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Systematics and Genetic Structure of Washoe Pine: Applications in Conservation Genetics

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Abstract

Independent studies of seedling populations of Washoe *(Pinus washoensis)* and ponderosa *(P. ponderosa)* pines grown in common gardens demonstrated that: (1) the systematic relationship between Washoe pine and the North Plateau race of ponderosa pine is close and (2) the allocation of genetic variability among and within populations of the narrow endemic, Washoe pine, is similar to that of the broadly dispersed ponderosa pine. The results from this quantitative analysis of adaptive traits thus support previous works involving morphology, terpene chemistry, allozyme variation, mating systems, DNA biochemistry, and classical taxonomy that lead to a conclusion of synonymity for Washoe pine and ponderosa pine. The results also provide no genetic evidence that small population sizes and isolated distributions have had deleterious genetic consequences. Populations of Washoe pine nevertheless have unique characteristics that may be worthy of conservation. Programs should concentrate on habitat preservation and range expansion.

Key words: Pinus washoensis, quantitative traits, genetic structure, systematics, conservation biology.

Introduction

Loss of habitat, restricted distributions, isolation, and inbreeding lead toward the deterioration of genetic variability, loss of genes, extinction of populations, and, ultimately, endangerment of species. Estimating the risk before endangerment challenges conservationists to accumulate and synthesize disparate sources of information. Besides demographics, phylogenetic and systematic relationships must be considered together with the factors molding the system of genetic variability (Avise,

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1996). Topics of particular importance to understanding the genetic system include mating systems and genetic diversity, both of which are readily addressed with molecular techniques (AVISE, 1996). Equally important, however, are evolutionary potentials which reflect the ability of an organism to adjust genetically to change and which are assessed only from quantitative analyses of adaptive traits (LYNCH, 1996).

Washoe pine *(Pinus washoensis* H. MASON and STOCKW.) is a decidedly lesser member of the *Ponderosae* subsection of genus *Pinus* (CRITCHFIELD and LITTLE, 1966), which is dominated by the widespread ponderosa pine *(Pinus ponderosa* LAWS.). Washoe pine occurs at three high-elevation (>2200 m) locations *(Fig. 1)*: in numerous populations and subpopulations in the southern tip of the Warner Mountains; in a single population at Babbitt Peak; and in two small populations on Mt. Rose (CRITCHFIELD and ALLENBAUGH, 1965). Individual trees, however, may occur infrequently in surrounding forests (HALLER, 1961; GRIFFIN and CRITCHFIELD, 1976) composed primarily of ponderosa pine and Jeffrey pine *(Pinus jeffreyii* GREV. and BALF.), another of the *Ponderosae,* and even as far north as British Columbia (HALLER, 1965).

Among the three *Ponderosae* taxa endemic to the mountains of northeastern California and adjacent Nevada, reproductive incompatibilites are nearly complete between Washoe and Jeffrey pines (CRITCHFIELD, 1984), the two of which co-occur at all locations supporting Washoe pine *(Fig. 1)*. However, incompatibilities are only partial between Washoe pine and the Pacific race of ponderosa pine (see *Fig. 1*), which occurs on the lower slopes of Mt. Rose and Babbitt Peak but does not co-occur with Washoe pine. Only in the Warner Mountains, an area of transition between the Pacific and North Plateau races of ponderosa pine *(Fig. 1;* CRITCHFIELD, 1984), do Washoe and ponderosa pines occur together, thereby facilitating hybridization.

The ability of Washoe pine to persist naturally has long been questioned, largely because of its limited distribution (CRITCHFIELD, 1984). As a result, Washoe pine has been the subject of independent studies of morphology (HALLER, 1957), terpene chemistry (SMITH, 1967, 1977), crossability (CRITCHFIELD, 1984), allozyme diversity (NIEBLING and CONKLE, 1990; CONKLE, 1992), mating systems (MITTON *et al.,* 1997), and DNA biochemistry (PATTEN, 1999). Together these studies have demonstrated a close systematic relationship of Washoe pine with its cohort, the broadly dispersed ponderosa pine. The relationship, however, seems much closer with northern populations of the North Plateau race (see *Fig. 1;* CONKLE and CRITCHFIELD, 1988; PATTEN, 1999) than with neighboring populations of either the Pacific race of California or the North Plateau race of southern Oregon (CRITCHFIELD, 1984). The fact that WELLS' (1964) analyses of genetic variation in ponderosa pine are not necessarily compatible with these conclusions supports the need to thoroughly consider the evolutionary potential of Washoe pine with quantitative traits.

In this paper, quantitative analyses of traits that reflect the adaptation of populations to different climates (see REHFELDT, 1991) are used to assess the genetic structure of Washoe pine. The results pertain directly to conservation issues. Throughout this paper, the term 'provenance' is used to designate a geographic location; 'population' refers to the individuals at that location and their natural, wind-pollinated descendants; and 'family' refers to the offspring of a single tree.

Materials and Methods

Two studies were established with independent samples of wind-pollinated cones collected from natural populations *(Fig. 1).* The first concentrated on (1) genetic variation among and within populations of Washoe pine from the three locations where the species occurs and (2) systematic relationships between Washoe, ponderosa, and Jeffrey pines. The second was designed and implemented after the completion of the first and considered genetic variation among and within the many populations and subpopulations in the Warner Mountains. Analyses of both studies used the algorithms of SAS (1996).

Study 1

Mature cones were collected from 10 Washoe pines in the Warner Mountains (elevations from 2200 m to 2300 m); 9 trees at Babbitt Peak (elevations from 2500 m to 2550 m); 22 trees at Hidden Lake (elevations from 2350 m to 2400 m) on Mt. Rose; and 18 trees at Grass Lake (elevations from 2200 m to 2300 m) also on Mt. Rose. In addition, cones from at least 10 trees were bulked as population samples for two provenances of the Pacific race of ponderosa pine, one from Mt. Rose (elevation = 2000 m) and one from near Truckee (elevation = 1900 m); a provenance of Jeffrey pine (elevation = 2050 m) from near Truckee; and eight North Plateau provenances (elevations from 400 m to 1900 m), two of which were from British Columbia.

In total, therefore, the experimental materials included seedlings derived from 59 single trees and 11 populations, each of which is referenced subsequently as a seedlot. Seedlings from these seedlots were grown in independent tests conducted in greenhouses and shadehouses at Moscow, Idaho (latitude = 46.7°, longitude = 117°) and a field site at the Priest River Experimental Forest near Priest River, Idaho (latitude = 48.5°, longitude = 116.7°).

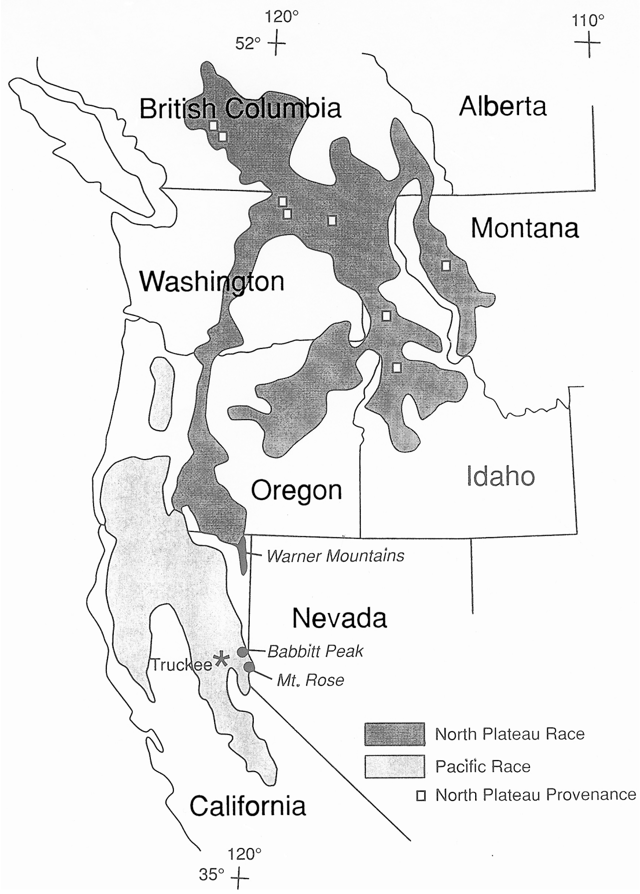

Fig. 1. – Locations where Washoe pine populations occur (Warner Mountains, Babbitt Peak, and Mt. Rose) within the general distribution (after LITTLE and CRITCHFIELD, 1966) of the Pacific and North Plateau races of ponderosa pine var. *ponderosa* (from CONKLE and CRITCHFIELD, 1988). Also located are the North Plateau provenances sampled in Study 1.

Greenhouse tests followed procedures used effectively for studying genetic variation among families and populations of the *Ponderosae* for traits reflecting adaptation to natural environments (*e. g.,* REHFELDT, 1991, 1999). Nine seedlings from each seedlot were grown in plastic containers (740 cm^3) in each of three blocks of a randomized complete block design. The containers were arranged in trays that held three seedlots. Seeds were sown in May, 1992, and the seedlings were grown for 6 mo in a shadehouse (50% shade), were transferred to a greenhouse for the winter months where temperatures were maintained above –2 °C, and, beginning on March 3 of the second growing season, were exposed to a daytime temperature of 25 °C which was allowed to cool to a minimum of 13 °C at night. After shoot elongation was completed, the seedlings were moved back to the shadehouse for the duration of the summer.

Shoot elongation of each seedling during yr 2 was measured at approximately 3-d intervals and was modeled with a modified logistic function (REHFELDT and WYKOFF, 1981). Regression statistics produced by the logistic function were used to estimate the (1) start of shoot elongation, the day on which 2 mm of elongation had occurred; (2) initiation of rapid elongation, the day on which 8 mm of elongation had occurred; (3) cessation of elongation, the day on which all but 2 mm of elongation had occurred; (4) duration of elongation, the number of days between the start and cessation; and (5) rate of elongation, the amount of elongation per day over the period during which 20% to 80% of the annual elongation had occurred. Total elongation and 2-yr height also were measured, and the number of needles per fascicle was estimated from the average of 10 fascicles distributed along the 2-yr shoot.

The field test also was established in a randomized complete block design that provided a maximum of 42 seedlings for each seedlot. As implemented several times previously for studies of ponderosa pine (*e. g.,* REHFELDT, 1991), the test was established at close spacing (35 cm between rows and 27 cm between seedlings within rows), cultured intensively to eliminate extraneous environmental effects, and terminated after four years. Three variables were either scored or measured on each seedling: 4-yr height; needle length, the average of 5 needles dispersed along the second year shoot; and color (value of 0 = green or 1 = waxy blue-green) of the succulent 4-yr shoot.

Statistical analyses included a canonical discriminant analysis for separating four taxonomic groups: Washoe pine families, the Jeffrey pine provenance, provenances of the Pacific race of ponderosa pine, and provenances of the North Plateau race of ponderosa pine. The analyses used the correlation matrix of seedlot means for 11 variables.

A second set of analyses was used to assess the genetic structure of Washoe pine. These analyses used data on individual trees for only the 59 families classified as Washoe pine. A separate analysis was made for each variable under a model of random effects that extracted variance due to blocks, populations, families in populations, an experimental error consisting of the interactions involving blocks, and a sampling error consisting of variance within family plots. Results are presented as intraclass correlations — the ratio of the variance component (σ^2) of an effect to the sum of the components. The total variance and variance components associated with the effects of populations and families within populations are designated subsequently by σ_T^2, σ_P^2, and σ_F^2, respectively.

Study 2

Mature cones were collected from 51 Washoe pines distributed across 20 populations (elevations from 1900 m to 2400 m) within the continuous forests of the species in the southern tip of the Warner Mountains *(Fig. 1)*. One to seven trees were sampled in each population. Cones were also collected from 11 ponderosa pines in the Warner Mountains (elevations from 1650 m to 2000 m), 6 Washoe pines growing within the general distribution of ponderosa pine near Truckee, and 4 ponderosa pines from Truckee. Also included in the study were the two provenances of the Pacific race of ponderosa pine that were used in study 1. In total, study 2 included 74 seedlots.

The seedlots were tested in the greenhouses at Moscow, Idaho as described for study 1; field tests were not established. Although study 2 was initiated in 1996, four years after study 1, an attempt was made to repeat experimental conditions of the previous study. Thus, the experimental design was the same, seeds were sown on May 1, shoot elongation was measured at 3-d intervals beginning on March 3 during the second growing season, and daytime temperatures of about 25 °C were allowed to cool to a minimum of 13 °C at night.

The procedures yielded seven variables for analyses: six variables that described shoot elongation plus 2-yr height. Statistical analyses included a canonical discriminant function developed from the correlation matrix of seedlot means for separating five groups: Washoe pine families from the Warner Mountains, ponderosa pine families from the Warner Mountains, Washoe pine families from Truckee, ponderosa pine families from Truckee, and provenances of the Pacific race of ponderosa pine.

The genetic structure of the 51 Warner Mountain Washoe pine families was assessed from analyses of variance as described for study 1.

Results

Study 1

The canonical discriminant analysis provided three multivariate vectors that sequentially accounted for 67%, 27%, and 6% of the variance among the four groups. Ordination of group means according to the first two vectors *(Fig. 2)* provided a discrete separation of all groups. The third vector provided additional separation of the Jeffrey pine population from the ponderosa and Washoe pine seedlots and is not presented. *Figure 2* also illustrates continuous variation between the Washoe pine families and the North Plateau populations along the first vector. This variation is largely in response to growth potential: mean 4-yr height of the Washoe pine families was 61 cm; that of the British Columbia populations was 77 cm; and those of the North Plateau race of the United States varied from 83 cm to 108 cm in direct association with increasing values of vector 1.

Analyses of variance using only Washoe pine data detected differences among populations for seven of the 12 variables *(Table 1)*. These effects accounted for as much as 15% of the total variance while averaging about 7%. Variance among families within populations was statistically significant for all variables and on average accounted for about 13% of the total variance. Population means showed that the two populations from Mt. Rose performed similarly and exhibited together the highest growth potentials (greatest amount of shoot elongation, 2-yr height, and 4-yr height); latest cessation and longest duration of shoot elongation; and fewest needles per fascicle (3.00). Trees from the Warner Mountains had a significantly lower growth potential than those from Mt. Rose and also had the earliest cessation and shortest duration of shoot elongation. The trees from Babbitt Peak, the population at the highest elevation, had the lowest growth potential that resulted from an extremely slow rate of elongation. When all traits are

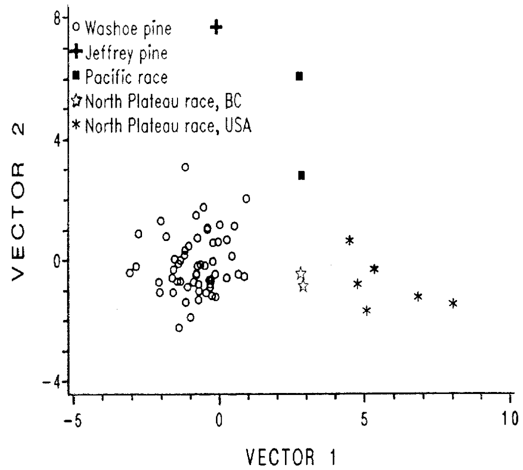

Fig. 2. – Ordination of seedlot means according to the first and second canonical vectors produced in study 1 of 59 Washoe pine families, one Jeffrey pine population, two Pacific populations of ponderosa pine, two North Plateau ponderosa pine populations from British Columbia (BC), and six North Plateau ponderosa pine populations from the Inland Northwest of the United States (USA).

considered, therefore, populations from Mt. Rose, Babbitt Peak, and the Warner Mountains all tend to have a different assortment of genotypes.

Study 2

The canonical discriminant function produced four multivariate vectors for separating the five groups. Of these vectors, the first accounted for 81% of the variance among groups, the second 12%; and each of the remaining less than 5%. Ordination of seedlot means *(Fig. 3a)* tended to separate Washoe pine and ponderosa pine seedlots along the first vector, but none of the groups separated discretely. Neither the third, fourth nor fifth vectors provided for additional separation of groups.

Figure 3b illustrates that much of the variation associated with the first canonical vector is related to the elevation of the seed origin ($R^2 = 0.62$). As demonstrated by this figure, a single cline includes ponderosa pine families that tend to inhabit low elevations and the Washoe pine families from high elevations *(Fig. 3b)*.

Analyses of variance for only those seedlots originally classified as Washoe pine detected significant effects of populations and families within populations for all variables *(Table 1)*. On average, effects of populations accounted for nearly 14% of the total variance while effects of families accounted for about 12%. A portion of the population effects was related to the elevation of the seed source by statistically significant ($P < 0.01$) simple correlations *(r)* of –0.68 for 2-yr height, –0.60 for rate of shoot elongation, and –0.58 for the amount of elongation. Applying the techniques of REHFELDT (1991) showed that populations would have to be separated by about 335 m in elevation before one could be reasonably confident of genetic differentiation.

Discussion

Since its naming, the taxonomic identity of Washoe pine has been questioned periodically (HALLER, 1965; NIEBLING and CONKLE, 1990; BRAYSHAW, 1997; LAURIA, 1997). To be sure, the taxon is identified by characters that tend to be indefinite or ephermal: cone size, number of cone scales, and purple-black submature cones (MASON and STOCKWELL, 1945; CRITCHFIELD, 1984). Cone size and scale numbers, for instance, are highly variable (see BRAYSHAW, 1997), and cone color is apparent for only a brief period in only those years when cones are being produced. The questionable diagnostic value of these characters along with a comprehensive review of the taxonomic history of Washoe pine led LAURIA (1997) and BRAYSHAW (1997) to propose synonymity for *P. washoensis* and *P. ponderosa*.

The present results dealing with quantitative traits of adaptive value demonstrate a close relationship between

Table 1. – Variance components for the total variance (σ_T^2) and intraclass correlations for the effects of populations ($\sigma_P^2/\ \sigma_T^2$) and families (σ_F^2/σ_T^2) within populations of Washoe pine for variables tested in study 1 and 2.

Variable	Study 1			Study 2		
	σ_T^2	σ_P^2/σ_T^2	σ_F^2/σ_T^2	σ_T^2	σ_P^2/σ_T^2	σ_F^2/σ_T^2
2-yr shoot elongation	856.88	0.12**	0.14**	598.16	0.16**	0.12**
Initiation of elongation	0.57	0.00	0.17**	0.56	0.04*	0.09**
Start of elongation	2.82	0.01	0.20**	2.76	0.07*	0.11**
Cessation of elongation	24.16	0.15**	0.09**	29.02	0.21**	0.09**
Duration of elongation	24.97	0.14**	0.08**	29.38	0.19**	0.09**
Rate of elongation	2.26	0.08**	0.16**	1.76	0.10**	0.11**
2-yr height	2023.84	0.08**	0.16**	2226.87	0.18**	0.19**
Needles per fascicle	0.02	0.02**	0.05**	–	–	–
Stem color	0.11	0.04	0.09**	–	–	–
4-yr height	16997.90	0.07**	0.14**	–	–	–
Needle length	388.14	0.01	0.12**	–	–	–

*) Significance of F value at $0.05 \leq P \leq 0.01$.

**) Significance of F value at $P \leq 0.01$.

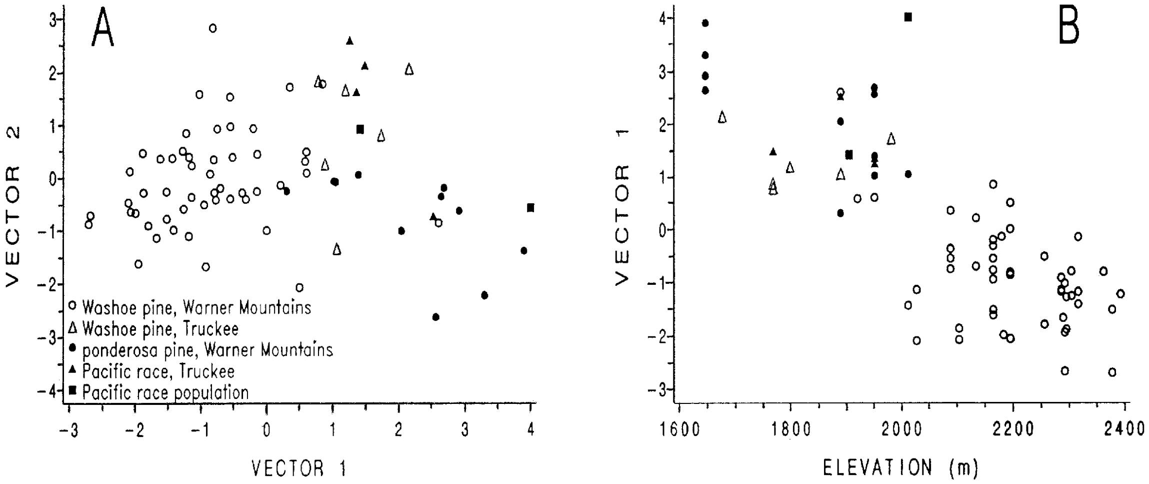

Fig. 3. – Study 2 results showing (A) ordination of mean values according to the first two canonical vectors for 51 Washoe pine and 11 ponderosa pine families from the Warner Mountains, six Washoe pine and four Pacific race ponderosa pine families near Truckee, and the two Pacific race populations of ponderosa pine used in study 1, and (B) seedlot means for the first canonical vector plotted by the elevation of the seedlot origin.

Washoe pine and the North Plateau race of ponderosa pine, and, more specifically, to British Columbia populations of that race. The results thus add corroborative support to the many experimental works dealing with the systematics of this species but provide no evidence to refute the views of LAURIA and BRAYSHAW. Washoe pine indeed seems to be synonymous with ponderosa pine.

Persuasive arguments of CRITCHFIELD (1984) and later NIEBLING and CONKLE (1990) present Washoe pine as a recent derivative of the North Plateau race of ponderosa pine whose natural history is intertwined with recent glacial epics. The argument begins with a distribution of the North Plateau race that was more southerly during the Wisconsin episode. As post Wisconsin emigrants tracked their optimal climates, Washoe pine became stranded at high elevations while (1) the bulk of the race advanced northward, (2) the Pacific race of ponderosa pine immigrated into much of the territory formerly occupied by the North Plateau race, and (3) competition from Jeffrey pine and the Pacific race excluded Washoe pine from middle and low elevation sites. As a result of parallel selection in severe environments, Washoe pine retained greater genetic similarity with North Plateau populations far to the north in British Columbia than with neighboring ponderosa pine populations. Although CRITCHFIELD (1984) considered Washoe pine as an unsuccessful experiment in speciation, NIEBLING and CONKLE (1990) view the taxon as merely a relic of a formerly widespread forest.

Nevertheless, of the characteristics listed by MASON and STOCKWELL (1945), purple-black submature cones and purple catkins and strobili distinguish Washoe pine from all of the North Plateau populations I have encountered during extensive work with this race in particular and with the pines of the *Ponderosae* in general. Because these characters are ephermal and difficult to observe, I conducted an informal survey among researchers, taxonomists, and tree breeders of western North America. The views were conflicting, contradictory, confused, and, therefore, inconclusive. Although it is possible that Washoe pine deserves a sub-specific rank within *Pinus ponderosa,* this study and its discussion once again (LAURIA, 1991; REHFELDT, 1999) demonstrate the need for a comprehensive taxonomic re-evaluation of the pines of the *Ponderosae* subgroup.

Without systematics as a primary justification, programs in conservation genetics should be commensurate with levels of genic diversity, characteristics of the mating system, and the evolutionary potential of the taxon. Of these factors, Washoe pine exhibits a robust mating system (MITTON *et al.,* 1997) and high levels of diversity (NIEBLING and CONKLE, 1990; CONKLE, 1992) that not only are similar to the broadly dispersed ponderosa pine but also are typical of forest trees as a whole. The only matter unresolved, therefore, is whether the taxon's genetic structure for quantitative traits of adaptive value reflects an evolutionary vulnerability not otherwise evident.

Because genetic variances in adaptive traits reflect evolutionary potentials (LYNCH, 1996), the vulnerability of Washoe pine's genetic system can be assessed by comparing its genetic structure to that of the North Plateau race of ponderosa pine. However, making such comparisons requires, first, merging the results of studies 1 and 2 to estimate the genetic structure of Washoe pine, and, second, comparing those results with a third study (REHFELDT, 1992) dealing with the genetic structure of the North Plateau race. These steps require, therefore, comparing variance components and intraclass correlations obtained from studies that have been subjected to different environmental effects. In the present case, the risk inherent in making such comparisons is alleviated by having studies of the same design conducted under similar conditions and yielding the same variables not only for Washoe pine but also for the North Plateau race of ponderosa pine.

For Washoe pine, study 1 considered populations from geographically dispersed localities and attributed about 8% of the total variance to the effects of populations and about 14% to the effects of families within populations for the seven traits in common with the second study. Study 2 considered numerous populations from only the Warner Mountains and attributed 14% of the total variance to the effects of populations and 11% to the effects of families. While the estimates of genetic variance between families within populations were similar for the two studies, estimated variances among populations within the Warner Mountains were larger than those among populations from geographically dispersed localities.

Of the explanations for larger population effects in study 2 than in study 1, inbreeding already has been eliminated by MITTON *et al.* (1997) who found no difference in outcrossing rates among populations at the Warner Mountains, Mt. Rose, or Babbitt Peak. However, not to be discounted is the fact that provenance elevation is ordinarily related to genetic variation in conifers of western North America in general and for Washoe pine in particular *(Fig. 3b),* and the range in elevation of sampled populations in study 2 was 300 m larger than for study 1. Other contributors may include statistical errors arising from the disparity in degrees of freedom available for estimating population effects for the two studies or sampling errors that could have arisen from inadvertent misclassification of trees in those areas of the Warner Mountains where Washoe pine and ponderosa pine are sympatric.

Still, the most probable contributor to the disparity in population effects estimated in studies 1 and 2 is interbreeding between Washoe and ponderosa pine that quite likely is taking place at low elevations in the Warner Mountains. In the vicinity of these mountains, the Pacific and North Plateau races of ponderosa pine are in transition (CRITCHFIELD, 1984), and only in these mountains do Washoe and ponderosa pines co-occur. In both the present results and those involving terpene analyses (SMITH, 1971), separating ponderosa pine from Washoe pine in the Warner Mountains was much more difficult than separating the same taxa at Mt. Rose and Babbitt Peak (compare *Fig. 2* with *Fig. 3a).* It is likely, therefore, that hybridization and introgression with ponderosa pine has enriched the germplasm of Washoe pine in the Warner Mountains and this enrichment was primarily responsible for the larger population effects estimated in study 2 than in study 1. SORENSEN (1994a) has similarly suggested that unusually low growth potentials of North Plateau populations of ponderosa pine in southern Oregon reflects introgression with the slow-growing Washoe pine. (Note that if one accepts synonymity for Washoe pine and ponderosa pine, hybridization in the Warner Mountains would be intraspecific but would nevertheless result in an enrichment of the Washoe pine germplasm.)

Even though study 1 included 10 Washoe pine families from the Warner Mountains, they were fortuitously located at a distance of about 20 km from the closest ponderosa pine. As a result, hybridization has probably had less of an effect on the population effects estimated in study 1 than for study 2. It seems reasonable to conclude, therefore, that at least 8% but less than 10% of the total phenotypic variance in Washoe pine occurs among populations, while as much as 14% occurs among families within populations. By comparison, in studies of three widely dispersed North Plateau populations of ponderosa pine conducted similarly to the present, 6% of the phenotypic variance in traits describing shoot elongation of 2-yr trees was apportioned to the effects of populations and 14% to the effects of families within populations (REHFELDT, 1992). The similarity in genetic structure of these two taxa is unmistakable.

Genetic variation among the Warner Mountain populations and subpopulations was arranged along clines associated with elevation. Such clines also have been documented for both the Pacific (CALLAHAM and LIDDICOET, 1961) and the North Plateau races (REHFELDT, 1991; SORENSEN, 1994a, b) of ponderosa pine var. *ponderosa,* ponderosa pine var. *scopulorum* (REHFELDT, 1993), and for species of the *Ponderosae* in general (REHFELDT, 1999). The slope of the cline for Washoe pine suggested that populations needed to be separated by about 335 m of elevation before one could be reasonably certain of genetic differentiation. This interval corresponds closely with those of 300 m estimated for North Plateau populations of ponderosa pine in southern Oregon (SORENSEN, 1994a) and 400 m for populations of the same race in Idaho and Montana (REHFELDT, 1991).

The present results thus join with many previous to show that in terms of systematics, phenotypic variation, characteristics of the mating system, levels of genic diversity, genetic structure for adaptive traits, geographic clines, and evolutionary potentials, Washoe pine is remarkably similar to the North Plateau race of ponderosa pine. Despite occurring in disjunct, geographically isolated populations, no evidence suggests deterioration of genetic variability, accumulated deleterious effects of inbreeding, or loss of evolutionary potential. To the contrary, the evidence suggests that the genetic system of Washoe pine is robust and resilient. From the genetic viewpoint, the taxon is neither threatened, vulnerable, nor endangered.

Still, Washoe pine has unusual and distinguishing characteristics that can be used to justify the conservation of the taxon and its individual populations. Although the taxonomic significance of purple-black submature cones and deep purple catkins and strobili are not yet known, the taxon has an interesting natural history and exhibits adaptive characteristics that position it near the terminus of a cline that relates genetic variation among North Plateau populations to the severity of the climate. This position is evidenced by the taxon's occurrence at high elevations, slow growth, and close similarity with the high latitudinal populations of British Columbia.

The primary threats to Washoe pine include the destruction of existing populations by fire and the loss of habitat from either human encroachment or a changing climate. In addition, if Washoe pine is considered as a subspecific entity within ponderosa pine, then contamination of the genetic system from introgression with ponderosa pine is also a threat to the persistence of the taxon. Of these factors, introgression already seems to be occurring in the Warner Mountains. Fire is a threat to the small populations on Mt. Rose and Babbitt Peak, and the two populations on Mt. Rose are the most vulnerable to human encroachment. Mt. Rose is located between two population centers and is well developed recreationally. It is an unfortunate coincidence, therefore, that genotypes with the highest growth potentials tend to be on Mt. Rose. To protect the genetic system of these populations as well as that of the taxon as a whole, three conservation measures seem appropriate: (1) silvicultural treatments to reduce the fire hazard, (2) planting programs to expand distributions, and (3) establishment of reserves.

A conservative approach to maintaining current levels of adaptedness would demand that planting programs are consistent with patterns of genetic variation. This would mean that seeds should not be transferred between Mt. Rose, Babbitt Peak, and the Warner Mountains. In addition, the clines also would suggest that seeds from a given population should not be transferred more than ±170 m in elevation. If conservation includes objectives requiring the establishment of plantings in foreign environments, control of competition from Jeffrey pine and the Pacific race of ponderosa pine quite likely will be necessary.

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Multilocus Analysis of *Pinus halepensis* Mill. from Spain: Genetic Diversity and Clinal Variation

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Abstract

Fifteen populations of *Pinus halepensis* from Spain were analysed employing 5 polymorphic loci by starch gel electrophoresis. A multilocus approach was used, in order to detect changes in the level of polymorphism and to reveal the pattern of variation of the species. The multilocus analyses show that large differences exist when island, inland and marginal populations are considered. These differences are quite high in terms of the effective number of genotypes, and in subpopulation differentiation. The multilocus differentiation is mainly caused by relations of allelic frequencies among populations, the most likely cause being that, by chance, alleles were associated differently in the refugia of the species. The population differentiation is large in comparison with that observed in other conifers (F_{ST} = 0.11; CF_{ST} = 0.17). The canonical variate corresponding to the largest differentiation among populations is positively correlated to the frequency of the most common allele of Mdh4 and Aco and negatively to the frequency of Hk and Pgm2. A clear pattern of variation related to latitude and longitude has been detected by the high correlation with the first canonical variate (r = –0.907 and r = –0.832 respectively). In conclusion, the clinal variation could be attributed to a

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