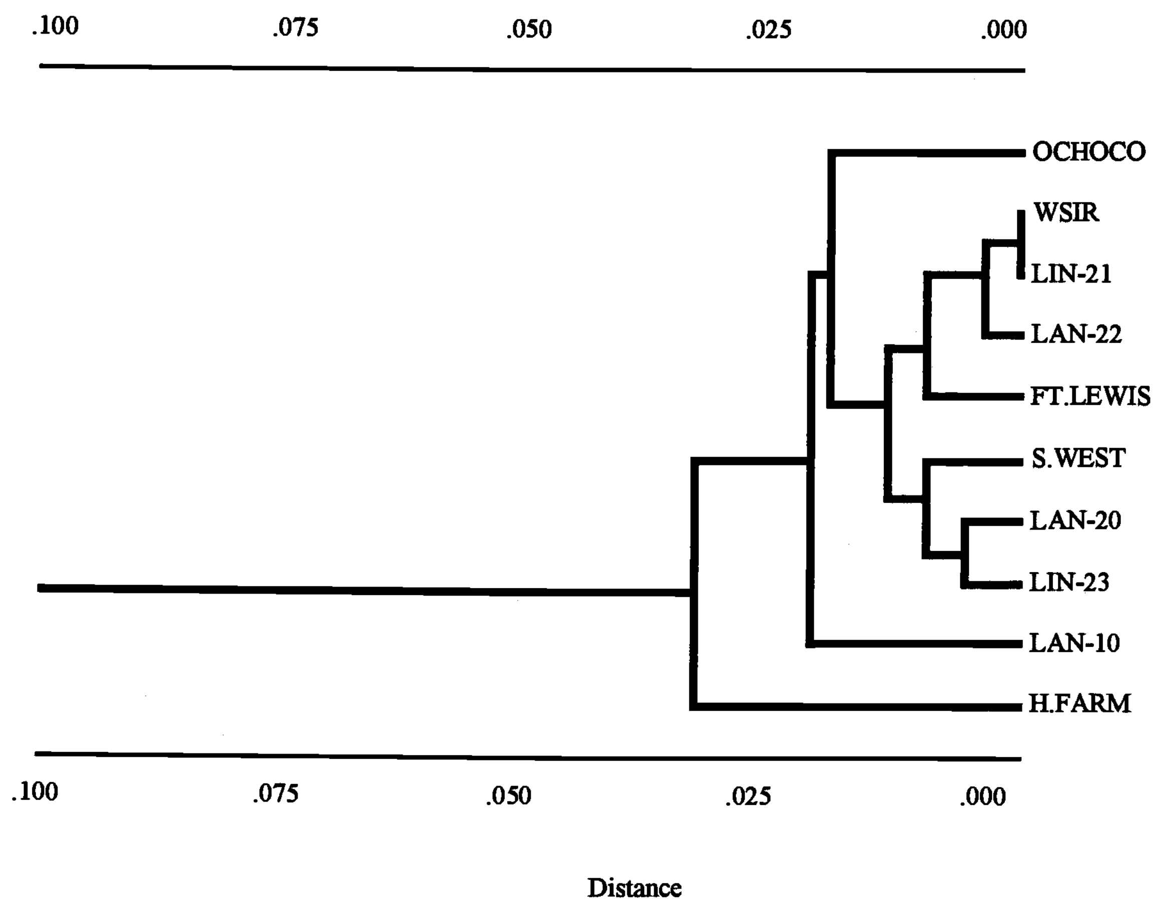


Figure 2.4. Population clustering based on Nei's unbiased genetic distance using the UPGMA method (parent tree data)

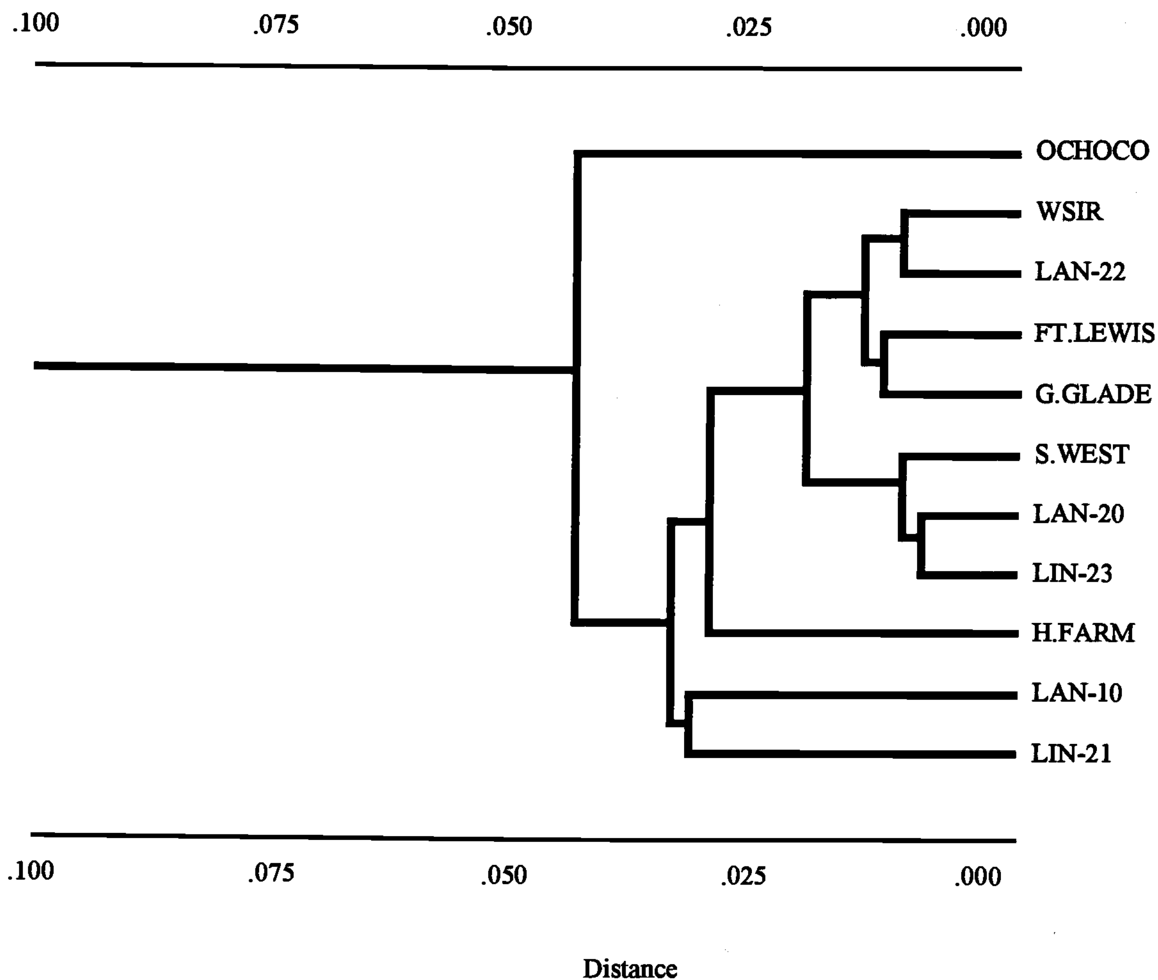


Figure 2.5. Population clustering based on Nei's unbiased genetic distance using the UPGMA method (pollen data)

Table 2.9. Contingency chi-square analysis testing variation in allele frequency in progeny data at all polymorphic loci among stands within the Willamette Valley region.

Locus	Number of Alleles	Chi-square	D.F.	P
PEP-1	3	88.908	12	**<0.0001**
PEP-2	3	155.506	12	**<0.0001**
PEP-4	2	159.979	6	**<0.0001**
LAP-2	3	82.558	12	**<0.0001**
LAP-3	3	202.353	12	**<0.0001**
PGM-1	4	230.52	18	**<0.0001**
GOT-1	5	660.388	24	**<0.0001**
GOT-2	5	177.996	24	**<0.0001**
GOT-3	5	85.901	24	**<0.0001**
ACP-1	4	92.036	18	**<0.0001**
GDH-1	3	355.044	12	**<0.0001**
IDH-1	3	111.645	12	**<0.0001**
SDH-1	4	139.044	18	**<0.0001**
SDH-2	2	102.148	6	**<0.0001**
PGI-1	4	157.745	18	**<0.0001**
MDH-1	3	76.321	12	**<0.0001**
MDH-3	4	170.772	18	**<0.0001**
ADH-1	3	103.158	12	**<0.0001**
FUM-1	4	94.894	18	**<0.0001**
Totals		**3246.916**	**288**	**<0.0001**

Table 2.10. Contingency chi-square analysis testing variation in allele frequency in progeny data at all polymorphic loci among stands within the East Cascades region.

Locus	**Number of Alleles**	**Chi-square**	**D.F.**	**P**
PEP-1	2	6.554	1	**0.0105**
PEP-2	2	7.268	1	**0.0070**
PEP-4	2	146.446	1	**<0.0001**
LAP-2	4	4.005	3	0.2609
LAP-3	3	24.893	2	**<0.0001**
PGM-1	4	44.364	3	**<0.0001**
GOT-1	4	9.642	3	**0.0219**
GOT-2	3	4.844	2	0.0887
GOT-3	4	15.977	3	**0.0012**
ACP-1	4	11.134	3	**0.0110**
GDH-1	3	10.693	2	**0.0048**
IDH-1	2	1.451	1	0.2284
SDH-1	3	2.552	2	0.2792
SDH-2	2	0.052	1	0.8190
PGI-1	4	54.253	3	**<0.0001**
MDH-1	2	2.736	1	0.0981
MDH-3	4	9.543	3	**0.0229**
ADH-1	3	25.921	2	**<0.0001**
FUM-1	3	55.065	2	**<0.0001**
Totals		**3246.916**	**288**	**<0.0001**

Table 2.11. Measures of genic diversity for progeny samples among and within seven stands of ponderosa pine within the Willamette Valley region.

Locus	H_S	H_T	D_{ST}	G_{ST}
PEP-1	0.108	0.113	0.005	0.045
PEP-2	0.110	0.117	0.007	0.056
PEP-4	0.106	0.121	0.015	0.121
LAP-2	0.128	0.132	0.004	0.032
LAP-3	0.203	0.220	0.017	0.078
PGM-1	0.346	0.366	0.020	0.054
GOT-1	0.302	0.362	0.060	0.166
GOT-2	0.316	0.325	0.009	0.029
GOT-3	0.171	0.179	0.008	0.042
ACP-1	0.288	0.297	0.009	0.031
GDH-1	0.147	0.168	0.021	0.123
IDH-1	0.292	0.311	0.019	0.062
SDH-1	0.357	0.363	0.006	0.018
SDH-2	0.316	0.353	0.037	0.106
PGI-1	0.303	0.310	0.007	0.022
MDH-1	0.034	0.035	0.001	0.020
MDH-3	0.403	0.457	0.054	0.118
ADH-1	0.423	0.433	0.010	0.023
FUM-1	0.229	0.240	0.011	0.045
Mean [1]	0.241	0.258	0.017	0.063

H_T = Total genic diversity.

H_S = Average genic diversity within stands.

D_{ST} = Average genic diversity among stands.

G_{ST} = Proportion of total genic diversity due to differences among stands.

[1] Mean is unweighted for 19 polymorphic loci.

Table 2.12. Measures of genic diversity for progeny samples among and within eleven stands of ponderosa pine.

Locus	H_S	H_T	D_{ST}	G_{ST}
PEP-1	0.096	0.100	0.004	0.037
PEP-2	0.117	0.124	0.007	0.055
PEP-4	0.157	0.196	0.039	0.200
LAP-2	0.087	0.091	0.004	0.045
LAP-3	0.174	0.188	0.013	0.070
PGM-1	0.328	0.354	0.026	0.074
GOT-1	0.273	0.315	0.042	0.134
GOT-2	0.270	0.281	0.011	0.038
GOT-3	0.132	0.139	0.007	0.053
ACP-1	0.273	0.280	0.007	0.024
GDH-1	0.117	0.132	0.015	0.110
IDH-1	0.336	0.372	0.035	0.095
SDH-1	0.334	0.340	0.006	0.017
SDH-2	0.295	0.323	0.028	0.086
PGI-1	0.281	0.288	0.007	0.025
MDH-1	0.032	0.033	0.000	0.012
MDH-3	0.411	0.449	0.038	0.086
ADH-1	0.425	0.445	0.020	0.044
FUM-1	0.257	0.274	0.017	0.061
Mean [1]	0.231	0.249	0.017	0.067

H_T = Total genic diversity.

H_S = Average genic diversity within stands.

D_{ST} = Average genic diversity among stands.

G_{ST} = Proportion of total genic diversity due to differences among stands.

[1] Mean is unweighted for 19 polymorphic loci.

genic diversity due to among stand variation for the entire sample is slightly higher (G_{ST} = 0.067) than for the Willamette Valley region only (Table 2.12).

Discussion

Levels of genetic variability found in Willamette Valley ponderosa pine (WVPP) indicate that this population is not genetically distinct in terms of allozyme composition from populations in Eastern Oregon, Southwest Oregon or Fort Lewis, Washington. By far the bulk of genetic variation found in all of the analyzed samples is within stands, not among them (G_{ST} = 0.067). While there are some differences in estimates of genetic parameters among WVPP stands compared to the other stands in this analysis, these differences are for the most part minor and suggest that WVPP has not been reproductively isolated from the surrounding area for a long period. Observed differences would are not large enough to support an argument for the long-term isolation of WVPP.

The three types of data used (parent trees, progeny, and pollen) were all used in the calculation of genetic diversity statistics, but this discussion is based mostly on the progeny data. These different types of data were similar in terms of allele frequency (appendices A, B, and C). The parent tree sample provides an accurate estimation of gene diversity measures for the sampled trees, because they are averaged over many loci. Unfortunately, the number of parent trees sampled (mean 15.7, range 10-26) was small in several stands, so there is some loss of precision applying these estimates to the entire

stand. However, a large number of progeny were analyzed for each stand (mean 119.4, range 100-156), and this data includes the contribution of some pollen alleles from other trees in the stand. The progeny sample may not provide a completely random sample of the entire offspring generation in these stands, because it is based on a relatively small number of parent trees. However, the agreement between parent tree and progeny data was high enough to allow the use of progeny data in the calculation of genetic diversity statistics.

The parent tree and pollen gamete data may be biased because of high selfing rates (see Chapter 3). Because WVPP appears to undergo a great deal of selfing, the sample of pollen gametes may be biased by the small number of parent trees, because a large proportion of pollen comes from these parents. I compared the observed pollen gamete allele frequencies with allele frequencies calculated by the mating system analysis program, which is adjusted for the frequency of selfing. In all cases, the deviation between observed and adjusted pollen gamete allele frequencies was less than or equal to 3%. This difference is low enough that the observed parent tree and pollen allele frequencies may be used in place of the adjusted frequencies for the estimation of population genetic parameters.

Average observed heterozygosities within the Willamette Valley for progeny data (range = 0.149-0.236) were slightly higher than the average heterozygosity (H=0.14) for 36 alleles found in a study of the Pacific region of ponderosa pine by Niebling and Conkle (1990). The earlier study used 21 enzyme systems, including 11 of the 12

enzymes used in the current study, so the results should be comparable. All of the values observed in the current study fall within the range reported for other conifer species (0.00-0.34, Ledig 1986). The range of heterozygosities in stands outside the Willamette Valley (0.124-0.209) overlapped most of the range of values for stands within the Willamette Valley. This suggests no significant population differentiation.

It has been suggested that Native American tribes brought ponderosa pine seed across the Cascade Range and established the Willamette Valley population because they used the seed for food. It is possible that this alleged transfer, combined with the planting of non-local sources of ponderosa pine in the Valley by modern residents, could account for the relatively high number of alleles per locus in WVPP. Non-local sources of ponderosa pine have been widely planted in the valley, and pollen contamination by planted trees is the most likely explanation for the high number of semi-private alleles present in the pollen sample. However, most of this is fairly recent activity, and it is unlikely that this activity was common enough before the last century to result in widespread shifts in allele frequency in the modern population of WVPP.

Nei's unbiased genetic distance values for progeny samples were wide ranging (0.007-0.049), but there was no apparent association between genetic distance and geographical distance, indicating little geographic structure to genetic variation. The lowest distance value found, 0.007, was observed between W.S.I.R., an Eastern Oregon stand, and Grassy Glade, a stand between the Willamette Valley and Oakridge, on the western slope of the Cascades. The lowest distance value was therefore observed

between stands occupying different geographical regions, on opposite sides of the Cascade Range. High genetic distance values of above 0.040 were observed not only between W.S.I.R. and Lan-10, two stands in different regions, but also between Lan-10 and Lan-22, two stands that are not only both in the Willamette Valley but in the same county (Lane), approximately 10 km apart. This does not suggest any consistent relationship between genetic differentiation and physical separation.

The lack of geographic pattern to genetic distance was confirmed in the average genetic distance values between regions. The average distance between stands within the Willamette Valley is 0.024. This is higher than the average distance between WVPP stands and the Southwest Oregon stand (0.020) and the average distance between WVPP stands and the Fort Lewis stand (0.019), and only slightly less than the average distance between WVPP stands and Eastern Oregon stands (0.026). It is important to note, however, that all of these genetic distance estimates are small.

The cluster diagrams reconfirm the absence of a correlation between geographic and genetic distance. There is no tendency for WVPP stands to be more related to one another than to stands in other regions. The figures do not suggest any relationship between the degree of genetic relatedness and the degree of physical proximity between any two stands. This is consistent for progeny, parent tree and pollen data alike.

One notable feature of the cluster diagrams is the position of the Hort. Farm stand. Hort. Farm is an outlier in the cluster diagrams constructed from progeny data (Fig. 2.3)

and parent tree data (Fig. 2.4), but not for pollen data (Fig. 2.5). Genetic distance estimates among stands within the Willamette Valley are, on average, highest for Hort. Farm (Table 2.7). Several other characteristics differentiate the Hort. Farm from the other Willamette Valley stands. Hort. Farm has a relatively high number of private and semi-private alleles (Table 2.6). Hort. Farm is an outlier for parent tree allele frequencies, but not for pollen. Heterozygosity estimates also reflect this pattern: average observed heterozygosity for the Hort. Farm stand is above average for progeny data (Table 2.3) and much higher than any other sampled stand for parent tree data (Table 2.4), but consistent with other stands for pollen data (Table 2.5).

These patterns suggest that the trees at Hort. Farm have a different origin from the other stands within the Willamette Valley. There is a strong possibility that this stand was planted from non-local seed, especially considering that it the stand is located on a horticultural farm. It is also possible that the Hort. Farm trees are the progeny of non-local, planted stock and native WVPP. The fact that pollen data from Hort. Farm is consistent with other WVPP stands for most population genetic statistics suggests that the differences described above are very localized, or else they would also be seen in the pollen cloud. It is therefore more likely that differences between Hort. Farm and the other stands are due to a non-local origin for the parent trees, rather than geographic differences within the Willamette Valley. However, because only eleven mother trees were sampled for this stand, the differences seen could also be due to sampling error.

It is apparent from the consistent differences between observed and expected heterozygosities that some factor is affecting genotype frequencies in the Willamette Valley samples. The difference between expected and observed heterozygosity in progeny samples of Willamette Valley stands ranges between 0.001 and 0.065, with an average value of 0.035. This difference is most likely the result of widespread assortative mating, or mating among relatives. One result of inbreeding is that more homozygotes are produced at each locus than would be expected under random mating. Inbreeding in ponderosa pine significantly reduces height growth and survival to ten years of age (Sorenson and Miles 1982). The progeny samples used in this study may therefore contain a much larger proportion of inbred genotypes than the parent trees, which have been subject to years of selection pressure, effectively removing most of the inbred genotypes from their ranks. This is supported by the smaller deficiency in heterozygotes found in parent trees (range –0.051 - 0.096, average 0.015).

It is also possible that inbreeding has increased since the parent tree generation was established, due to fragmentation of populations and reduced effective population sizes in the last century. A detailed analysis and discussion of the mating system of WVPP is presented in Chapter 3 of this thesis.

Sampling error may affect the results in this study. Unfortunately, some of the sample sizes in this study are small and the number of parent trees sampled is not the same in each stand. The scattered distribution of trees and stands in the Willamette Valley, as well as the poor cone crop typically produced by WVPP made a large and

balanced cone collection difficult. The low percentage of filled seeds and low germination rates that many seedlots exhibited further limited sample size. If I were to repeat this study, I would consider collecting foliage or bud samples instead of seed, and assessing more variable markers, such as microsatellite DNA markers. Isozyme analysis of seed is advantageous in that it allows determination of the pollen parent and mating system analysis, but a larger, better-distributed collection could have been obtained with foliage samples.

Although sampling error is likely to have affected the results of this study to some extent, it is extremely unlikely that it could result in the sizable deficit of heterozygotes observed in progeny samples compared to parent trees. The consistency of this deficit suggests that inbreeding affects genotype frequencies of WVPP progeny, and selection against inbreds causes the difference between parent trees and progeny in terms of heterozygosity.

The results of this study indicate that WVPP possesses a high degree of genetic variability and diversity, and that it is not significantly different from ponderosa pine in Eastern Oregon, Southwest Oregon, and the Puget Sound Basin in Washington in terms of allozyme composition. Estimates of allele frequencies, observed and expected heterozygosity, genetic distance and other population genetic parameters suggest that WVPP is not a genetically unique population in terms of allozyme composition.

Woodland owners in the Willamette Valley who wish to plant ponderosa pine would be advised to plant locally obtained seed, as this study did not make any attempt to analyze variation in adaptive traits within the Willamette Valley. Collections should be made from stands known to be native, if possible. Seed origin in planted stands is unknown, and not necessarily from within the Willamette Valley. Given the typically poor cone crop produced by WVPP each year, it is possible that seed used for planting within the Willamette Valley came from another population, perhaps from eastern or southwest Oregon. Anecdotal evidence strongly suggests that local seed is better adapted to the conditions in the Willamette Valley than seed from other geographic areas. I found no evidence of an association between genetic variation and geographic proximity within the Willamette Valley population of ponderosa pine. It appears that trees in the valley can be treated as members of a single population for genetic conservation purposes. Because there is no strong genetic differentiation between WVPP stands, most of the genetic variation present in the population could be captured by collecting seed from a large number of individual trees, with little regard to their distribution. Such a collection would not have to focus on collecting cones from a large number of separate stands across the entire length and breadth of the Willamette Valley.

In order to conserve ponderosa pine effectively in the Willamette Valley, a large number of trees will need to be protected to ensure a sufficiently large effective population size continues to exist and reproduce. It is possible that common-garden studies investigating adaptive differences among stands may reveal geographic differentiation of this species within the Willamette Valley, and until the results of these

studies are known, the possibility that there is some geographic component to patterns of genetic variation cannot be discounted. Conservation strategies for Willamette Valley ponderosa pine should focus on preserving large remaining stands and using local valley seed sources for afforestation purposes, preferably from a recently established seed orchard or from large natural stands. Several stands should be preserved as a form of insurance against catastrophic loss due to fire or agricultural or urban encroachment. The stands should be large to minimize inbreeding, which could reduce fecundity and fitness of future generations of WVPP. By preserving the remaining native stands, conservation of the full gamut of genetic diversity represented by WVPP is assured. By using only Willamette Valley seed sources when planting, the dilution of the WVPP pollen pool with non-local, possibly maladapted stock can be avoided.

CHAPTER 3: THE MATING SYSTEM OF WILLAMETTE VALLEY PONDEROSA PINE

Abstract

Multilocus and single-locus estimates of outcrossing were determined for six stands of ponderosa pine within the Willamette Valley of Oregon. Outcrossing rates were estimated for seven polymorphic loci using MLTR, a mating system analysis software package. Multilocus estimates of the outcrossing rate for these stands averaged 64%, substantially lower than most estimates previously reported for other populations of ponderosa pine, other pine species, and other species in the family Pinaceae. No significant differences were detected among stands for outcrossing rate. High levels of inbreeding did not appear to be related to stand density, stand size, or degree of isolation from other stands of ponderosa pine in the region. There was also no apparent association between level of inbreeding and amount of genetic variation present within a stand. Inbreeding depression has previously been shown to reduce survival and growth in ponderosa pine. The high level of inbreeding in this population could be a result of population fragmentation due to human activities, or to a reduced level of pollen production in this population compared to other populations of ponderosa pine. Genetic conservation strategies for this population should include the protection of the large native stands that remain and the use of local, adapted sources for afforestation.

Introduction

It is important to estimate of the amount of outcrossing and inbreeding that occurs within a stand to understand the causes of its genetic structure. Most conifer species have a high rate of outcrossing, but individual stands may differ markedly in outcrossing rate from species-wide estimates because of varying local conditions (Adams and Birkes 1988). Good information about the mating system of a population is a necessary prerequisite to make a wide variety of management decisions, including genetic conservation strategies, seed collection protocols, and seed transfer guidelines. With an understanding of the mating system, problems related to inbreeding depression can be avoided in breeding, deployment, or conservation populations.

Conifers are especially convenient among plant species for mating system studies using isozyme analysis. Embryo and megagametophyte tissues of seeds can be analyzed separately, allowing direct determination of the isozyme genotype of the pollen that fertilized that seed. The megagametophyte in a conifer seed is haploid and its genotype is the same as the genetic contribution of the maternal parent to the embryo. The embryo is diploid, resulting from fertilization of an egg cell by a pollen grain. By “subtracting” the alleles present in the haploid megagametophyte from the genotype of the diploid embryo, the pollen grain haplotype can be determined. Using this information, one can estimate the outcrossing rate in a stand. For example, if the maternal parent could not have produced the pollen gamete that was successful in fertilization, the offspring must therefore be outcrossed.

Because of the ability to easily determine the pollen haplotype of conifer seeds, many conifer species have been the subject of mating system studies using isozyme data. Most studies have indicated that the outcrossing rate, t, is high, usually above 0.85 (Adams and Birkes 1988). Studies of Douglas-fir (Shaw and Allard 1982; Neale and Adams 1985) have found multilocus outcrossing rates ranging from 0.86 to 1.00. A study of Jeffrey pine populations found multilocus outcrossing rates ranging from 0.80 to 0.95 (Furnier 1984). High estimates of outcrossing have been reported for other species, including lodgepole pine (0.94 to 0.99), loblolly pine (0.94), and balsam fir (0.89) (summarized in Adams and Birkes 1988).

One notable exception to high estimates of outcrossing in conifers is western red cedar (Thuja plicata D. Don), a very widespread species, in which an outcrossing rate of 0.32 was found in one seed-orchard population. This high level of inbreeding is thought to be tolerated because of the relatively low level of genetic variability in the species, possibly the result of a population bottleneck during which recessive deleterious alleles were purged (El-Kassaby *et al.* 1994). Another exception is the rare Mexican endemic Chihuahua spruce (Picea chihuahuana Martínez), in which multilocus outcrossing rates of 0.153 and 0.0 were found in two small stands. This high level of inbreeding is thought to result from genetic drift after fragmentation of the species into small, relictual populations due to climate change (Ledig *et al.* 1997).

Results from a limited number of mating system studies have been reported for populations of ponderosa pine. An average outcrossing rate of 0.96 was estimated in an

intensively studied Rocky Mountain population of ponderosa pine (Mitton *et al.* 1981). A later study of the same population estimated outcrossing rates ranging from 0.81 for low-density stands to 0.96 for stands of average density (Farris and Mitton 1984). The high level of outcrossing found in ponderosa pine agrees with the relatively high estimates of heterozygosity and other measures of genetic diversity that have been made at the species and below-species levels, based on variation in xylem monoterpenes, growth, and allozymes (Conkle and Critchfield 1988).

Objectives

The objective of this study is to estimate the amount of outcrossing and inbreeding in natural stands of ponderosa pine in the Willamette Valley. These stands are relatively small and somewhat isolated compared to the larger and more continuous populations found in Southwest Oregon and on the east side of the Cascade Range. Stands in the Willamette Valley have been fragmented by human activities (agriculture, forestry and urbanization) in the past century. Information about the mating system of Willamette Valley ponderosa pine will be useful in determining a genetic conservation strategy for the population based on the minimization of inbreeding. Additionally, information on how to reduce inbreeding will be helpful in the development of seed collection and transfer guidelines for reforestation purposes.

If Willamette Valley ponderosa pine is in general highly inbred, or if large differences in mating system characteristics are found among different stands within the

valley, it would suggest that seed produced by some stands may show high levels of inbreeding depression. If the mating system of WVPP is characterized by a high degree of selfing, or if levels of selfing vary greatly among stands, special care would be indicated in conserving what stands remain and in choosing seed sources for planting. Specifically, it would be important to avoid promoting survival of inbreds in plantations and collecting seed from stands with high levels of inbreeding, because of growth and survival problems related to inbreeding depression (Sorenson and Miles 1982). Conversely, high levels of outcrossing across stands within the valley may indicate that the population is relatively homogeneous and that seedlots collected from stands will contain little inbred seed. Outcrossing rates also have implications for seed orchard management, where outcrossed seed is the desired product.

Materials and Methods

Cones were collected in August and September, 1994, from several stands in the Willamette Valley. Stands were deemed appropriate for analysis if at least two mature cones could be collected from each of at least ten trees. Seed from collected cones was extracted by hand and stored below 0°C. Seed was kept separate by mother tree. Samples of seed were X-rayed and the proportion of filled seed was estimated. Due to the relatively poor cone crop in 1994 (typical for this species in this area) and the low proportion of filled seed per cone (52%), only six stands produced enough seed to be used in the analysis. The stands used were denoted as follows: Hort Farm, Lan-10, Lan-20, Lan-22, Lin-21 and Lin-23 (see Chapter 2). Seed were germinated and isozyme

genotypes were determined for 20 loci using starch-gel electrophoresis. Between six and ten seeds per tree were analyzed so that isozyme genotypes were determined for no less than 100 seeds per stand. Detailed procedures for cone collection, seed processing and isozyme methods are discussed in Chapter 2 of this thesis.

Seven of 20 loci, representing four enzyme systems, were used in the mating system analysis: three loci from the peptidase system (PEP-1, -2 and -4); two from the leucine aminopeptidase system (LAP-2 and -3); one from the isocitrate dehydrogenase system (IDH-1); and one from the malic dehydrogenase system (MDH-1). Loci were selected for use in the mating system analysis based on polymorphism and consistency of results in the isozyme analysis. Staining was inconsistent in some cases for most loci so that genotypes could not be determined for every seed at every locus. The seven loci used in the mating system analysis had the most consistent results in staining so that missing data were minimized.

Single and multilocus estimates of outcrossing (t_s and t_m, respectively) were calculated for each of the six stands used in this study. These estimates were made using the DOS-based computer program MLTR (Ritland 1990). The estimation methods used in this program are based on the mixed mating model. This model assumes that all viable progeny are the result of either a random outcross (with probability t) or self-fertilization (with probability s). The probability of observing an outcrossed progeny is assumed to be independent of the genotype of the maternal parent, and allele frequencies in the outcross pollen pool are assumed to be homogeneous among maternal parents.

Additionally, it is assumed that genotypes of progeny are selectively neutral between fertilization and isozyme analysis. This assumption may be violated to some extent by polyembryony, common in conifers, where several egg cells are fertilized by male gametes but usually only one embryo develops. Selection against inbreeding may operate at this point. For the purposes of this study, however, selection is presumed to be negligible so that the mixed-mating model can be used.

MLTR uses a maximum-likelihood procedure for estimating multilocus outcrossing rates. Using this model, the probability that progeny are the result of self-fertilization can be estimated at each locus. In cases of crosses between closely related individuals (siblings, cousins, or parents and offspring) estimates of selfing will be upwardly biased. Because multilocus estimates of outcrossing (t_m) are less influenced by this bias than single-locus estimates (t_s), the difference between single-locus (t_s) and multilocus outcrossing rates (t_m) can separate uniparental inbreeding (selfing) from biparental inbreeding (mating among relatives). If (t_m - t_s) is significantly greater than zero, biparental inbreeding is likely.

Isozyme genotypes for the seven loci used were entered into progeny arrays, kept separate by mother tree and stand identity. The program was run for the entire data set, with stands marked as separate groups in the progeny arrays. Individual stand analyses were also performed to see if estimates would differ between results for each stand analyzed separately and the entire population analyzed as one group. Both population estimates and stand estimates were obtained for single locus and multilocus outcrossing

rates. Individual family estimates are not reported due to the small sample size per population and the resulting large standard errors. When running the program for each set of population estimates, no constraints were made on values for t (outcrossing rate) or F (average single-locus inbreeding coefficients of maternal parents) (Ritland 1990). The program estimated outcross pollen and ovule allele frequencies. For each estimated parameter (t and F) the Newton-Raphson method of iteration was used (Ritland 1990). A starting value for F was set at 0.1, while t was given a starting value of 0.9. The number of bootstrap estimates was set at 100 for each run of the program (Ritland 1990).

Two types of analyses were performed. In the grouped analysis, all data were used, separated into groups by stand. This analysis assumes that all stands have the same outcrossing rate, but allele frequencies are different in each stand. The "individual stand" analysis treated each stand separately. This analysis assumes that outcrossing rate and allele frequencies differ among stands. The two analyses were compared using log-likelihood ratios (Zar 1984). The log-likelihood test compared the grouped analysis with the aggregate of the individual stand analyses to see if the models differed significantly. The null hypothesis in this test was that there is no difference in outcrossing rates among stands.

In order to relate mating system estimates to stand characteristics, each of the six stands was qualitatively characterized as high or low in size, density, and isolation. Stand size was rated "high" or "low" based on an estimate of the number of trees in the stand (above or below 50 trees). Stand density was rated "high" for a dense forest with little

light reaching the forest floor, or "low" for an open, savanna-type forest. Stand isolation was rated "high" or "low" based on a rough estimate of the distance to the nearest ponderosa pine stand (above or below five miles). Quantitative estimates of these parameters would have been preferable, but unfortunately the stands were not mapped or surveyed. Because these estimates were subjectively assessed, they are only used for broad generalizations.

Results

For the "grouped" analysis, multilocus estimates of outcrossing (t_m) ranged from 0.388 to 0.879, with an average of 0.642 (S.E.= 0.149) (Table 3.1). Mean single locus estimates of outcrossing (t_s) ranged from 0.351 to 0.842, with an average of 0.612 (S.E.=0.137). Estimates of t_m were slightly larger than estimates of mean t_s for the same stand in all stands except Lan-20, in which the difference was –0.012. The value of the difference (t_m - t_s) was generally small, with an average of 0.030 (S.E.= 0.040) and range of -0.012 to 0.063. The estimate of F (average single locus inbreeding coefficient) for all the grouped analysis was 0.092 (S.E.=0.052).

The individual stand analysis yielded similar results; thus, only summary statistics are presented for this analysis (Table 3.1). Estimates of t_m ranged from 0.389 to 0.941, with an average of 0.696 (S.E.= 0.205). Mean t_s estimates ranged from 0.353 to 0.917, with an average of 0.671 (S.E.= 0.201). Estimates of t_m were larger than mean estimates

Table 3.1. Multilocus and single locus outcrossing rates for grouped and individual stand analyses (standard errors in parentheses).

Grouped analysis (log-likelihood = -1322.989):

Stand	Loci	Progeny	Families	tm	SE	ts	SE	Diff tm-ts	SE
Hort Farm	7	101	11	0.667	(0.125)	0.604	(0.096)	0.063	(0.052)
Lan-10	7	113	12	0.388	(0.115)	0.351	(0.106)	0.034	(0.025)
Lan-20	7	120	20	0.365	(0.170)	0.377	(0.151)	-0.012	(0.037)
Lan-22	7	100	10	0.760	(0.192)	0.733	(0.192)	0.027	(0.032)
Lin-21	7	110	11	0.879	(0.177)	0.842	(0.176)	0.038	(0.048)
Lin-23	7	132	22	0.794	(0.115)	0.765	(0.101)	0.029	(0.045)
Mean	**7**	**112.7**	**14.3**	**0.642**	**(0.149)**	**0.612**	**(0.137)**	**0.030**	**(0.040)**

Mean results for individual stand analyses (sum of log-likelihoods = -1255.752):

Stands	Loci	Progeny	Families	tm	SE	ts	SE	Diff tm-ts	SE
7	**7**	**112.7**	**14.3**	**0.696**	**(0.205)**	**0.671**	**(0.201)**	**0.025**	**(0.045)**

of t_s for four out of six stands, the exceptions being Lan-20 and Lan-22, in which the difference was very small (–0.009 and –0.002, respectively). The average value of the difference (t_m - t_s) was 0.025 (S.E.= 0.045) and ranged from –0.009 to 0.056. F estimates were highly variable (range from -0.270 to 0.173) in individual stand analyses.

Stand rankings for mean estimates of t_s were stable for the grouped analysis and the individual stand analyses. Estimates of t_m for the grouped analyses had the same ranks as estimates of mean t_s with the exception of Lan-10 and Lan-20, which switched ranks in the individual stand analysis.

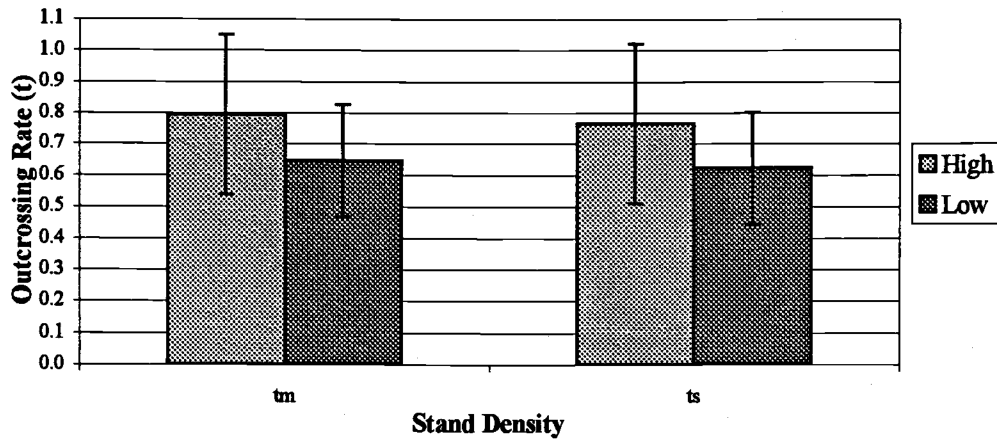
The log-likelihood ratio test showed no significant difference ($p>0.05$, based on Chi-square distribution with five degrees of freedom) between the grouped analysis and the individual stand analyses. This shows that the assumption that one outcrossing rate can be applied to all stands. Because there is no significant difference among stands in outcrossing rate, the estimate obtained from the grouped analysis can be used.

The effect of qualitative stand characteristics, including relative size, density, and isolation, and their effect on outcrossing rates (from the grouped analysis) is shown in Figure 3.1. Table 3.2 shows how individual stands were characterized.

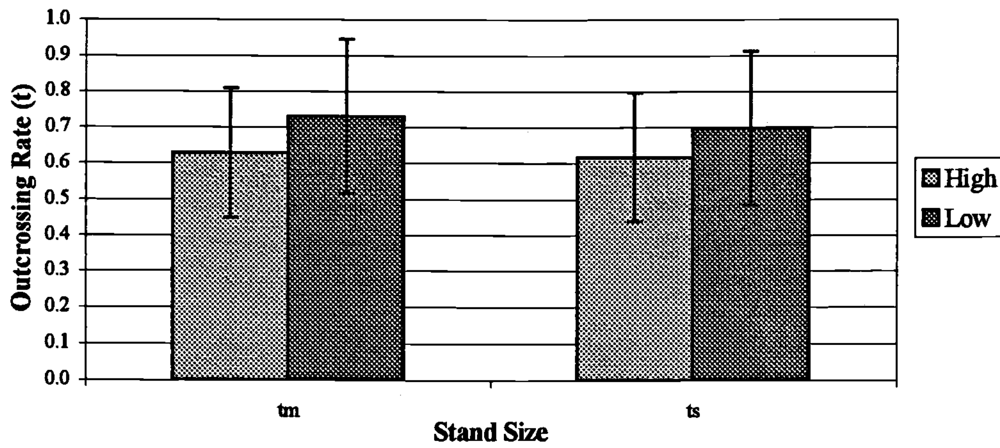
Table 3.2. Stand characteristics for the six stands used in the mating system analysis.

	HF	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**
Isolation:	High	Low	Low	Low	High	High
Size:	Low	Low	High	Low	Low	High
Density:	High	Low	Low	High	Low	Low

a



b



c

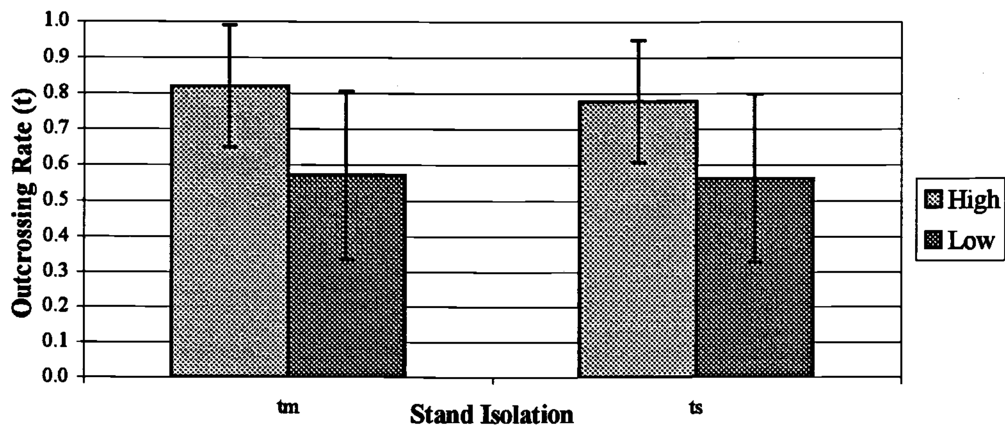


Figure 3.1. Multilocus (t_m) and single-locus (t_s) outcrossing rates as a function of (a) stand density; (b) stand size; and (c) stand isolation.

Discussion

Both single- and multi-locus estimates of outcrossing in WVPP are generally lower than previously reported for stands of ponderosa pine (Farris and Mitton 1984, Mitton *et al.* 1981). In the grouped analysis, mean estimates of t_m (0.642) and t_s (0.612) are considerably lower than the average values for conifers. In pines these values are usually close to 0.90 (Rudin at al. 1974, Furnier 1984, Roberds and Conkle 1984, El-Kassaby *et al.* 1987). Exceptions to this general pattern of high outcrossing rates have been reported for some conifers (El-Kassaby *et al.* 1994, Ledig *et al.* 1997).

Single-locus outcrossing rates were calculated separately for each locus to see if one locus was responsible for the low overall multilocus outcrossing rate. This could be the case if the scoring process was biased in favor of homozygotes for one locus. For example, if a locus stains poorly and the resolution of bands is poor, scoring an embryo as homozygous can be more likely because the band indicating heterozygosity is difficult to see. Individual locus outcrossing rates varied widely (range 0.309–1.204, mean 0.666, median 0.591), but it did not appear that one locus had especially high selfing rates compared to the others. All but one of the loci were well below the expected value in conifers of $t_s = 0.90$, indicating that bias in scoring was not responsible for the observed low outcrossing rates.

The low levels of outcrossing observed in this study suggest that stands of WVPP may be highly inbred. It would take many generations for a change in the mating system,

from primarily outcrossing to a high proportion of selfing, to result in observable differentiation of local populations as a result of genetic drift. The lack of large differences among WVPP stands according to the genetic diversity analysis in Chapter 2 of this thesis suggests that populations are not highly differentiated, and the low F value (0.092) calculated in the grouped analysis indicates that the parent trees are probably not highly inbred. However, a change in the mating system could have profound effects on the fitness, vigor and level of inbreeding depression in Willamette Valley stands in only one generation. Seed produced by even one generation of inbreeding can be greatly reduced in fitness, compared to outcrossed seed. Inbreeding depression has been shown to cause significant reductions in height, height growth, and survival in ponderosa pine (Sorenson and Miles 1982). The high degree of inbreeding seen in this study suggests that WVPP is unique and that a need exists to conserve what stands of WVPP remain. Care must be taken in choosing seed sources for small woodland owners interested in planting ponderosa pine in the Willamette Valley, so that the deployment population is not highly inbred, leading to problems related to inbreeding depression.

The effect of stand density, size and isolation on outcrossing rates was investigated to see if the level of inbreeding could be predicted by superficial stand characteristics. If there is a reliable correlation between stand characteristics and outcrossing rates, recommendations could be made to avoid the collection and planting of more inbred and likely less vigorous seedlots. There is no strong relationship apparent between t_m or mean t_s and any of the qualitatively estimated stand characteristics (Figure 3.1). While the absolute values of the estimates appear to be different for the differing

stand characteristics, no statistical differences were detected among stands by the log-likelihood ratio test.

Stand density was not very effective in explaining differences in outcrossing rates. In the current study, both the second lowest and highest estimates of t_m were found in Lan-10 and Lin-21, two of the four stands characterized as "low density", However, there is a general trend indicated by Figure 3.1(a) showing outcrossing rate decreasing as density decreases. The observed difference is small, but it is consistent for both single-locus and multilocus outcrossing.

Stand size did not have any observable effect on outcrossing rate. In the absence of other factors, a stand of many trees might have a higher outcrossing rate than a stand with few trees, because self-pollen would make up a greater proportion of available pollen in the small stand. In the current study the lowest estimate of t_m was for Lan-20, which had a relatively large population size. The highest estimate of t_m was for Lin-21, which had a relatively small population size. The other stands do not appear to follow any trend, neither increasing nor decreasing outcrossing rates with increasing population sizes. The overall trend indicated in Figure 3.1(b) is that outcrossing rates increase with decreasing stand size, but this is not a strong relationship.

The third characteristic, stand isolation, was also ineffective at explaining differences in outcrossing rates. The two stands with the lowest observed values of mean t_s and t_m (Lan-20 and Lan-10) are both characterized as stands with low isolation, which

is counterintuitive. The expectation is that a tree in an isolated stand is surrounded by a pollen cloud containing a larger proportion of self-pollen, because there are fewer trees rpresent to contribute pollen. This could result in a lower level of outcrossing. In the current study, the two stands with the highest observed values of mean t_s and t_m were characterized as relatively isolated stands, which does not confirm this expectation. The general trend in Figure 3.1(c) is that outcrossing rates increase with increasing stand isolation, but again, this is not a strong relationship.

This analysis might be more powerful if the sampled stands had been rigorously mapped and surveyed in order to provide precise quantitative estimates of size, density and isolation. However, even precise estimates of these characteristics could have indicated only general trends, at best, because there were no significant differences among stands in outcrossing rate.

There is no apparent relationship between outcrossing rate and level of genetic variation for the WVPP stands analyzed. Lan-10 and Lan-20, the stands with the lowest level of outcrossing, are not less genetically diverse than the other stands in the study (Table 2.7). Lan-22 has the lowest level of genetic diversity of any Willamette Valley stand, as estimated by mean number of alleles per locus, percent polymorphic loci, and mean observed and expected heterozygosity. In the mating system analysis, however, Lan-22 had the third highest single- and multi-locus outcrossing rates.

It is possible that the low level of outcrossing seen in WVPP is due to stand isolation, although that is not seen in figure 3.1(c). These stands may have been isolated from one another for some time, and it is possible that the parent trees sampled in this study are the result of related matings among trees that were then, as now, members of relatively small and isolated stands. However, the lack of large differences in allele frequencies among stands described in Chapter 2 of this thesis do not support this theory. This suggests that there may have been a recent change in mating system not yet reflected in genetic diversity measures.

It is possible that the high degree of inbreeding in WVPP is a result of human impacts. Human activities have certainly reduced the number and size of stands of native ponderosa pine in the Willamette Valley over the last 150 years. Direct effects include the removal of individual trees and entire stands, either to harvest lumber or clear land for development. Indirect effects include fire prevention, which has increased the relative competitive ability of Douglas-fir. This is evident when observing how dense Douglas-fir stands now occupy fallow land on the fringes of the valley that was formerly an open savanna (Towle 1982). The result of these human impacts has been to fragment the population of WVPP, resulting in a patchwork of small, isolated stands that was once far more widespread and continuous across the valley floor. This reduced population produces a smaller pollen cloud than stands of ponderosa pine elsewhere, which may contribute to the low estimates of outcrossing for WVPP.

Another factor that may contribute to the low outcrossing rate observed in this study is reduced pollen production. The seed used to estimate outcrossing rates is all from one collection year, 1994. It may have been a poor year for pollen production, and the parent trees used in this study may have produced more pollen than other trees. This could result in a higher proportion of self-pollination in 1994 than in other years. It is also possible that WVPP always produces less pollen than other populations of ponderosa pine, and that self-pollination is usually high. The bulk of the species range is in arid, mountainous environments. The wet, maritime climate of the Willamette Valley is atypical for ponderosa pine and may not be conducive to high pollen production in most years. Pollen management in seed orchards may be the best method of ensuring quality seed production in WVPP.

The results of this study indicate that the level of inbreeding in Willamette Valley ponderosa pine is generally higher than that reported for other populations of the same species, and that outcrossing rate varies substantially among stands. This suggests that stands of WVPP are highly fragmented, which would indicate a need for gene conservation efforts, as well as the use of local seed sources in afforestation efforts. It also suggests that inbreeding is common in WVPP, which may cause reductions in population fitness due to inbreeding depression. Specifically, these results imply a need to: 1) conserve some of the larger stands; 2) establish seed orchards with unrelated individuals and use pollen management techniques to promote outcrossing; and 3) use nursery culling of wild seedlots to eliminate individuals exhibiting poor growth that may be due to inbreeding depression.

It is clear that the population of Willamette Valley ponderosa pine is shrinking as human population pressures in the valley increase. Hopefully all land management decisions that affect WVPP will make use of the best genetic and ecological information available to ensure the continued survival and growth of this population.

CHAPTER 4: CONCLUSIONS

Results

This study investigated the population structure genetic structure of ponderosa pine (Pinus ponderosa Dougl.) in the Willamette Valley of Oregon. In order to describe the amount and distribution of genetic variation present in Willamette Valley ponderosa pine (WVPP), cones were collected from native stands of ponderosa pine in the Willamette Valley, eastern Oregon, southwest Oregon, and the Puget Sound Basin of Washington. The total sample size was 1242 seeds from 157 parent trees found in 11 stands, including seven stands from the Willamette Valley.

All seeds were germinated and subjected to isozyme analysis. Results were obtained for 12 enzyme systems comprising 20 different loci. Population genetic parameters for progeny, parent trees and pollen were estimated from isozyme data using BIOSYS-2, a population genetic statistical software package. For progeny data, total gene diversity (H_T) was 0.249; average genic diversity within stands (H_S) was 0.231; average genic diversity among stands (D_{ST}) was 0.017; and the proportion of total genic diversity due to differences among stands (G_{ST}) was 0.067.

No substantial differences in gene diversity statistics, including number of alleles per locus, percent polymorphic loci, and average and observed heterozygosity were found among Willamette Valley stands and between the Willamette Valley region and the other

sampled regions of Oregon and Washington. This suggests that individual stands of WVPP do not differ substantially from one another, and that WVPP is not genetically different from other populations of ponderosa pine in Washington and Oregon. There is no evidence that the amount of genetic diversity in WVPP is less than in other populations of ponderosa pine examined in this study. The amount of diversity found in WVPP is consistent with what has been observed in previous studies of other conifers, including ponderosa pine.

This study found no apparent geographic structure to genetic variation in WVPP. Genetic distances among stands, calculated from allele frequencies, were generally small. There was no tendency for stands within the Willamette Valley to be more closely related to one another than to stands in eastern Oregon, southwest Oregon, or the Puget Sound Basin of Washington.

Multilocus and single-locus estimates of outcrossing rate were determined for six stands within the Willamette Valley. Outcrossing rates were estimated for seven polymorphic loci using MLTR, a mating system analysis software package. Estimates of multilocus outcrossing for these stands (mean 0.642; range 0.365 to 0.879) were substantially lower than estimates previously reported for other populations of ponderosa pine, other pine species, and other species in the family Pinaceae.

High levels of inbreeding in WVPP did not appear to be related to stand density, stand size, or degree of isolation from other stands in the Willamette Valley. There was

also no apparent association between level of inbreeding and amount of genetic variation present within a stand. The high level of inbreeding observed in this population is unusual and could be attributable to population fragmentation due to human activities, or a reduced level of pollen production in this population compared to other populations of ponderosa pine.

Recommendations

The lack of a relationship between genetic distance and geographic distance suggests WVPP is not highly differentiated. Protection of the genetic resource represented by WVPP can be accomplished by conserving a large number of individuals in a few large stands or many small stands. Genetic conservation strategies for this population should include the protection of the large native stands that remain, and the use of local, adapted sources for afforestation. Ponderosa pine seed sources outside the Willamette Valley may be maladapted to local conditions and should not be used for planting programs in the Valley.

The high degree of inbreeding is of concern because ponderosa pine is known to exhibit inbreeding depression, resulting in reductions in growth and survival. Management techniques can reduce the level of inbreeding in a deployment population. Unrelated individuals can be used to establish a WVPP seed orchard. Pollen management techniques in a seed orchard can be used to maximize pollen production, and controlled crossing or controlled-mass pollination can be used to produce a highly

outcrossed deployment population. The use of these techniques should improve the genetic quality of seed over wild stand seed by reducing inbreeding levels. Additionally, culling of nursery-grown seedlings that exhibit poor growth can further decrease inbreeding in the deployment population by eliminating inbred individuals from the population before outplanting.

This study has provided valuable information on the population genetic structure and mating system of WVPP. However, additional studies are needed. The stands used in this study were concentrated in the central and southern parts of the Willamette Valley, and the cones used in the study came from only one collection year. It would be useful to make a cone collection over several years, to reduce year-to-year variation in cone and pollen production. It would also be useful to sample stands from ponderosa pine stands elsewhere in the Valley, particularly in northern and on the west side of the Willamette River. A collection over a wider portion of the Willamette Valley would provide a larger sample size, and a wider distribution of stands could possibly result in the detection of stand-to-stand variation not seen in the current study.

Another useful follow-up study would be an investigation of genetic variation in adaptive traits in WVPP. There may be genetic differences among WVPP stands, or between the Willamette Valley population and other populations of ponderosa pine that are not apparent by looking at allozyme variation. A study of adaptive traits would also provide valuable information on growth and yield, and would certainly be useful to local small woodland owners who wish to grow Willamette Valley ponderosa pine.

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APPENDICES

Appendix A. Estimated allele frequencies at 20 allozyme loci for progeny from 11 ponderosa pine stands, two in Eastern Oregon, one at the Fort Lewis Army Base in Washington, one in Southwest Oregon, and seven in the Willamette Valley of Oregon.

	Region/Population [1]										
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley						
Locus/ Allele	**Ochoco**	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**	**Grassy Glade**
PEP-1											
(N) [2]	*132*	*118*	*130*	*80*	*101*	*114*	*101*	*100*	*70*	*132*	*48*
A	0.962	0.996	0.942	0.944	0.936	0.987	0.861	1.000	0.864	0.985	0.948
B	0.038	0.004	0.058	0.019	0.045	0.013	0.129	0.000	0.136	0.015	0.052
C	0.000	0.000	0.000	0.038	0.020	0.000	0.010	0.000	0.000	0.000	0.000
PEP-2											
(N)	*132*	*118*	*130*	*80*	*101*	*112*	*120*	*100*	*70*	*132*	*48*
A	0.970	1.000	0.885	0.856	0.886	0.893	0.996	1.000	1.000	0.943	0.854
B	0.030	0.000	0.019	0.000	0.094	0.107	0.004	0.000	0.000	0.042	0.021
C	0.000	0.000	0.096	0.144	0.020	0.000	0.000	0.000	0.000	0.015	0.125
PEP-4											
(N)	*132*	*119*	*130*	*80*	*101*	*113*	*120*	*100*	*70*	*132*	*48*
A	0.519	0.992	0.915	0.813	0.738	1.000	0.950	0.990	1.000	0.913	0.958
B	0.481	0.008	0.085	0.188	0.262	0.000	0.050	0.010	0.000	0.087	0.042
LAP-1											
(N)	*132*	*48*	*130*	*100*	*101*	*113*	*120*	*100*	*90*	*114*	*48*
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2											
(N)	*132*	*66*	*130*	*100*	*101*	*113*	*120*	*100*	*110*	*120*	*48*
A	0.985	0.992	0.996	1.000	0.950	0.965	0.975	0.955	0.923	0.896	0.844
B	0.000	0.008	0.004	0.000	0.050	0.000	0.004	0.000	0.000	0.025	0.000
C	0.011	0.000	0.000	0.000	0.000	0.035	0.021	0.045	0.077	0.079	0.156
D	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LAP-3											
(N)	*132*	*66*	*130*	*100*	*101*	*99*	*120*	*99*	*110*	*120*	*48*
A	0.951	0.886	0.988	0.905	0.881	0.682	0.883	0.995	0.936	0.921	0.854
B	0.042	0.008	0.000	0.095	0.079	0.045	0.046	0.005	0.009	0.058	0.146
C	0.008	0.106	0.012	0.000	0.040	0.273	0.071	0.000	0.055	0.021	0.000
PGM-1											
(N)	*132*	*84*	*130*	*100*	*101*	*94*	*104*	*60*	*110*	*113*	*48*
A	0.784	0.988	0.819	0.630	0.762	0.713	0.654	0.967	0.814	0.717	0.854
B	0.030	0.000	0.177	0.300	0.139	0.048	0.197	0.025	0.009	0.133	0.052
C	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.094
D	0.186	0.000	0.004	0.070	0.099	0.239	0.149	0.008	0.177	0.150	0.000
GOT-1											
(N)	*128*	*140*	*130*	*100*	*101*	*106*	*101*	*83*	*70*	*132*	*48*
A	0.926	0.882	0.785	0.910	0.366	0.915	0.757	0.801	0.886	0.958	0.844
B	0.020	0.032	0.042	0.025	0.421	0.005	0.000	0.018	0.079	0.011	0.052
C	0.004	0.043	0.000	0.000	0.000	0.042	0.035	0.024	0.021	0.004	0.052
D	0.051	0.043	0.173	0.065	0.054	0.038	0.208	0.157	0.014	0.027	0.052
E	0.000	0.000	0.000	0.000	0.158	0.000	0.000	0.000	0.000	0.000	0.000

Appendix A. (continued).

Locus/ Allele	Region/Population [1]										
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley						
	Ochoco	W.S.I.R.	Fort Lewis	SW	Hort. Farm	Lan-10	Lan-20	Lan-22	Lin-21	Lin-23	Grassy Glade
GOT-2											
(N)	*101*	*114*	*130*	*100*	*101*	*102*	*102*	*93*	*50*	*126*	*48*
A	0.881	0.917	0.808	0.970	0.777	0.735	0.848	0.731	0.820	0.857	0.885
B	0.064	0.022	0.046	0.000	0.000	0.167	0.000	0.032	0.020	0.099	0.000
C	0.054	0.061	0.146	0.030	0.213	0.098	0.152	0.237	0.160	0.020	0.115
D	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000
GOT-3											
(N)	*132*	*120*	*130*	*100*	*101*	*113*	*101*	*96*	*70*	*131*	*48*
A	0.992	0.925	0.988	0.960	0.812	0.850	0.936	0.823	0.986	0.931	0.979
B	0.008	0.029	0.004	0.040	0.168	0.150	0.064	0.146	0.014	0.042	0.010
C	0.000	0.008	0.008	0.000	0.010	0.000	0.000	0.000	0.000	0.011	0.010
D	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.026	0.000	0.015	0.000
ACP-1											
(N)	*118*	*48*	*130*	*100*	*101*	*68*	*96*	*28*	*72*	*109*	*48*
A	0.873	0.792	0.904	0.860	0.950	0.875	0.781	0.839	0.896	0.674	0.771
B	0.127	0.167	0.088	0.140	0.020	0.125	0.156	0.161	0.104	0.211	0.167
C	0.000	0.031	0.008	0.000	0.030	0.000	0.063	0.000	0.000	0.096	0.063
D	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
GDH-1											
(N)	*132*	*60*	*130*	*100*	*101*	*113*	*102*	*60*	*110*	*125*	*48*
A	0.932	0.992	1.000	0.940	0.975	0.704	0.975	1.000	0.914	0.928	0.875
B	0.068	0.000	0.000	0.055	0.000	0.296	0.000	0.000	0.009	0.008	0.000
C	0.000	0.008	0.000	0.005	0.025	0.000	0.025	0.000	0.077	0.064	0.125
IDH-1											
(N)	*132*	*148*	*130*	*100*	*101*	*113*	*119*	*100*	*110*	*131*	*48*
A	0.568	0.618	0.562	0.890	0.946	0.770	0.929	0.830	0.618	0.802	0.760
B	0.432	0.382	0.438	0.110	0.054	0.221	0.071	0.165	0.382	0.198	0.240
C	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.005	0.000	0.000	0.000
SDH-1											
(N)	*132*	*84*	*130*	*100*	*101*	*113*	*102*	*99*	*70*	*131*	*48*
A	0.848	0.833	0.762	0.840	0.757	0.801	0.755	0.854	0.700	0.679	0.823
B	0.129	0.161	0.238	0.160	0.243	0.199	0.157	0.146	0.293	0.321	0.177
C	0.023	0.006	0.000	0.000	0.000	0.000	0.088	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000
SDH-2											
(N)	*132*	*84*	*130*	*100*	*101*	*81*	*102*	*60*	*48*	*131*	*48*
A	0.894	0.887	0.838	0.760	0.757	0.630	0.819	1.000	0.542	0.866	0.781
B	0.106	0.113	0.162	0.240	0.243	0.370	0.181	0.000	0.458	0.134	0.219

Appendix A. (continued).

Locus/ Allele	Region/Population [1]										
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley						
	Ochoco	W.S.I.R.	Fort Lewis	SW	Hort. Farm	Lan-10	Lan-20	Lan-22	Lin-21	Lin-23	Grassy Glade
PGI-1											
(N)	*132*	*153*	*130*	*100*	*101*	*77*	*97*	*98*	*109*	*129*	*48*
A	0.909	0.873	0.862	0.800	0.812	0.883	0.794	0.827	0.784	0.907	0.740
B	0.000	0.111	0.004	0.025	0.099	0.045	0.031	0.000	0.000	0.008	0.146
C	0.000	0.010	0.038	0.000	0.010	0.000	0.021	0.071	0.000	0.000	0.000
D	0.091	0.007	0.096	0.175	0.079	0.071	0.155	0.102	0.216	0.085	0.115
MDH-1											
(N)	*132*	*156*	*130*	*100*	*101*	*113*	*120*	*60*	*110*	*132*	*48*
A	0.992	0.974	1.000	0.975	0.995	0.960	0.971	1.000	1.000	0.992	0.958
B	0.008	0.026	0.000	0.000	0.005	0.040	0.029	0.000	0.000	0.008	0.000
C	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042
MDH-3											
(N)	*113*	*138*	*130*	*100*	*101*	*68*	*119*	*20*	*60*	*132*	*48*
A	0.761	0.797	0.654	0.695	0.718	0.471	0.622	1.000	0.792	0.519	0.802
B	0.058	0.011	0.227	0.170	0.104	0.037	0.227	0.000	0.008	0.223	0.094
C	0.181	0.188	0.119	0.135	0.178	0.493	0.151	0.000	0.200	0.246	0.104
D	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000
ADH-1											
(N)	*114*	*113*	*130*	*100*	*101*	*104*	*120*	*20*	*110*	*132*	*48*
A	0.566	0.677	0.892	0.645	0.827	0.750	0.729	0.775	0.618	0.636	0.729
B	0.140	0.217	0.108	0.355	0.153	0.250	0.150	0.225	0.145	0.235	0.167
C	0.294	0.106	0.000	0.000	0.020	0.000	0.121	0.000	0.236	0.129	0.104
FUM-1											
(N)	*72*	*137*	*130*	*100*	*101*	*113*	*116*	*30*	*70*	*126*	*42*
A	0.646	0.916	0.873	0.755	0.950	0.894	0.832	0.767	0.929	0.734	0.940
B	0.333	0.058	0.127	0.245	0.050	0.058	0.129	0.233	0.071	0.222	0.060
C	0.021	0.026	0.000	0.000	0.000	0.049	0.026	0.000	0.000	0.044	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000

[1] Refer to Figures 2.2 and 2.3 for stand locations.

[2] Number of trees sampled in each population for the locus.

Appendix B. Estimated allele frequencies at 20 allozyme loci for parent trees from 11 ponderosa pine stands, two in Eastern Oregon, one at the Fort Lewis Army Base in Washington, one in Southwest Oregon, and six in the Willamette Valley of Oregon.

	Region/Population [1]									
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley					
Locus/ Allele	**Ochoco**	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**
PEP-1										
(N) [2]	*22*	*21*	*13*	*8*	*11*	*12*	*20*	*10*	*7*	*22*
A	0.955	0.976	0.923	0.938	0.864	1.000	0.825	1.000	0.857	0.977
B	0.045	0.024	0.077	0.000	0.091	0.000	0.150	0.000	0.143	0.023
C	0.000	0.000	0.000	0.063	0.045	0.000	0.025	0.000	0.000	0.000
PEP-2										
(N)	*22*	*19*	*13*	*8*	*10*	*12*	*20*	*10*	*7*	*22*
A	0.955	1.000	0.846	0.875	0.800	0.875	1.000	1.000	1.000	0.955
B	0.045	0.000	0.038	0.000	0.100	0.125	0.000	0.000	0.000	0.045
C	0.000	0.000	0.115	0.125	0.100	0.000	0.000	0.000	0.000	0.000
PEP-4										
(N)	*22*	*20*	*13*	*8*	*11*	*12*	*20*	*10*	*7*	*22*
A	0.500	1.000	0.923	0.813	0.773	1.000	0.950	0.950	1.000	0.886
B	0.500	0.000	0.077	0.188	0.227	0.000	0.050	0.050	0.000	0.114
LAP-1										
(N)	*22*	*8*	*13*	*10*	*10*	*12*	*20*	*10*	*9*	*19*
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2										
(N)	*22*	*11*	*13*	*10*	*10*	*12*	*20*	*10*	*11*	*20*
A	0.955	0.955	0.962	1.000	0.850	0.917	0.950	0.900	0.864	0.850
B	0.000	0.045	0.038	0.000	0.150	0.000	0.000	0.000	0.000	0.025
C	0.045	0.000	0.000	0.000	0.000	0.083	0.050	0.100	0.136	0.125
LAP-3										
(N)	*22*	*11*	*13*	*10*	*10*	*12*	*20*	*10*	*11*	*19*
A	0.932	0.818	1.000	0.900	0.850	0.667	0.825	1.000	1.000	0.974
B	0.045	0.000	0.000	0.100	0.100	0.083	0.050	0.000	0.000	0.026
C	0.023	0.182	0.000	0.000	0.050	0.250	0.125	0.000	0.000	0.000
PGM-1										
(N)	*22*	*14*	*13*	*10*	*11*	*10*	*19*	*6*	*11*	*19*
A	0.795	1.000	0.692	0.600	0.773	0.650	0.632	1.000	0.773	0.737
B	0.023	0.000	0.308	0.350	0.136	0.050	0.263	0.000	0.000	0.132
D	0.182	0.000	0.000	0.050	0.091	0.300	0.105	0.000	0.227	0.132
GOT-1										
(N)	*21*	*19*	*13*	*10*	*10*	*12*	*16*	*9*	*7*	*22*
A	0.976	0.947	0.846	0.950	0.350	0.958	0.938	0.944	0.929	1.000
B	0.000	0.053	0.038	0.000	0.450	0.042	0.000	0.056	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.071	0.000
D	0.024	0.000	0.115	0.050	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.000	0.000

Appendix B. (continued).

Locus/ Allele	Region/Population [1]									
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley					
	Ochoco	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**
GOT-2										
(N)	*16*	*18*	*13*	*10*	*11*	*12*	*17*	*10*	*7*	*21*
A	0.875	0.833	0.769	0.950	0.727	0.625	0.735	0.550	0.857	0.810
B	0.063	0.056	0.038	0.000	0.000	0.208	0.000	0.050	0.000	0.119
C	0.063	0.111	0.192	0.050	0.273	0.167	0.265	0.400	0.143	0.048
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024
GOT-3										
(N)	*22*	*20*	*13*	*10*	*11*	*12*	*17*	*10*	*7*	*22*
A	0.977	0.850	0.962	0.950	0.773	0.750	0.912	0.700	0.929	0.864
B	0.023	0.050	0.000	0.050	0.182	0.250	0.088	0.150	0.071	0.068
C	0.000	0.025	0.038	0.000	0.045	0.000	0.000	0.000	0.000	0.045
D	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.023
ACP-1										
(N)	*19*	*11*	*13*	*10*	*11*	*7*	*16*	*3*	*7*	*18*
A	0.789	0.773	0.923	0.850	0.864	0.786	0.719	0.833	0.857	0.583
B	0.211	0.182	0.077	0.150	0.045	0.214	0.219	0.167	0.143	0.278
C	0.000	0.045	0.000	0.000	0.091	0.000	0.063	0.000	0.000	0.111
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028
GDH-1										
(N)	*13*	*14*	*13*	*10*	*10*	*12*	*17*	*6*	*11*	*21*
A	0.769	0.964	1.000	1.000	1.000	0.667	1.000	1.000	1.000	1.000
B	0.231	0.036	0.000	0.000	0.000	0.333	0.000	0.000	0.000	0.000
IDH-1										
(N)	*22*	*25*	*13*	*10*	*10*	*12*	*20*	*10*	*11*	*21*
A	0.614	0.600	0.500	0.900	0.950	0.667	0.925	0.800	0.591	0.762
B	0.386	0.400	0.500	0.100	0.050	0.292	0.075	0.150	0.409	0.238
C	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.050	0.000	0.000
SDH-1										
(N)	*22*	*14*	*13*	*10*	*10*	*12*	*17*	*10*	*7*	*22*
A	0.886	0.857	0.769	0.850	0.850	0.708	0.765	0.850	0.643	0.795
B	0.091	0.143	0.231	0.150	0.150	0.292	0.118	0.150	0.357	0.205
C	0.023	0.000	0.000	0.000	0.000	0.000	0.118	0.000	0.000	0.000
SDH-2										
(N)	*22*	*14*	*13*	*10*	*10*	*8*	*17*	*6*	*5*	*21*
A	0.977	0.929	0.962	0.950	0.850	0.938	0.971	1.000	0.800	1.000
B	0.023	0.071	0.038	0.050	0.150	0.063	0.029	0.000	0.200	0.000

Appendix B. (continued).

	Region/Population [1]									
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley					
Locus/ Allele	**Ochoco**	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**
PGI-1										
(N)	*22*	*26*	*13*	*10*	*11*	*8*	*20*	*10*	*11*	*21*
A	1.000	0.904	0.923	0.950	0.818	0.938	0.775	0.800	0.909	1.000
B	0.000	0.058	0.000	0.050	0.182	0.063	0.125	0.000	0.000	0.000
C	0.000	0.019	0.077	0.000	0.000	0.000	0.025	0.100	0.000	0.000
D	0.000	0.019	0.000	0.000	0.000	0.000	0.075	0.100	0.091	0.000
MDH-1										
(N)	*22*	*26*	*13*	*10*	*10*	*12*	*20*	*6*	*11*	*22*
A	0.977	0.942	1.000	0.950	0.950	0.958	0.950	1.000	1.000	0.977
B	0.023	0.058	0.000	0.000	0.050	0.042	0.050	0.000	0.000	0.023
C	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000
MDH-3										
(N)	*19*	*23*	*13*	*10*	*11*	*7*	*20*	*2*	*6*	*22*
A	0.711	0.761	0.731	0.800	0.500	0.429	0.575	1.000	0.750	0.523
B	0.079	0.022	0.154	0.050	0.136	0.071	0.200	0.000	0.000	0.227
C	0.211	0.217	0.115	0.150	0.364	0.500	0.225	0.000	0.250	0.227
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023
ADH-1										
(N)	*19*	*20*	*13*	*10*	*7*	*11*	*20*	*2*	*11*	*22*
A	0.632	0.700	0.885	0.500	0.929	0.818	0.700	0.750	0.636	0.636
B	0.132	0.175	0.115	0.500	0.071	0.182	0.175	0.250	0.182	0.250
C	0.237	0.125	0.000	0.000	0.000	0.000	0.125	0.000	0.182	0.114
FUM-1										
(N)	*12*	*26*	*13*	*10*	*10*	*12*	*21*	*2*	*7*	*21*
A	0.792	0.865	0.885	0.600	0.900	0.833	0.786	0.750	0.857	0.738
B	0.167	0.115	0.115	0.400	0.100	0.083	0.190	0.250	0.143	0.238
C	0.042	0.019	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.024
D	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.000

[1] Refer to Figures 2.2 and 2.3 for stand locations.

[2] Number of trees sampled in each population for the locus.

Appendix C. Estimated allele frequencies at 20 allozyme loci for pollen component in seed from 11 ponderosa pine stands; two in Eastern Oregon, one at the Fort Lewis Army Base in Washington, one in Southwest Oregon, and seven in the Willamette Valley of Oregon.

	Region/Population [1]										
	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley						
Locus/ Allele	**Ochoco**	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**	**Grassy Glade**
PEP-1											
(N) [2]	*132*	*118*	*130*	*80*	*101*	*113*	*120*	*100*	*70*	*132*	*48*
A	0.970	1.000	0.946	0.913	0.921	0.982	0.933	1.000	0.871	0.992	0.938
B	0.030	0.000	0.054	0.050	0.059	0.018	0.067	0.000	0.129	0.008	0.063
C	0.000	0.000	0.000	0.038	0.020	0.000	0.000	0.000	0.000	0.000	0.000
PEP-2											
(N)	*132*	*118*	*130*	*80*	*101*	*113*	*120*	*100*	*70*	*132*	*48*
A	0.970	1.000	0.931	0.913	0.901	0.912	0.992	1.000	1.000	0.917	0.875
B	0.030	0.000	0.008	0.087	0.099	0.088	0.008	0.000	0.000	0.053	0.125
C	0.000	0.000	0.062	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000
PEP-4											
(N)	*132*	*118*	*130*	*80*	*101*	*113*	*120*	*100*	*70*	*132*	*48*
A	0.523	0.983	0.892	0.837	0.733	1.000	0.950	1.000	1.000	0.955	0.917
B	0.489	0.017	0.108	0.162	0.267	0.000	0.050	0.000	0.000	0.045	0.083
LAP-1											
(N)	*132*	*66*	*130*	*100*	*101*	*113*	*120*	*100*	*90*	*117*	*48*
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-2											
(N)	*132*	*66*	*130*	*100*	*101*	*113*	*120*	*100*	*110*	*120*	*48*
A	0.992	1.000	1.000	1.000	0.970	0.982	0.992	0.980	1.000	0.958	1.000
B	0.000	0.000	0.000	0.000	0.030	0.018	0.008	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.042	0.000
D	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LAP-3											
(N)	*132*	*66*	*130*	*100*	*101*	*113*	*120*	*99*	*110*	*120*	*48*
A	0.947	0.894	0.977	0.940	0.861	0.699	0.892	0.990	0.873	0.892	0.938
B	0.053	0.015	0.000	0.060	0.069	0.044	0.058	0.010	0.018	0.067	0.063
C	0.000	0.091	0.023	0.000	0.069	0.257	0.050	0.000	0.109	0.042	0.000
PGM-1											
(N)	*132*	*84*	*130*	*100*	*101*	*94*	*104*	*60*	*110*	*125*	*48*
A	0.765	0.976	0.862	0.610	0.752	0.777	0.663	0.933	0.818	0.728	0.792
B	0.030	0.000	0.131	0.280	0.158	0.043	0.173	0.050	0.018	0.112	0.104
C	0.000	0.024	0.000	0.000	0.089	0.000	0.000	0.017	0.000	0.000	0.104
D	0.205	0.000	0.008	0.110	0.000	0.181	0.163	0.000	0.164	0.160	0.000
GOT-1											
(N)	*132*	*151*	*130*	*100*	*101*	*103*	*102*	*94*	*70*	*132*	*48*
A	0.856	0.861	0.715	0.880	0.347	0.825	0.578	0.660	0.800	0.917	0.792
B	0.038	0.046	0.069	0.050	0.475	0.000	0.000	0.021	0.157	0.023	0.083
C	0.076	0.086	0.000	0.000	0.000	0.078	0.010	0.043	0.014	0.008	0.021
D	0.098	0.007	0.215	0.070	0.109	0.073	0.044	0.277	0.029	0.053	0.104
E	0.000	0.000	0.000	0.000	0.069	0.029	0.000	0.000	0.000	0.000	0.000

Appendix C. (continued).

Locus/ Allele	Eastern Oregon: Ochoco	Eastern Oregon: W.S.I.R.	Fort Lewis: Fort Lewis	Southwest Oregon: SW	Willamette Valley: Hort. Farm	Willamette Valley: Lan-10	Willamette Valley: Lan-20	Willamette Valley: Lan-22	Willamette Valley: Lin-21	Willamette Valley: Lin-23	Willamette Valley: Grassy Glade
GOT-2											
(N)	*132*	*135*	*130*	*100*	*101*	*111*	*102*	*99*	*50*	*132*	*48*
A	0.909	0.941	0.854	1.000	0.921	0.748	0.980	0.929	0.860	0.886	0.979
B	0.076	0.015	0.077	0.000	0.000	0.189	0.000	0.030	0.040	0.091	0.021
C	0.015	0.044	0.069	0.000	0.079	0.063	0.020	0.030	0.100	0.008	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000
GOT-3											
(N)	*132*	*138*	*130*	*100*	*101*	*113*	*101*	*96*	*70*	*141*	*48*
A	1.000	0.993	0.992	0.970	0.822	0.920	0.931	0.865	1.000	0.887	1.000
B	0.000	0.007	0.008	0.030	0.149	0.080	0.069	0.135	0.000	0.085	0.000
C	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.014	0.000
E	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.014	0.000
ACP-1											
(N)	*118*	*48*	*130*	*100*	*101*	*68*	*96*	*28*	*72*	*109*	*48*
A	0.958	0.771	0.900	0.820	0.970	0.956	0.865	0.893	0.917	0.761	0.813
B	0.042	0.208	0.085	0.180	0.010	0.044	0.073	0.107	0.083	0.138	0.146
C	0.000	0.000	0.015	0.000	0.020	0.000	0.063	0.000	0.000	0.101	0.042
D	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GDH-1											
(N)	*132*	*60*	*130*	*100*	*101*	*113*	*102*	*60*	*110*	*125*	*48*
A	1.000	0.983	1.000	0.880	0.950	0.761	0.951	1.000	0.827	0.856	0.750
B	0.000	0.000	0.000	0.110	0.000	0.239	0.000	0.000	0.018	0.056	0.250
C	0.000	0.017	0.000	0.010	0.050	0.000	0.049	0.000	0.155	0.088	0.000
IDH-1											
(N)	*132*	*152*	*130*	*100*	*101*	*113*	*119*	*99*	*110*	*131*	*48*
A	0.538	0.671	0.669	0.880	0.931	0.832	0.924	0.808	0.618	0.863	0.771
B	0.462	0.329	0.331	0.120	0.069	0.168	0.076	0.192	0.382	0.137	0.229
SDH-1											
(N)	*132*	*84*	*130*	*100*	*101*	*113*	*102*	*99*	*70*	*131*	*48*
A	0.735	0.762	0.754	0.780	0.604	0.832	0.735	0.788	0.743	0.618	0.792
B	0.258	0.226	0.246	0.220	0.396	0.168	0.186	0.212	0.243	0.382	0.208
C	0.008	0.012	0.000	0.000	0.000	0.000	0.078	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000
SDH-2											
(N)	*132*	*84*	*130*	*100*	*101*	*80*	*102*	*60*	*48*	*131*	*48*
A	0.795	0.833	0.708	0.580	0.614	0.412	0.676	1.000	0.250	0.733	0.646
B	0.205	0.167	0.292	0.420	0.386	0.587	0.324	0.000	0.750	0.267	0.354

Appendix C. (continued).

Locus/ Allele	Eastern Oregon		Fort Lewis	Southwest Oregon	Willamette Valley						
	Ochoco	**W.S.I.R.**	**Fort Lewis**	**SW**	**Hort. Farm**	**Lan-10**	**Lan-20**	**Lan-22**	**Lin-21**	**Lin-23**	**Grassy Glade**
PGI-1											
(N)	*132*	*154*	*130*	*100*	*101*	*77*	*105*	*98*	*109*	*131*	*48*
A	0.818	0.870	0.769	0.640	0.743	0.857	0.800	0.796	0.633	0.809	0.667
B	0.000	0.117	0.000	0.010	0.079	0.143	0.029	0.000	0.000	0.015	0.146
C	0.000	0.006	0.123	0.000	0.020	0.000	0.000	0.071	0.000	0.000	0.000
D	0.182	0.006	0.108	0.350	0.158	0.000	0.171	0.133	0.367	0.176	0.188
MDH-1											
(N)	*132*	*156*	*130*	*100*	*101*	*113*	*120*	*60*	*110*	*132*	*48*
A	1.000	1.000	1.000	1.000	1.000	0.965	0.983	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.035	0.017	0.000	0.000	0.000	0.000
MDH-3											
(N)	*114*	*138*	*130*	*100*	*101*	*68*	*119*	*20*	*60*	*132*	*48*
A	0.781	0.826	0.623	0.610	0.832	0.456	0.613	1.000	0.883	0.500	0.917
B	0.070	0.007	0.238	0.310	0.089	0.059	0.252	0.000	0.017	0.227	0.000
C	0.149	0.159	0.138	0.080	0.079	0.485	0.134	0.000	0.100	0.258	0.042
D	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000
ADH-1											
(N)	*114*	*131*	*130*	*100*	*101*	*104*	*120*	*20*	*110*	*132*	*48*
A	0.526	0.718	0.946	0.810	0.832	0.673	0.725	0.750	0.627	0.629	0.833
B	0.158	0.206	0.054	0.190	0.129	0.327	0.150	0.250	0.118	0.220	0.083
C	0.316	0.076	0.000	0.000	0.040	0.000	0.125	0.000	0.255	0.152	0.083
FUM-1											
(N)	*72*	*137*	*130*	*100*	*101*	*113*	*116*	*20*	*70*	*126*	*48*
A	0.500	0.912	0.869	0.900	0.960	0.956	0.784	1.000	0.914	0.730	0.885
B	0.486	0.051	0.131	0.100	0.040	0.035	0.155	0.000	0.086	0.198	0.115
C	0.014	0.036	0.000	0.000	0.000	0.009	0.052	0.000	0.000	0.071	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000

[1] Refer to Figures 2.2 and 2.3 for stand locations.

[2] Number of trees sampled in each population for the locus.