

# Role of selection versus historical isolation in racial differentiation of ponderosa pine in southern Oregon: an investigation of alternative hypotheses

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**Abstract:** Continuous populations identified as Pacific and North Plateau races of ponderosa pine (*Pinus ponderosa* P. Laws. ex C. Laws.) are parapatric along the crest of the Cascade Range in southern Oregon. A 3-year common-garden study of bud phenology and seedling vigor was performed to estimate the nature and magnitude of differentiation between races, to characterize the transition zone between them, and to relate responses between and within races to topography and climate. Principal component (PC) analyses identified two significant character complexes, PC-1 (phenological traits) and PC-2 (size traits), that explained 73% of the geographic race-related variation. The races were differentiated in two regards. First, PC-1 scores, which were highly correlated with frost-free season and summer-winter temperature differential, displayed a sharp discontinuity in the transition zone. Second, PC-2 scores were significantly correlated with physiographic and climatic variables in the North Plateau but not in the Pacific race, even though these variables had greater ranges in the latter. The data supported a narrow, adaptive transition between races for a complex of traits probably related to cold hardiness, and provided evidence that plant vigor traits were more closely adapted to environments in the North Plateau than in the Pacific region, possibly as a consequence of past climatic-stress selection in the former and competitive-stress selection in the latter.

**Résumé :** Les races du Pacifique et du Plateau du Nord qu'on distingue parmi les populations continues de pin ponderosa (*Pinus ponderosa* P. Laws. ex C. Laws.) sont parapatriques le long de la crête de la chaîne des Cascades dans le Sud de l'Oregon. À l'aide d'une étude en plantation comparative menée pendant 3 ans, les auteurs ont étudié la phénologie du bourgeon et la vigueur des semis afin d'évaluer la nature et l'ampleur de la différenciation des races, de caractériser la zone de transition entre ces dernières, et d'établir le lien entre d'une part le climat et la topographie et d'autre part les différences de réponse au sein des races et entre ces dernières. L'analyse en composantes principales (CP) a permis d'identifier deux groupes de caractères significatifs, CP-1 (les caractères phénologiques) et CP-2 (les caractères de dimension), qui expliquent 73% de la variation géographique liée aux races. Les races sont différenciées de deux manières. D'une part, les valeurs obtenues sur CP-1, qui sont fortement corrélées avec la longueur de la période sans gel et la différence de température entre l'été et l'hiver, montrent une forte discontinuité dans la zone de transition. En second lieu, les valeurs obtenues sur CP-2 sont corrélées significativement avec les variables physiographiques et climatiques chez la race du Plateau du Nord, ce qui n'est pas le cas pour la race du Pacifique et ce, même si ces variables montrent de plus grands écarts chez cette dernière. Les données supportent le concept d'une zone de transition étroite liée à l'adaptation entre ces deux races et ce, pour un ensemble de caractères associés probablement à l'endurcissement au froid. Les données supportent également l'idée voulant que les caractères liés à la vigueur de la plante soient adaptés de façon plus étroite à l'environnement dans la région du Plateau du Nord que dans la région du Pacifique, vraisemblablement en raison d'une sélection passée liée au stress climatique dans la région du Plateau du Nord, comparativement à une sélection liée au stress de compétition dans la région du Pacifique.

[Traduit par la Rédaction]

## Introduction

Intraspecific variation across the landscape can be either continuous (clinal; Huxley 1939) or discontinuous (ecotypic; Turesson 1922). A long history of provenance research in

widespread conifer species has indicated predominantly clinal variation (e.g., Schott 1904; Engler 1908; Langlet 1938; Squillace 1966; Campbell 1986, 1991; Ying 1991). Some examples of ecotypic or racial patterns have been observed, but these were usually explained by historical sepa-

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ration and secondary contact following migration (Aagaard et al. 1995; Schmidting and Hipkins 1998; Newton et al. 1999).

An exception in the conifer literature has been the variation pattern in ponderosa pine (*Pinus ponderosa* P. Laws. ex C. Laws.), which has been described as ecotypic (Wells 1964; Wright et al. 1969) or as having geographic races (Conkle and Critchfield 1988) that are relatively discrete (Burdon and Low 1991). Within races or portions of races, clinal patterns are well documented in seedling common garden studies (e.g., Rehfeldt 1986a, 1986b; Sorensen and Weber 1994) and in isozyme analyses (Westfall and Conkle 1992; Rotach 1997). Transition zones between races, particularly between the Pacific and North Plateau races in Oregon, are pictured as narrow (Conkle and Critchfield 1988), but in fact "the abruptness of the transition...is largely unknown" (Rehfeldt 1993) and may be more a reflection of poor sampling than a true discontinuity (Langlet 1959, 1963); that is, variation across the racial "boundary" might be clinal, but sampling has been too sparse or too clumped to detect the continuity. Alternatively, past separation and migration could bring together populations that differed for historical reasons (Latta and Mitton 1999), in which case the discontinuity might be real but not adaptive. Ponderosa pine is apparently a recent colonizer throughout much of its present range (Conkle and Critchfield 1988), and Lauria (1991) suggests that time has been insufficient and climatic changes too rapid for "present variability [...to be] an expression of active evolutive processes" in the phylogeny of ponderosa pine and its near relatives.

The distribution of ponderosa pine in southern Oregon extends from relatively mild, near-maritime climate in the western Siskiyou Mountains to a continental climate in the Warner Mountains about 300 km east (Johnsgard 1963). Species range in the west appears limited primarily by interspecific competition, as ponderosa pine disappears from the mixed conifer forest as the land mass approaches the ocean and as moisture stress lessens and temperatures moderate (Waring 1969). In the east, ponderosa is the dominant coniferous species and its range appears more determined by climatic factors, particularly at lower elevation, where ponderosa forest grades into the shrub steppe or *Juniperus occidentalis* Hook. open woodland (Franklin and Dyrness 1973).

The north-south oriented Cascade Range is approximately in the middle of this distribution. The crest of the Cascades, which is relatively low at this latitude, forms the presumed boundary between two races, designated North Plateau on the east and Pacific on the west. Sampling for some traits (monoterpenes, immature cone color) in natural stands in this area has shown a discontinuity between the two races (Smith 1977, 1981; Sturgeon 1979). Significant racial differences have been observed in common garden tests in stem taper (La Farge 1974), fall and winter cold hardiness (Kempff 1928; Weidman 1939; Haller 1961; Wells 1964; Larson 1966; Wright et al. 1969), foliage color (Wells 1964; Burdon and Low 1991), resistance to *Dothistroma* needle blight (*Dothistroma pini*) (Burdon and Low 1991), frequency of lammis growth (Wright et al. 1969), internal structure of needles (Weidman 1939), and needle thickness (Haller 1961). In most of these studies, however, samples were too dis-

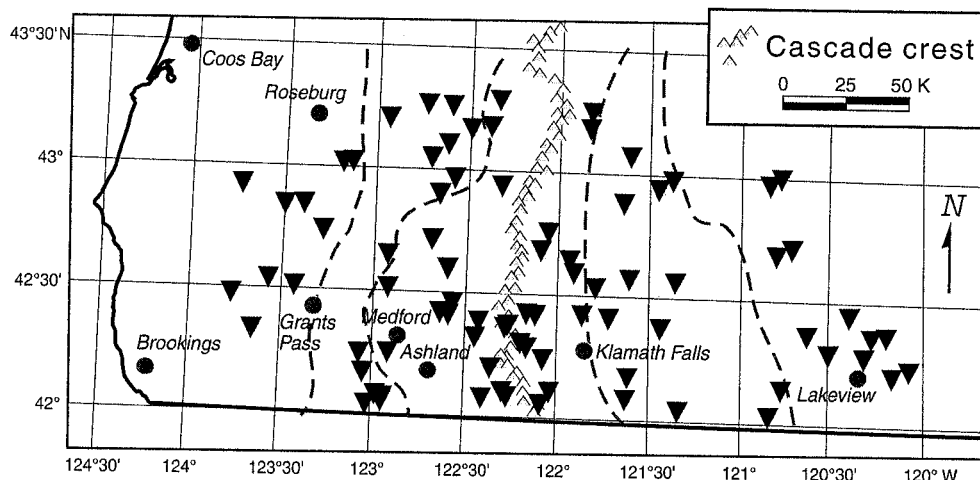
persed to identify the variation pattern as clearly clinal or ecotypic. From denser sampling, Burdon and Low (1991) note abrupt differences in several traits (noted above) but a clinal trend in height growth. Genetic distance based on allozymes indicates "a close genetic relationship between Pacific and North Plateau ponderosa pines" (Conkle and Critchfield 1988; also Niebling and Conkle 1990). Similarly, after analyzing a large number of samples in southwestern and central Oregon, Rotach (1997) found allozyme differentiation between the regions (races) as being "in the range of distances commonly found between populations (within races)" ( $F_{ST} = 0.01$ ).

Potential for gene migration and selection could influence the variation pattern across the transition zone. Ponderosa pine is anemophilous and mixed mating with generally high rates of outcrossing (Mitton et al. 1981; Farris and Mitton 1984; Sorensen 1994a). Pine pollen is about 50% air-filled bladders by volume, is of low density (Owens and Simpson 1986), and has extensive dispersal (McAndrews and Wright 1969). Ponderosa pine seeds are large and dispersal is localized (Latta et al. 1998). Most wind-dispersed seeds fall within 50 m of the source, and only small amounts are carried more than 150 m (Barrett 1966; McCaughy et al. 1986). Animals disperse seeds and place them favorably for germination, but this dispersal also appears to be local (West 1968; Vander Wall 1992). Longer distance dispersal on snow is possible (Heikinheimo 1932, also see Delcourt and Delcourt (1991) and Kullman (1998)). In good seed years, mature stands can produce several hundred thousand viable seeds per hectare (Dahms and Barrett 1975). It has been estimated that 1 seed in 20 to 1 seed in 100 becomes established in favorable and moderately favorable conditions (Schubert 1974; Pearson 1950), and seedling survival decreases by 75% or more by age 10 years (Fowells and Schubert 1956). Potentials for gene dispersal and selection both appear large.

In this study our goal was to evaluate patterns of adaptive variation within and between races in the area of the transition zone in southern Oregon. Recent climatic maps indicated that several growing season variables changed quite abruptly at the Cascade Crest in southern Oregon (maps generated by PRISM model, Daly et al. 1994; [www.ocs.orst.edu/prism](http://www.ocs.orst.edu/prism)). One of the major challenges for plants, particularly long-lived plants, is synchronizing growth and hardiness with the appropriate parts of the annual climatic cycle. Given the mating system (wind pollination, pollen widely dispersed) and reproductive system (large seed fall, very low mature tree:seed ratio) of ponderosa pine, what adaptive pattern might be expected where there is an abrupt climate change, and how might the pattern differ among traits?

Second, as noted earlier, the Pacific and North Plateau populations or races, although adjoining have been subject to different fitness stresses. In the more moderate Pacific region, the species usually grows in mixture with other conifers, and its range often appears to be limited by competitors. In the more climatically severe North Plateau, stands are often purely or predominantly ponderosa, and the species range in many places limited by climate (Cochran and Berntsen 1973; Steele 1988). How might genecological variation within races have been affected by these contrasting selective regimes?

**Fig. 1.** Distribution of parent tree locations (solid inverted triangles) in southwest Oregon. Pacific and North Plateau regions or races are separated by the crest of the Cascade Range; subregions within races are separated by thin broken lines. Large circles and names are town locations.



## Materials and methods

### Locations

Cones were collected from one or two trees (108 trees total) at 80 locations between 42°00' and 43°15'N, and from 150 km west to 160 km east of the Cascade crest (Fig. 1). Elevation gradually increases from west to east across the entire sample range. Special effort was made to obtain a good elevational distribution of locations. The Siskiyou Mountains west of the Cascades have deep valleys, and a wide range of elevations was sampled (245–1675 m); east of the Cascades the topography is more rolling, valleys are more shallow, and the elevational range of samples is corresponding less (1175–2165 m). Similarly, estimated climatic variables were more diverse in the Pacific than the North Plateau region (e.g., range in frost free season 69.3 days west vs. 40.9 days east, range in annual precipitation 2705 mm west vs. 1188 mm east). All parent trees were mature individuals growing in naturally regenerated stands.

### Nursery culture and experimental design

Seedlings were raised for 3 years in a common garden (Corvallis, Oreg.; 44°34'N, 123°27'W, 75 m elevation). Prior to sowing, seeds were placed by family in small net bags, soaked for 24 h in aerated water, water drained off, and packets put in a cooler at 2–3°C for 9 weeks. Stratified seeds were surface dried and sown, four per spot, in a raised nursery bed at 10 cm (between rows) by 7.5 cm (within rows) spacing in four-spot family row plots in a randomized complete block design with five replications. Two border rows were sown along each edge of the bed and eight buffer rows at each end. Seed spots were thinned systematically by direction (i.e., no selection for form or vigor) to three seedlings per spot after seed coat shed, to two seedlings at the end of the first growing season, and to one seedling at the beginning of the second growing season. Three traits were recorded prior to final thinning. Emergence variables were based on all germinant seedlings per spot. Height-1 and budset-1 were taken on the more western seedling in the spot and that seedling was left at the final thinning. Traits that were measured are listed in Table 1.

### Statistical models and analyses

Because we were comparing alternative hypotheses of stepped cline versus continuous cline, data were analyzed both with regression models using the  $R^2$  selection method of the SAS regression

procedure (SAS Institute Inc. 1990, p. 1339) and with a classification model (hierarchical analysis of variation; Snedecor and Cochran 1967, p. 285). Regression models used both physiographic and climatic information for independent variables.

Mean values for family row plots were the experimental units. Components of variance for each trait were estimated from a preliminary analysis of the form,  $Y_{ijk} = \mu + B_i + L_j + F_{jk} + \epsilon_{ijk}$ , where  $\mu$  is the grand mean,  $B$  is the replication effect ( $i = 1, 2, \dots, 5$ ),  $L$  is the random location effect ( $j = 1, 2, \dots, 80$ ),  $F$  is the random family-in-location effect ( $k = 1$  or  $2$ ), and  $\epsilon$  is the experimental error. Traits were deleted from further analysis if location variance was nonsignificant ( $p > 0.10$ ) or if two traits were highly correlated ( $r > 0.90$ ). In the latter case, the trait with the lower value of  $\sigma_L^2 / (\sigma_L^2 + \sigma_{\epsilon/L}^2)$  was deleted. For the remaining eight variables (Table 1, asterisks), principal components were calculated from the location mean correlation matrix by inputting location means into PROC PRINCOMP (SAS Institute Inc. 1990, p. 1241). The first two components particularly were biologically meaningful in that PC-1 loaded heavily for phenological variables, and PC-2 for vigor related traits. Factor scores for the principal components and some individual traits were analyzed.

For the physiographic regression model, latitude (LA), longitude (LO), and elevation (EL) were determined for each of the 80 locations. Selected trait values and factor scores of the PCs were fit to a physiographic model including linear through quartic terms of LO and EL, linear and quadratic terms of LA, and linear by linear interactions,  $LA \times LO$ ,  $LA \times EL$ , and  $LO \times EL$ . Cubic and quartic terms, which indicate a higher order change in the dependent variable than does the quadratic term, were included because the sample locations spanned a transition zone of unknown width between two "races." As it turned out, the higher order terms for longitude were retained in the physiographic models. Latitudinal distribution of locations was relatively narrow ( $1^\circ 15'$ ), but a preliminary test indicated LA to be generally significant; therefore, it was included. Regressions, using location means, were run for all combinations of 1–13 terms. The model selected for each PC was based primarily on  $R^2$  (Neter and Wasserman 1974, pp. 375–382), but we also took into consideration number and simplicity of terms. Lack of fit to the selected equations was tested by using as repeats family values from the 28 locations represented by two families each (Neter and Wasserman 1974, pp. 113–121). Because the classification analysis indicated a "step" at the Cascade crest, regressions also were run separately for the west (38 locations) and east (42 locations) regions.

**Table 1.** Size and other traits measured in the nursery test.

Trait No.	Variable	Description
<b>Size traits</b>		
1	H1 <sup>a</sup>	Final height, year 1 (cm)
2	H2E	Height measured early in elongation season, year 2
3	H2L	Height measured late but before elongation concluded, year 2
4	H2	Final height, year 2
5	H3	Final height, year 3
6	Hother <sup>a</sup>	Height, other (H3 - (H1 + (H2 - H2L)))
7	EI2	Early height increment, year 2 (H2E - H1)
8	LI2 <sup>a</sup>	Late height increment, year 2 (H2 - H2L)
9	D2	Diameter, year 2 (mm)
10	D3 <sup>a</sup>	Diameter, year 3
11	TW3	Top fresh mass at time of lifting, year 3 (g)
12	RW3	Root fresh mass at time of lifting, year 3
13	RGR23	Relative height increment, years 2 and 3 (ln H3 - ln H1)/2
14	NL	Needle length, year 2, taken 2-3 cm below highest needles on first flush (mm)
<b>Form traits</b>		
15	HD2	Height/diameter ratio, year 2
16	HD3	Height/diameter ratio, year 3
17	TWRW3 <sup>a</sup>	Top/root fresh mass ratio, year 3
<b>Phenological traits</b>		
18	EMERGMN <sup>a</sup>	Mean days to emergence for plot, observed daily from first emergence; day 1, April 23
19	EMERGSD	Within-plot standard deviation of emergence days
20	BS1 <sup>a</sup>	Date of bud set, year 1, observed weekly; day 1, July 27
21	BF3 <sup>a</sup>	Date of bud flush, year 3, observed half weekly; day 1, March 3, pm (date that bud scales on terminal buds reflexed was called date of bud flush)
22	SF2	Frequency of second flushing, year 2
<b>Other traits</b>		
23	SNS1	Secondary needle score, number of secondary fascicles on 1-year-old seedlings (0, no fascicles; 1, 1-5; 2, 6-10; 3, 11-15; 4, >15).

<sup>a</sup>Traits selected for principal component analysis.

**Table 2.** Description of variables used in the climatic regression model.

Variable	Description
TAVER	Mean annual temperature
TSUMWDIF	Difference between mean summer (June, July, August) and mean winter (December, January, February) temperatures
TSPDEDF	Difference between mean spring (March, April) and mean December temperatures
MINMIN	Mean minimum temperature of coldest month
FRSTFREE	Frost-free period
PTOTAL	Total annual precipitation
PM6M7TOT	Total June + July precipitation
PM6M7DF	June-July precipitation difference
PAUG	Mean August precipitation

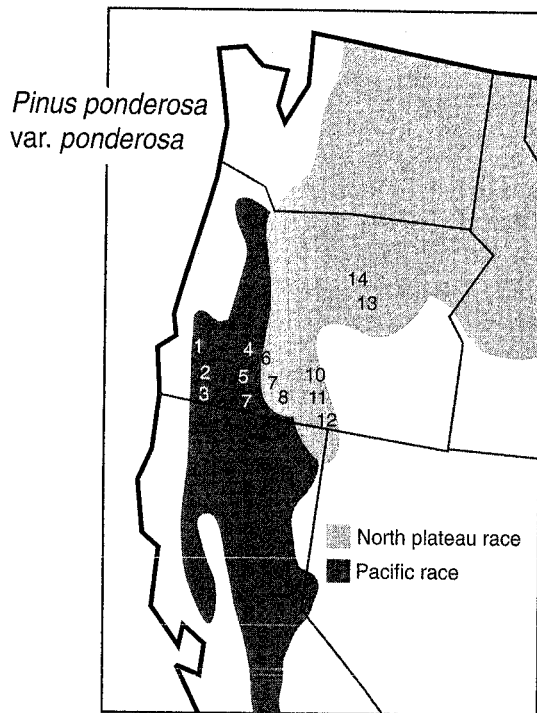
The climatic regression model was based on several temperature and precipitation variables that varied strongly across the landscape and were weakly correlated or not correlated one with another (J.B. St. Clair and K. Vance-Borland, unpublished climatic maps). These variables were derived from monthly mean, maximum, and minimum temperature (*T*) and precipitation (*P*) values for the parent tree locations, as determined by using the PRISM climate model (Daly et al. 1994). Principal component scores from the nursery test were regressed against several estimated climatic vari-

ables (Table 2) in a multiple regression analysis. This also was done for the whole area and separately for each region.

Because of the putative taxonomic distinction in the vicinity of the Cascade crest (Conkle and Critchfield 1988), a classification model was used to partition location variation according to a geographical hierarchy. The longitudinal range was first divided into the two regions (Pacific west and North Plateau east of the Cascade crest). Each region was further divided into three "physiographic subregions" (Fig. 1). Subregions were separated by more or less distinct north-south oriented topographic features and were included on the premise that if the Cascade crest was associated with a step in a longitudinal cline, similar topographic features within regions also might be associated with steplike changes. West of the Cascade crest, the subregions corresponded closely to the western Siskiyou, eastern Siskiyou, and Cascades as outlined in Waring (1969, Fig. 1). East of the crest, subregions were (i) east slopes of the Cascades down to the Klamath Lake basin, (ii) the Basin and Range Province of Franklin and Dyrness (1973, Fig. 2) and (iii) east of this province to the east edge of the range of ponderosa pine in south-central Oregon (east side of Warner Mountains). Finally, elevation was subdivided into 300-m bands (Sorensen 1994b). There were 9-16 sample locations (mean 13.3) per subregion.

Variance components in the classification analyses were estimated from a random nested model,  $Y_{ijklm} = \mu + R_i + S_{ij} + E_{ijk} + L_{ijkl} + F_{ijklm}$  where  $\mu$  is the grand mean,  $R$  is the effect of region,  $S$  is the effect of subregion within region,  $E$  is the effect of 300-m elevation band within subregion,  $L$  is the effect of location within elevation band within subregion, and  $F$  is the effect of family within

**Fig. 2.** Distribution of parent tree locations sampled for the RAPD analysis.



location. A significant  $L$  term represents lack of fit to the classification model. A large difference (step) between regions compared with the differences between subregions within regions would be evidence for a racial division (large  $R$  compared with  $S$ ) (Sorensen et al. 1990).

### Molecular markers (RAPDs)

Random amplified polymorphic DNA (RAPD) markers, a type of selectively neutral DNA-based marker (Williams et al. 1990), were used to assess molecular evidence for past geographic separation. One seed from a single maternal tree from each of seven locations per race (14 maternal trees total; Fig. 2) was used for total genomic DNA extractions from seed embryos. This sampling strategy was effective for identifying narrow racial boundaries in another conifer species in Oregon (Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco; see Aagaard et al. 1995).

Allelic frequencies within races for 20 RAPD markers (loci) were calculated from null homozygote frequencies assuming panmixis and corrected for dominance according to Lynch and Milligan (1994). Allelic frequency data were used to calculate Nei's (1973) measure of genetic differentiation between regions ( $G_{ST}$ ) and corrected for small sample size by using GENESTAT-PC 3.31 (Lewis 1994). Measurement of genetic differentiation between regions using Nei's  $G_{ST}$  is equivalent to the familiar  $F_{ST}$  of Wright (1965), and the two may be treated interchangeably in most cases (Nei 1977).

RAPD data also were analyzed using the analysis of molecular variation (AMOVA) approach of Excoffier et al. (1992). A distance matrix was constructed from the RAPD markers by using a metric distance measure ( $1 - (\text{fraction of matches})$ ). Simple dissimilarity has been employed previously as a measure of genetic distance with RAPD markers (e.g., Apostol et al. 1993) and has been used for similar studies in Douglas-fir (Aagaard et al. 1998). AMOVA provides an  $F_{ST}$  analog ( $\Phi_{ST}$ ) without the assumptions underlying allelic frequency calculations (e.g., dominance, panmixia). Permutation tests measure the statistical significance of genetic differenti-

ation (Excoffier et al. 1992). The computer program WINAMOVA (Excoffier 1993) was used for all AMOVA calculations.

### Flowering phenology

To determine if pollen exchange between races was restricted by flowering phenology, female flower receptivity and pollen shed were recorded every 4 days in May and June 1997 at 12 locations between  $122^{\circ}30'$  and  $121^{\circ}56'W$  (46 km lineal distance). These locations spanned the Cascade crest (Fig. 1). Longitudinal distance between observation points ranged from 0.6 to 7.7 km (mean 4.1 km). At each location, dates when 50% of cone buds were receptive and 50% of trees were shedding pollen were estimated. The phenology criteria were arbitrary; the goal was to use a stage that could be estimated consistently across locations.

## Results

### Principal component analysis (PCA) of seedling traits

The first three principal components explained 83.5% of the location variance associated with the eight traits entered into the PCA (Table 3). PC-1 loaded heavily for phenological traits and explained 42.6% of the location variation. Large PC-1 scores represented locations where phenological development started late and continued relatively late in the season. PC-2 loaded heavily for size traits and explained 30.1% of the location variation. Large PC-2 scores represented locations with large seedlings. PC-3 explained an additional 10.8% of the variation, and large scores indicated locations where seedlings had large top-to-root ratios and early bud flush in year 3. Because PC-3 scores had non-significant or weakly significant relationships with physiographic and climatic variables, this component is not included in most of the subsequent discussion.

### Physiographic regression model

Scores for PC-1 (phenology complex) were closely related to the physiographic variables for the entire sample area and also for each region separately (Table 4,  $R^2 \geq 0.69$ ,  $p < 0.0001$ ). For PC-2 (the vigor complex), the model for the North Plateau region had higher  $R^2$  than did the model for the entire area (Table 4), even though the latter included much greater elevational and longitudinal range. Lack of fit to the models was generally nonsignificant (Table 4, columns on right) thereby indicating that the models were adequate.

Two interesting regional contrasts were identified. First, the  $r$  value between PC-1 scores and elevation was greater in the Pacific ( $r = -0.723$ ;  $p < 0.001$ ) than in the North Plateau region ( $r = -0.524$ ;  $p < 0.001$ ). This was probably due to greater elevational range in the former region. Second, PC-2 scores responded strongly to elevation and longitude in the North Plateau but not at all in the Pacific region (Table 5). The contrast for PC-2 is particularly striking, because the range of sampled elevations was greater in the Pacific (245–1675 m) than in the North Plateau (1175–2165 m).

### Climatic regression model

Fits to the climatic model generally were a little lower (Table 6) than to the physiographic model (Table 4). (One weakness with a climatic model is that climate is not constant (Davis 1986; Lamb 1995). Selection is probably strongest during the regeneration phase which, in a long-lived

**Table 3.** Results of principal component analysis of eight independent seedling traits with loadings, eigenvalues, and percentage of location variance explained by the first three principal components.

Trait <sup>a</sup>	Loadings		
	PC-1	PC-2	PC-3
1. Height-1	0.015	0.601	-0.011
6. Height, other	0.190	0.565	0.027
8. Late height increment-2	0.476	-0.066	-0.040
10. Diameter-3	0.340	0.464	-0.085
17. Top/root fresh mass ratio-3	0.287	-0.066	0.834
18. Mean emergence date	0.479	-0.163	0.031
20. Date of bud set-1	0.442	-0.227	-0.065
21. Date of bud flush-3	0.338	-0.135	-0.539
Eigenvalue	3.409	2.409	0.867
Variation explained (%) <sup>b</sup>	42.6	30.1	10.8

<sup>a</sup>Trait numbers are as in Table 1.

<sup>b</sup>Percentage of location-related variation in the eight traits explained by the principal components.

conifer, may be spread over decades and reflect temporal variation in climate (Hamrick et al. 1979). The model, on the other hand, uses a standard set of years to model climate.) The results of the climatic model (Table 6) mirrored those of the physiographic (Table 4) in both relative sizes of  $R^2$  values and magnitudes of lack of fit.

Temperature variables associated with growing season length and winter cold (FRSTFREE, MINMIN, TSUMWNUF) were more strongly correlated with PC-1 scores across the entire sample area than within regions (Table 7). Within regions, temperature variables (particularly TAVER) were more strongly correlated with PC-1 scores in the Pacific than in the North Plateau, whereas summer precipitation variables (PAUG and PM6M7TOT) were much more strongly correlated with PC-2 scores in the North Plateau than in the Pacific region. We note again that the ranges in the climatic variables were greater in the Pacific than in the North Plateau.

Plotting of residuals for the climatic regression did not indicate differences in deviations associated with regions or with the subregions bordering the Cascade crest.

### Classification model

Two points were of primary interest in the classification analysis. First was to identify which traits had their family variation primarily among locations versus within locations. Second, in light of the taxonomic separation near the Cascade crest was to determine if there was a distinct difference in expression of adaptive traits where the two taxa came together. The ratio, mean square<sub>locations</sub>/mean square<sub>within locations</sub>, was several times larger for PC-1 and associated phenology traits than for PC-2 and vigor traits (Table 8). This meant that PC-1 scores, compared with PC-2 scores, had much more of the family variation among than within locations. When this location variance was classified over the landscape, PC-1 scores differed primarily between races (Table 9,  $\sigma_{S/R}^2$ ), and PC-2 scores differed primarily among elevation bands and among locations within elevation bands within the subregions (Table 9,  $\sigma_{E/S/R}^2 + \sigma_{L/E/S/R}^2$ ). Very little variation was

associated with subregions within regions (Table 9,  $\sigma_{S/R}^2$ ) in any trait.

The PC-1 scores differed between races ( $F = 84.80$ ;  $df = 1, 4$ ;  $p = 0.0008$ ), and there was a gradual common longitudinal trend within both races (Table 9, Fig. 3). Scores for other PCs are not illustrated, because they did not show significant differences at any level of classification. Variation in PC-1 scores among subregions was not significant ( $F = 0.67$ ;  $df = 4, 17$ ;  $P = 0.6185$ ); variation among elevation bands within subregions was ( $F = 4.18$ ;  $df = 17, 57$ ;  $p < 0.0001$ ). Because the elevational band in which ponderosa pine occurs increases from west to east, clines within regions possibly reflected the longitudinal change in elevation. The sharp step between regions (Fig. 3) was not associated with a topographic change.

Lack of fit to the classification model is given by the significance of  $\sigma_{L/E/S/R}^2$ . This term was not significant for PC-1 ( $F = 1.31$ ;  $df = 37, 28$ ;  $p = 0.2305$ ) but was for PC-2 ( $F = 2.12$ ;  $p = 0.0209$ ). Because sample locations potentially extended into the distribution of *Pinus washoensis* Mason & Stockwell in the east (Haller 1965; Sorensen 1994b) and definitely into the distribution of *Pinus jeffreyi* (Grev. & Balf.) in the west, we calculated mean  $\sigma_{L/E/S/R}^2$  for each subregion. For all PCs, and particularly for PC-2 (the size component),  $\sigma_{L/E/S/R}^2$  was largest in the westernmost subregion (Table 6). Apparently, the lack of fit to the classification model was due primarily to variability in the western subregion of the Pacific region.

### RAPD analyses

Genetic differentiation between regions was low to moderate depending on the method of analysis. Based on AMOVA, 13% of RAPD diversity was located between, as opposed to within, regions ( $\Phi_{ST} = 0.13$ ;  $p = 0.002$ ). Nei's  $G_{ST}$  indicated a somewhat higher degree of differentiation ( $G_{ST} = 0.25$ ) owing in part to the small sample size correction used in the analysis (Nei's unmodified  $G_{ST} = 0.18$ ). Because of problems when allele frequencies are estimated with small sample sizes using dominant markers (Lynch and Milligan 1994) and simulation studies showing that  $G_{ST}$  values tend to be inflated at the sample sizes we used here (Aagaard et al. 1998), we placed greater emphasis on the AMOVA results.

### Phenological observations

Phenological observations were made along 33 min of longitude (46 km) spanning the summit of the Cascades. There was no correlation between elevation and west-east position within this distance ( $r = 0.037$ ;  $df = 10$ ;  $p > 0.05$ ). Female and male flowering dates correlated with elevation ( $r = 0.719$  and  $0.816$ , respectively;  $df = 10$ ;  $p < 0.01$ ) but not with longitude ( $r = 0.076$  and  $0.066$ , respectively). There was no phenological break or even gradient across the transition zone independent of local elevation effect, and thus, there was no indication of a barrier to gene flow between races.

### Discussion

#### Differentiation between regions

The two trait complexes had different patterns of varia-

**Table 4.** Coefficients of determination ( $R^2$ ) for regressions of factor scores of the first two principal components (PC) against physiographic variables.

Area (race) and PC	Regression model				Lack of fit		
	$R^2$	$F$	df	$p^a$	$F$	df	$p^a$
Entire area							
PC-1	0.835	51.96	7, 72	<0.0001	1.64	72, 28	0.0729
PC-2	0.279	3.97	7, 72	0.0010	2.06	72, 28	0.0174
Pacific							
PC-1	0.748	7.99	10, 27	<0.0001	1.08	27, 12	0.4645
PC-2	0.294	1.51	8, 29	0.1963	1.86	29, 12	0.1818
North Plateau							
PC-1	0.694	9.35	8, 33	<0.0001	1.92	33, 16	0.0834
PC-2	0.653	9.14	7, 34	<0.0001	1.51	34, 16	0.1892

Note: Regressions are based on the entire area and on the Pacific and North Plateau regions separately. Tests for lack of fit to the regression models are given in the columns to the right.  
<sup>a</sup>Probability that  $R^2$  or lack of fit test equals zero.

**Table 5.** Correlation coefficients ( $r$ ) for factor scores of the first two principal components (PC) regressed individually against elevation and longitude across the entire area and within the regions separately.

Physiographic variables	Correlation coefficient for PC-1			Correlation coefficient for PC-2		
	Entire area ( $n = 80$ )	Pacific ( $n = 38$ )	North Plateau ( $n = 42$ )	Entire area ( $n = 80$ )	Pacific ( $n = 38$ )	North Plateau ( $n = 42$ )
Elevation	-0.866****	-0.723****	-0.524***	-0.195	-0.041	0.706****
Longitude	0.730****	0.206	0.357*	0.168	0.000	0.465**

Note: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P > 0.0001$ .

**Table 6.** Coefficients of determination ( $R^2$ ) for regressions of factors scores of the first two principal components (PC) against climatic variables.

Area (race) and PC	Regression model				Lack of fit		
	$R^2$	$F$	df	$p^a$	$F$	df	$p^a$
Entire area							
PC-1	0.776	51.22	5, 74	<0.0001	2.27	74, 28	0.0087
PC-2	0.183	3.32	5, 74	0.0093	2.10	74, 28	0.0153
Pacific							
PC-1	0.608	8.03	6, 31	<0.0001	1.97	31, 12	0.2070
PC-2	0.393	2.77	7, 30	0.0239	1.54	30, 12	0.2176
North Plateau							
PC-1	0.402	3.92	6, 35	0.0042	1.54	35, 16	0.1770
PC-2	0.502	4.89	7, 34	0.0007	1.82	34, 16	0.1010

Note: Regressions are based on the entire area and on the Pacific and North Plateau regions separately. Tests for lack of fit to the regression models are given in the columns to the right.  
<sup>a</sup>Probability that  $R^2$  or lack of fit test equals zero.

tion. PC-1 scores differed significantly between regions indicating two races. PC-2 scores, RAPD markers, and allozymes (Rotach 1997) indicated little or no difference between regions. We will discuss the PC-1 pattern first.

For PC-1 scores, the classification analysis had a large variance component associated with races ( $\sigma_R^2 = 0.818$ ; Table 9). The physiographic regression model explained only slightly more of the variance ( $R^2 = 0.835$ , Table 4) than did regions in the classification model. Such close agreement between the two models indicated a steeply stepped cline (Stern 1964; Sorensen et al. 1990). Also, when region was included as a qualitative variable in the regression analysis,

region was significant ( $p < 0.001$ ), and region did not interact with any of the physiographic terms ( $p > 0.10$ ), i.e., slopes of the regression lines within regions were not significantly different and the lines did not meet at the crest (Fig. 3).

That the ponderosa races differed in a cluster of traits synchronizing growing-season cycle with annual temperature cycle agreed well with past field and nursery tests, which reported differences between the two races in autumn and winter cold hardiness (Kempff 1928; Weidman 1939; Haller 1961; Wells 1964; Larson 1966; Wright et al. 1969).

What was striking for a trait complex apparently adaptive

**Table 7.** Correlation coefficients ( $r$ ) for factor scores of the first two principal components (PC) regressed individually against four temperature and three precipitation variables for the whole area and for the two regions separately.

	Correlation coefficient for PC-1			Correlation coefficient for PC-2		
	Entire area ( $n = 80$ )	Pacific ( $n = 38$ )	North Plateau ( $n = 42$ )	Entire area ( $n = 80$ )	Pacific ( $n = 38$ )	North Plateau ( $n = 42$ )
TAVER	0.348**	0.455**	0.039	0.013	-0.046	0.119
FRSTFREE	0.831****	0.451**	0.333*	0.025	-0.003	0.090
MINMIN	0.836****	0.496**	0.404**	0.036	0.047	0.248
TSUMWNDF	-0.562****	-0.067	0.042	-0.037	0.074	-0.156
PAUG	0.192	-0.228	-0.546****	-0.037	0.155	-0.598****
PM6M7TOT	0.003	-0.365*	-0.137	-0.192	-0.062	-0.419**
PTOTAL	0.321**	-0.297	0.082	-0.043	-0.071	-0.028

Note: Description of the climatic variables is given in Table 2. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P > 0.0001$ .

**Table 8.** Mean squares associated with locations ( $MS_L$ ) and families in locations ( $MS_{F/L}$ ) for selected growth, phenology and form traits, and for the three principal components.

Trait	Classification level		
	$MS_L$	$MS_{F/L}$	$MS_L/MS_{F/L}$
PC-1	19.78****	2.43****	8.14
PC-2	14.39**	5.71****	2.52
PC-3	5.07*	2.46*	2.06
H1	34.32**	13.73****	2.50
H2	207.3****	52.05****	3.98
H3	983.3****	202.9**	4.85
EMERGMN	217 752****	29 321****	7.43
EI2	10.00**	4.22****	2.37
LI2	81.94****	8.88*	9.23
TW3/RW3	4.94	3.52****	1.41

Note: Trait abbreviations are as in Table 1. The two centre columns are type III mean squares from the general linear model analysis of variance. The right column gives the ratio between the two mean squares. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ .

to climate was the abruptness of the step, and the size of the break between races compared with the shallow gradients within races (Fig. 3). This was unexpected both because of the potential for gene flow in a large outcrossing species, and because "...climatic factors are generally highly variable in time, but less variable over short distances" (Snaydon 1970). There are exceptions (Aston and Bradshaw 1966), but usually, abrupt breaks are associated with edaphic or management variables (Jowett 1964; Snaydon and Davies 1976), which were not operating here.

Historical evidence indicates that ponderosa pine occupied its present range relatively recently (Conkle and Critchfield 1988; Lauria 1991). Therefore, the step at the Cascade crest could be due to an historical factor (e.g., migration of the races from separate refugia) or to ongoing selection. Three observations, taken together, provide evidence that selection has been important: (i) the presence of a step in one trait complex (PC-1) but not in others, (ii) the relatively high correlation between PC-1 scores and current temperature variables, and (iii) the lack of differentiation in putatively neutral markers (isozymes, RAPDs). If historical factors were the dominant basis for the step we would expect larger differentiation between the two regions in marker traits be-

cause of past restrictions on gene flow and drift (Aagaard et al. 1995; Latta and Mitton 1999).

Three other traits, resin composition, western pine beetle (*Dendroctonus brevicomis* LeConte) resistance, and immature cone color also show a steep cline or step near the Cascade crest (Smith 1977, 1981; Sturgeon 1979) that appears to be adaptive. Historically, the pine beetle has been more of a pest east than west of the crest (Eaton 1941; Miller and Keen 1960). Data collected by Sturgeon (1979) suggest that "...western pine beetle may be exerting a frequency-dependent selection pressure on chemically polymorphic populations..." Immature cone color of ponderosa changes from predominantly green (west) to predominantly purple (east) near the crest (Smith 1981). An increase in frequency of purple-coned trees in other species has been associated with increased elevation (Sturgeon and Mitton 1980; Pukacki and Modrzyński 1988). Pigmentation has been previously related to conelet internal temperature (Sturgeon and Mitton 1980) and to protection from high ultraviolet B radiation (Caldwell 1981). Although the adaptive significance is only anecdotal, the observed differences do provide additional evidence for a change in selection pressures immediately on opposite sides of the Cascade crest even though the crest is almost undefined at this location.

Where selection is important, the steepness of a step in a cline is determined by a balance between gene flow and selection (Jain and Bradshaw 1966; McNeilly 1968; Wright 1978). Ponderosa pine seed dispersal is limited, but pollen dispersal is potentially great and apparently not restricted in this area by phenological differences. Anemophilous large woody species generally reflect this dispersal potential by large genetic diversity within populations and low population differentiation (Hamrick and Godt 1996). The present results, therefore, indicate strong selective differential (Watson 1969; Bradshaw 1972; Endler 1973; Levin 1988) over a short distance. The slightly larger PC-1 values for  $\sigma^2_{L/E/S/R}$  in the two subregions adjoining the step (Table 10, Pacific subregion 3 and North Plateau subregion 1 compared with subregion 2 in both regions) are compatible with some gene exchange across the step (Antonovics and Bradshaw 1970).

#### Adaptive variation within regions

The second unexpected racial difference associated with the transition zone was the much higher physiographic coefficient of determination ( $R^2$ ) for PC-2 (vigor) scores in the

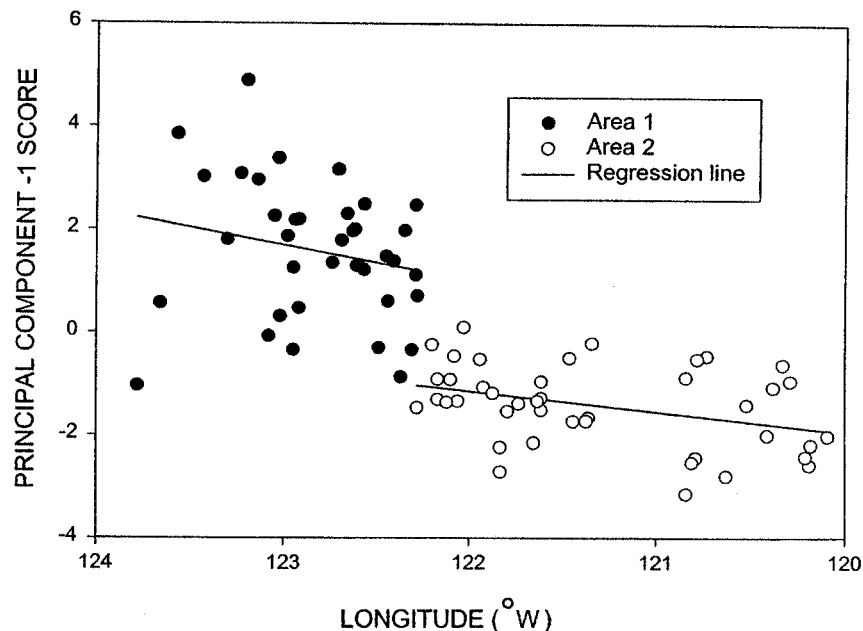


**Table 9.** Selected structural relations in the variability among seedlings illustrated by coefficients of variation ( $\times 100$ ) for families-in-locations and for plot error, and by components of variation ( $\times 100$ ) for the four classification levels for locations, regions ( $\sigma_R^2$ ), subregions in regions ( $\sigma_{S/R}^2$ ), 300-m elevation bands in subregions ( $\sigma_{E/S/R}^2$ ), and for locations in elevation bands in subregions ( $\sigma_{L/E/S/R}^2$ ).

Trait	$x$	Coefficients of variation		Classification levels			
		$\sigma_{F/L}/x$	$\sigma_p/x$	$\sigma_R^2$	$\sigma_{S/R}^2$	$\sigma_{E/S/R}^2$	$\sigma_{L/E/S/R}^2$
PC-1	0	—	—	81.8	0	14.2	4.0
PC-2	0	—	—	0	6.9	22.5	70.6
PC-3	0	—	—	0	0	0	100.0
H1	10.1	8.1	8.6	0	0.3	18.4	81.4
H2	24.7	5.3	9.4	37.1	9.1	18.3	35.5
H3	54.2	4.4	10.1	35.7	10.5	24.9	28.9
EMERGMN	678.0	10.3	15.1	83.3	0	2.2	14.5
EI2	3.39	13.1	15.6	46.4	0	0	53.6
LI2	3.88	11.0	30.8	65.9	0.1	20.3	13.7
TW3/RW3	4.81	7.2	13.3	70.9	0	2.8	26.3

**Note:** Representative size, phenology, and form traits and three principal components are used for illustration. Trait codes are given in Table 1. Components of variation were determined using restricted maximum likelihood estimation procedure and are given in percentages.

**Fig. 3.** Plot of principal component-1 scores against longitude and their within-region regression lines. Solid circles, Pacific region locations; open circles, North Plateau locations.



North Plateau than in the Pacific race (Table 5). This occurred in spite of a greater elevational range in the Pacific region (Table 11). Other conifers have also shown steeper elevational gradients for vigor traits inland than near the coast (Campbell and Sorensen 1978; Campbell 1986). One explanation is that there is greater directional movement of wind-dispersed pollen near the coast than inland. Extensive gene flow might counteract selection. This seems unlikely, because gene flow should have an homogenizing effect on all traits; however, in fact, source-related variation in PC-1 scores was as great or greater in the Pacific as in the North Plateau (Table 5, also compare  $R^2$ s for PC-1 in the two regions in Tables 4 and 6). A second possible explanation is that climatic gradients are not as steep near the ocean as they are inland (Manley 1945). This did not seem to be the

case for ponderosa pine, because ranges for estimated climatic variables for the sample sites also were greater in the Pacific region than in the North Plateau (Table 11). Nevertheless,  $R^2$ s for PC-2 scores regressed against climatic variables were larger in the North Plateau than in the Pacific race (Table 6). This suggests that the two regions differed either in the selective environment, in the ability of the races to respond to selection, or both.

As noted in the Introduction, the Pacific climate is relatively moderate, and ponderosa pine distribution is more often limited by interspecific competition, whereas the North Plateau climate is more continental and ponderosa's range climatically determined. Volis et al. (1998) found greater interpopulation variance among marginal than among central populations of *Hordeum spontaneum* Koch in a common

**Table 10.** Mean values for  $\sigma_{L/E/S/R}^2$  for each of the six subregions.

Region and subregion	N	$\sigma_{L/E/S/R}^2$		
		PC-1	PC-2	PC-3
Pacific				
1	3	1.66	14.15	1.82
2	5	0.46	1.33	1.09
3	5	0.68	2.04	1.00
North Plateau				
1	3	0.60	1.16	0.55
2	3	0.23	0.46	0.56
3	4	0.23	1.23	0.42

**Note:** Subregion 1 is the westernmost part of the Pacific region and is in the western Siskiyou Mountains; the other subregions are progressively eastward. N, number of elevation bands on which the mean value is based.

garden test, where marginal populations were defined as those native to arid locations and central populations were native to more mesic locations. This suggests that climatic-stress selective strategy, compared with competition strategy, may involve greater sensitivity to environmental cues for some traits (Hoffmann and Parsons 1991).

A second possibility involves the ecological aspects of genetic variances and heritability (Prout and Barker 1989). Mousseau and Roff (1987) and Hoffmann (2000) in surveys of literature on wild, outbred animals found that life-history traits had lower heritabilities than morphological traits. Following Hoffmann and Parsons (1991), we suggest that the contrast among traits reported by Mousseau and Roff (1987) may extend to the response of an individual trait in contrasting environments; that is, vigor may respond more like a morphological trait in a climatic-stress environment, more like a fitness trait in a competitive-stress environment. In other words, past competitive stress selection may have partially depleted additive genetic variance for vigor and reduced ability to respond to differences in climate (Bradshaw 1991). We sampled two families at several locations in each race. Pooled heritabilities for three vigor traits were all lower for the Pacific than for the North Plateau race, but family numbers were much too small to do more than indicate that further testing would be valuable.

Whatever the cause, PC-2 vigor scores of the two races have reacted differently to physiographic and climatic variability. There appears to be some connection between the ecological situation and genetic variances. Determination of this relationship is basic to interpreting past selective responses and predicting future response to management and to environmental change (Mitchell-Olds 1986; Mousseau 2000). Most important for widespread coniferous species would be the predictive aspect, which will involve a comparative evaluation of genetic variances and heritabilities both in a common garden setting and in the wild (Jenkins et al. 1997).

#### Local variation in the westernmost subregion

Local population variation, particularly for the seedling size PC, was much larger in the westernmost subregion than elsewhere (Table 10). Possible contributors to this were adaptation to the large climatic, topographic, and particularly

**Table 11.** Range of values in two physiographic and six climatic variables for the entire area and for the areas occupied separately by the Pacific and North Plateau regions.

Variable	Entire area	Pacific region	North Plateau region
Longitude (°)	3.69	1.50	2.19
Elevation (m)	1920	1432	987
TAVER (°C)	9.41	9.41	5.52
FRSTFREE (days)	115.6	69.3	40.9
TSUMWVDF (°C)	8.38	4.78	5.90
PAUG (mm)	46.4	43.2	18.3
PM6M7TOT (mm)	66.5	66.5	52.5
PTOTAL (mm)	2970	2705	1188

**Note:** Climatic variables are given in Table 2.

edaphic diversity of the western Siskiyou Mountains (Whittaker 1960; Jenkinson 1974), or varying levels of introgression with *Pinus jeffreyi*, which is slower growing than ponderosa pine (Burdon and Low 1991). The size of the contrast in  $\sigma_{L/E/S/R}^2$  values in Table 10 and the moderate physiographic and climatic  $R^2$  for PC-2 in the Pacific region (Tables 4 and 6) suggest that adaptive response to climatic variation may be of only minor importance. Jenkinson (1974) and Millar and Jenkinson (1995) report no growth difference between ponderosa pine families from granitic and ultramafic soils when compared on granitic nursery soils. The estimated crossability between *Pinus jeffreyi* and California ponderosa is low (<5%; Conkle and Critchfield 1988, Fig. 7). Natural crosses have been observed, but they are rare (Oliver and Ryker 1990). Thus, at present the evidence both for selection and for introgression is weak, and further testing is indicated.

## Conclusions

The geographic variation pattern in ponderosa pine has been described as ecotypic (Wells 1964) with narrow transitions between "races" (Conkle and Critchfield 1988) and clines within races (Rehfeldt 1986a, 1986b; Westfall and Conkle 1992; Sorensen and Weber 1994; Rotach 1997). A transition zone in central Montana was attributed to secondary contact after historical separation (Latta and Mitton 1999). In contrast to that, the abrupt transition in southern Oregon apparently has resulted from response to selection in contrasting temperature environments. The differentiation is primarily associated with a complex of traits probably related to cold hardiness. A second important racial contrast in southern Oregon was the different physiographic and climatic clines within races. The clines were steeper in the North Plateau than in the Pacific region, and we suggested that this might have been a consequence of climatic-stress selection in the former and competitive-stress selection in the latter.

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