Adaptation of *Picea engelmannii* populations to the heterogeneous environments of the Intermountain West

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Seedlings from 104 natural populations of Engelmann spruce (*Picea engelmannii*) and 10 blue spruce (*Picea pungens*) populations from the Intermountain West, U.S.A., were compared in common gardens. Comparisons involved 16 characters that described growth, development, and cold hardiness plus nine monoterpenes. Canonical discriminant analyses detected one population of blue spruce that had been misidentified, readily separated populations of blue and Engelmann spruce, suggested that three Southwest populations differed markedly from Intermountain populations of Engelmann spruce, but identified no Intermountain populations that reflected introgression with blue spruce. Genetic differences were detected among populations of Engelmann spruce for 16 of the morphometric characters, and multiple regression models accounted for as much as 70% of the variance among populations. The regressions described genetic variation occurring along clines that were dominated by elevational and latitudinal effects. The slope of the clines, however, was relatively gentle; populations located within the same physiographic province must be separated by at least 420 m in elevation before genetic differentiation becomes a reasonable possibility. By providing a means for assessing the degree by which similar genotypes recur across the landscape, the regression models have direct practical application in programs ranging from land management to conservation biology.

Key words: population differentiation, genetic variation, ecological genetics.

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L'auteur a comparé, dans un même jardin, des plantules d'épinette d'Engelmann (*Picea engelmannii*) et d'épinette bleue provenant respectivement de 104 et de 10 populations naturelles de la région d'Intermountain Ouest aux États-Unis. Ces comparaisons impliquent 16 caractères décrivant la croissance, le développement et la tolérance au froid, en plus de neuf monoterpènes. L'analyse par discrimination canonique a mis en évidence une population d'épinette bleue mal identifiée, permet de distinguer facilement les populations d'épinette bleue et d'Engelmann, et suggère que trois populations du sud-ouest diffèrent de façon marquées des populations d'Intermountain, mais ne permet pas d'identifier des populations d'Intermountain reflétant une introgression par l'épinette bleue. On décèle des différences génétiques dans les populations d'épinette d'Engelmann impliquant 16 des caractères morphométriques, et les modèles de regression multiple expliquent jusqu'à 70% de la variance entre les populations. Les regressions décrivent la variation génétique comme reliée aux pentes où dominent les effets de l'élévation et de la latitude. L'acuité des pentes est cependant relativement faible; les populations localisées dans une même province physiographique doivent être séparées par au moins 420 m d'élévation avant que la variation génétique devienne raisonnablement possible. En donnant un moyen d'évaluer le degré par lequel des génotypes similaires se retrouvent dans le paysage, les modèles de regression offrent une application pratique directe dans les programmes allant de l'utilisation des terres jusqu'à la biologie de la conservation.

Mots clés : différenciation des populations, variation génétique, génécologie.

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Introduction

Natural systems of genetic variability have been molded by spatial and temporal environmental heterogeneity to produce local populations (sensu Mayr 1970) composed of adaptively similar, interbreeding individuals. With the ecological optimum of each population at different points along the environmental gradient, the series of local populations thus describes the ecological amplitude of a species. The breadth of a population's ecological niche, however, depends on the degree to which the phenotype is under the predominant control of either the genotype or the environment (Levins 1968). While genetic differentiation is ideal for coping with the spatial heterogeneity that occurs across broad environmental gradients, phenotypic plasticity is absolutely necessary for accommodating temporal heterogeneity. Adaptedness thus reflects a balance between alternative mechanisms, with evolutionary history apparently determining whether the balance favors differentiation or plasticity (Via and Lande 1987).

Species of forest trees inhabiting the mountains of northern Idaho, for example, exhibit the different mechanisms by which

adaptedness can be achieved. Genetically differentiated populations of *Pseudotsuga menziesii* tend to occupy habitats that differ by at least 18 days in the mean frost-free period (Rehfeldt 1989). A corresponding interval is 20 days for *Pinus contorta* (Rehfeldt 1988), 35 days for *Pinus ponderosa* var. *ponderosa* (Rehfeldt 1991), 40 days for *Larix occidentalis* (Rehfeldt 1982), and 55 days for *Thuja plicata* (Rehfeldt 1994). In *Pinus monticola*, however, population differentiation has not been demonstrated even though the species has an ecological distribution that encompasses frost-free periods that differ by as much as 80 days (Rehfeldt et al. 1984; Steinhoff et al. 1983). Because all of these species frequently occur in the same plant associations (Daubenmire and Daubenmire 1968), there seems little doubt that similar environments are being responded to much differently. In *Pinus monticola* and *T. plicata*, adaptedness is provided predominantly by phenotypic plasticity, while in *Pinus contorta* and *Pseudotsuga menziesii*, populations are much more specialized.

The present study considers Engelmann spruce (*Picea engelmannii*), a subalpine species broadly distributed across 23° of
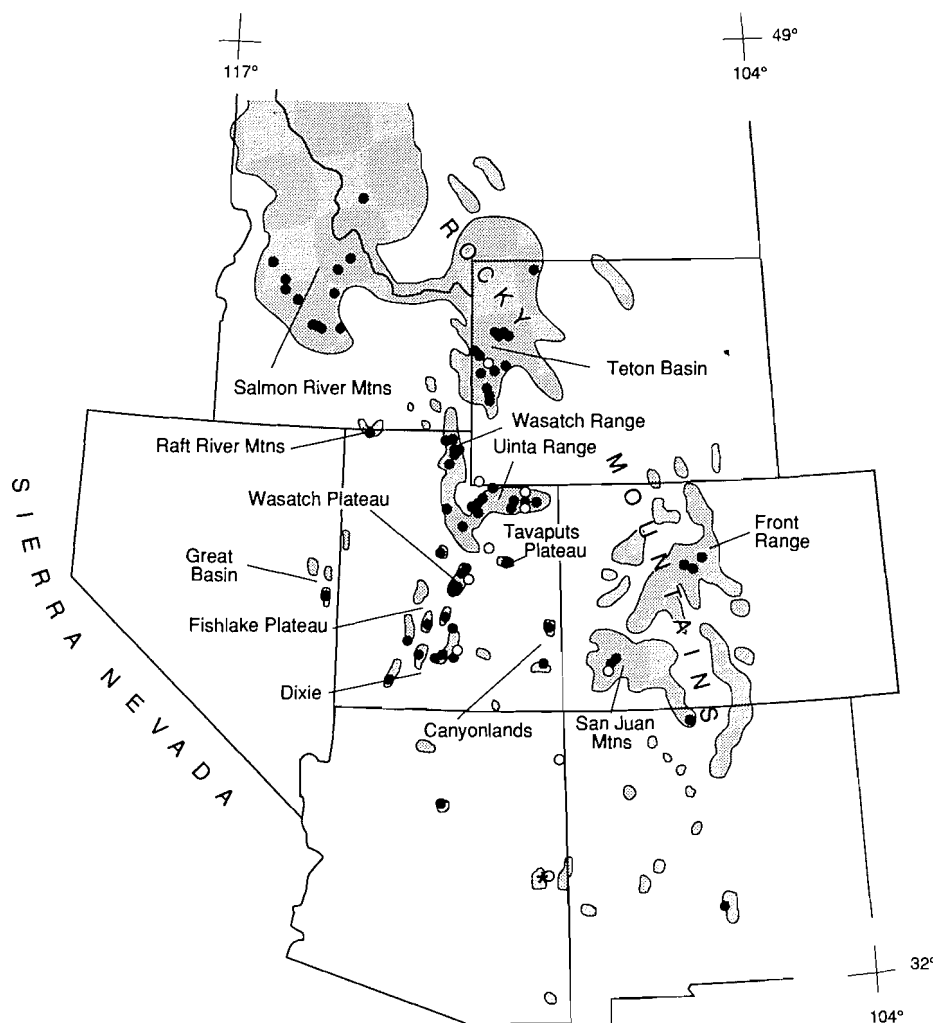
FIG. 1. Region of study locating the populations sampled within the distribution (shading, after Little 1971) of Engelmann spruce. ●, Engelmann spruce; ○, blue spruce; *, misidentified blue spruce.

latitude (Little 1971) and 3000 m of elevation (Alexander 1958). While population differentiation in this species has been documented (Roche 1969; Shepperd et al. 1981), little is known concerning the genetic structure of populations. The primary objective of this study was to contribute to an understanding of the system of genetic variability of populations from the Intermountain West, the vast area lying between the Sierra Nevada and the Rocky Mountains (Fig. 1). An understanding of the natural genetic system determines suitable approaches to programs ranging from land management to conservation biology. Future work will address populations in the Southwest and in the Inland Northwest.

Complicating genetic studies in the genus *Picea* are first, low levels of morphological and anatomical variation among species (Wright 1955) and second, tendencies toward introgression between species that are largely allopatric (see Sigurgeirsson 1992). The first of these factors makes individual species difficult to recognize, and the second confounds studies of intraspecific variation. In British Columbia, for instance, introgression among *Picea glauca*, *Picea sitchensis*, and *Picea engelmannii* is commonplace (see Daubenmire 1974); while clines in adaptive traits tend to follow environmental gradients (Roche 1969), the degree to which this variation reflects either introgression or microevolution is largely unknown (Daubenmire 1968; Fowler et al. 1988).

In the Intermountain region, the subalpine Engelmann spruce and the montane blue spruce (*Picea pungens*) co-occur in a narrow elevational band. Within this band, individuals occasionally occur that seem to be phenotypically intermediate between the species (Daubenmire 1972; Taylor et al. 1975). In some populations, species' identification has been so difficult that discriminant functions have been devised to aid in distinguishing species and putative hybrids (Schaefer and Hanover 1985, 1986). Even though genetic studies of natural populations suggest that Engelmann spruce and blue spruce are incompatible and do not hybridize (Fechner and Clark 1969; Kossuth and Fechner 1973; Mitton and Andalora 1981), artificial hybridization will yield some viable seeds when *Picea engelmannii* is the female parent (Ernst et al. 1990). These results, coupled with the introgression that has been documented within the genus (Roche 1969; Daubenmire 1974), suggest that hybridization cannot be ignored when studying genetic variability in natural populations of either Engelmann or blue spruce.

Materials and methods

The growth, development, and monoterpene composition of seedlings representing 114 spruce populations (Fig. 1) were compared in common environments. Of these populations, 104 were identified in their native habitat as Engelmann spruce, while 10 were identified as blue spruce. Identification was based on the morphological charac-

teristics that are used by field personnel: bark, twig, cone, and branching characters (Jones and Bernard 1977).

The Engelmann populations intensively sampled the ecologic, geographic, and elevational distribution of the species in the Intermountain region; additional populations from peripheral areas were included primarily to provide a link to future studies. Sampled populations were grouped into physiographic provinces (Table 1; Fig. 1), which will be referenced subsequently by the average latitude and longitude of the populations located therein. Note that the distribution of Engelmann spruce in these physiographic regions varies from broad and extensive in the Teton Basin and Salmon River Mountains to small and isolated in the Great Basin, the Raft River Mountains, and the Tavaputs Plateau. All populations were in mature forests with ages approaching or exceeding 100 years.

Populations of blue spruce broadly sampled the species distribution and were included to provide a basis for assessing hybridization and introgression.

In each population, wind-pollinated cones exhibiting a variety of morphologies, sizes, and colors were collected from several squirrel caches within an area of 5–10 ha. Seeds extracted from these cones thus provided a broad sample of the genetic diversity of each population. In this paper, populations reference groups of interbreeding individuals growing at the same locality.

Field tests

Seedlings from each population were grown in plastic containers (65 cm³) in a shadehouse at Moscow, Idaho (latitude 46.7°N, longitude 117°W, elevation 690 m), and 1-year seedlings were fall-planted in common gardens at three environmentally disparate sites: Moscow, 800-m elevation and 130-day frost-free season; Benton Flat, 720-m elevation and 90-day frost-free season; and Tarlac, 1500-m elevation with a frost-free season known only to be short. The latter two sites were at the Priest River Experimental Forest that is in northern Idaho, 190 km north of Moscow.

Field tests were established in a randomized, complete-block design that consisted of two blocks at Moscow and three blocks at both Priest River plantings. Each population was represented in each block by 10 trees planted in row plots. At Moscow, 12 cm separated rows and 10 cm separated seedlings within rows; at Benton Flat the corresponding spacings were 50 and 36 cm, and at Tarlac, 14 and 10 cm. The residual sandy-loam soil was used at Benton Flat, but at Moscow and Tarlac trees were planted in beds filled to a depth of 30 cm with a commercial mixture of peat and vermiculite. All sites were tilled and fenced before planting and were irrigated and weeded periodically. Testing was completed before competitive effects masked genetic differences: age 4 at both Benton Flat and Tarlac and age 3 at Moscow. Variations in experimental design between these sites allowed optimal utilization of the available growing space without confounding the statistical analyses.

Disparate environmental effects at the various planting sites meant that a different set and number of variables were required to describe growth and development. Field tests thus contributed a diverse array of 10 site-specific variables that included morphometric traits, spring and winter freezing damage, and leaf color (Table 2). Of these variables, note that the deviations are residuals from regressions that account for the autocorrelation of the annual shoot growth of trees. As such, they are relatively independent of prior effects and are capable of expressing adaptedness to a particular environment in a short time. Tree heights were not considered at Moscow and Benton Flat because of environmental effects that confounded the expression of growth potential. (The term growth potential is used here as the ability to produce photosynthate and assimilate wood in the absence of environmental effects that mask the genotype.) Trees at Moscow suffered freezing damage to terminal shoots during the second and third winters, while those at Benton Flat suffered so severely from frost heaving during the first winter that shoot elongation in the 2nd year became hopelessly confounded. In fact, data from 18% of the trees planted at Benton Flat were discarded because of the frost heaving.

Greenhouse tests

Seedlings from each population were grown for 6 months in plastic

TABLE 1. Number of populations sampled and altitudinal range of natural populations of Engelmann spruce according to physiographic region

Physiographic region	No. of populations	Altitudinal range (m)
West Salmon River Mountains	4	1800–2200
East Salmon River Mountains	6	2100–2600
North Teton Basin	9	1900–2800
South Teton Basin	6	2000–2800
Wasatch Range	8	2100–2800
Raft River Mountains	1	2700–2900
West Uinta Range	11	2500–3200
East Uinta Range	6	2600–3300
Great Basin	1	2900–3200
Wasatch Plateau	10	2600–3300
Tavaputs Plateau	1	2900–3000
Fishlake Plateau	9	2800–3400
Dixie	12	2800–3300
Canyonlands	5	2800–3200
Colorado Front Range	5	2800–3200
San Juan Mountains	4	2900–3600
Peripheral regions[a]	5	—

[a]Montana, Arizona, and New Mexico. Altitudinal ranges vary greatly among these regions and were not adequately sampled in this study.

containers (740 cm³) in a shadehouse at Moscow, Idaho. The experimental design consisted of nine seedlings from each population growing in row plots in each of three blocks. Trays of containers containing three plots were transferred into an unheated greenhouse for the winter months and in early March of the second growing season were exposed to a daytime temperature of about 24°C, which was allowed to cool to a minimum of 13°C at night. After bud burst was complete, all seedlings were measured twice each week until about 90% of the trees had completed shoot elongation. Thereafter, each seedling was measured once each week until elongation ceased.

Shoot elongation of individual trees was modeled with a modified logistic function (Rehfeldt and Wykoff 1981) from which rate and cessation of shoot elongation were estimated for each seedling. These two variables were combined with other morphometric and phenologic characters to provide six variables for describing the pattern and amount of shoot elongation of individual trees (Table 2).

Monoterpenes

Because of the success of Schaefer and Hanover (1985, 1986) in using terpenes to separate blue and Engelmann spruce, monoterpenes of each population were analyzed from 1-year seedlings that were surplus to the establishment of field tests. Terminal buds of dormant trees were excised, and the extruded oleoresin was drawn into a microcapillary pipet. The oleoresin from five trees was combined to form a conglomerate sample of 5–10 μL. Each population was represented by either four or five replicates.

The oleoresin samples were placed in air-tight vials and stored under refrigeration at 2°C. Before analysis, the samples were diluted to 2.5% with pentane. Various amounts of heptane were added as an internal standard, depending on the sample size. The samples were analyzed on an HP 5890 series II chromatograph equipped with a HP ultra 1 column (dimethylploysiloxane 50 m × 0.2 mm × 0.33 μm), a flame ionization detector, and an HP 7673A automatic injector. One microlitre of sample was injected with a spit ratio of 29:1. The temperature was programmed for an initial temperature of 70°C and maintained for 10 min with an increase rate of 10°C/min to a final temperature of 250°C, which was maintained for 22 min. Integration was done with an HP 3396A electronic integrator. Peak identification was achieved by comparison with the relative retention times of known standards. Standard curves of the response of known concentration of monoterpene standards were prepared for quantifying the monoterpenes. Analyses were performed sequentially by replication.

TABLE 2. Description of the variables analyzed

Variable[a]	Description
BDEV4	Deviation from regression of 4-year height on 2-year height
BWI2	Presence or absence of winter injuries to foliage in year 2
THT4	Age 4 height
TDEV4	Deviation from regression of 4-year height on 2-year height
TWI2	Presence or absence of winter injuries to foliage in year 2
TCL	Scores of leaf color (green, 0; blue, 1) in year 4
MDEV3	Deviation from regression of 3-year height on 2-year height
MDB2	Presence or absence of buds that died over the 2nd winter
MSF3	Presence or absence of spring frost damage to new shoots in year 3
MDT3	Presence or absence of winter injury to the terminal shoot in year 3
GEL	Length of the terminal shoot produced in year 2
GBB	Date on which developing leaves were first visible through the expanding 2-year bud
GEND	Cessation of elongation: the day by which all but 2 mm of elongation had occurred
GDUR	Duration of elongation: the number of days between bud burst and cessation
GRT	Elongation per day during the period for which 20–80% of the shoot elongated
GHT	Age 2 height

[a]First letter codes the test site: B, Benton Flat; T, Tarlac; M, Moscow; G, greenhouse.

TABLE 3. Mean values for species groups and results of analyses of variance (ANOVA) for Engelmann spruce populations

Variable	Mean values			Intraclass correlations		
	Blue spruce	Southwest spruce	Engelmann spruce	Populations	Experimental error	Sampling error
BDEV4 (cm)	9.7	2.8	−1.2	0.24**	0.03	0.74
BWI2 (%)	10.1	11.7	41.5	0.07**	0	0.93
THT4 (cm)	29.9	27.6	29.6	0.27**	0.09	0.64
TDEV4 (cm)	−1.9	−0.1	0.1	0.21**	0.11	0.68
TWI2 (%)	25.6	16.6	11.9	0	0.03	0.97
TCL (%)	16.2	65.3	44.1	0.17**	0.10	0.73
MDEV3 (cm)	7.3	0.9	−0.8	0.15**	0	0.84
MDB2 (%)	15.4	36.6	61.3	0.07**	0.02	0.91
MSF3 (%)	58.6	93.3	63.4	0.13**	0.04	0.82
MDT3 (%)	0	0	5.0	0.04**	0.04	0.92
GEL (cm)	7.7	6.6	5.1	0.17**	0.09	0.74
GBB (day)	15.2	13.5	12.9	0.07**	0.04	0.88
GEND (day)	54.5	49.9	35.3	0.19**	0.06	0.75
GDUR (day)	39.2	36.4	22.5	0.17**	0.06	0.77
GRT (mm/day)	2.7	2.5	2.9	0.11**	0.12	0.76
GHT (cm)	15.5	13.1	12.4	0.23**	0.07	0.69

NOTE: ANOVA results are presented as intraclass correlations, the ratio of the variance component for the indicated effects to the total phenotypic variance. To the total phenotypic variance is calculated as $\sigma^2_P + \sigma^2_E + \sigma^2_W$, as defined by eq. 1. Variables are defined in Table 2. **, statistical significance of the F-value at $p < 0.01$.

Nine monoterpenes were assayed: α-pinene, camphene, β-pinene, sabinene, myrcene, 3-carene, limonene, terpinolene, and bornyl acetate. Results were expressed as a proportion of the total monoterpene complement. As discussed by Birks and Kanowski (1988), expressing monoterpenes as a percentage destroys their independence and confounds their interpretation. While statistical tests are sensitive to assumptions concerning normality and independence, the algorithms themselves are not. Terpene compositions, therefore, were used only in multivariate analyses for assessing differentiation, hybridization, and introgression between blue and Engelmann spruce. No statistical tests were performed with these variables.

Statistical analyses

The series of statistical analyses described below all used software from the SAS Institute (1985). The first step of this series was canonical discriminant analysis (*i*) to produce functions that would separate populations of Engelmann, blue, and Southwest spruce and (*ii*) to allow the identification of populations that were intermediate between

Engelmann and blue spruce and therefore might be introgressed. The discriminant analyses used population means for the nine monoterpenes plus the 16 variables describing growth and development (Table 2). Because of the occurrence of phenotypically intermediate individuals in the field, it is important to note that none of the variables used in these discriminant analyses are used to identify the species in native habitats.

The second step of the series involved analyses of variance to assess intraspecific variation in Engelmann spruce. These and subsequent analyses used only those populations that were classified as Engelmann spruce by the discriminant functions. The field and greenhouse tests were analyzed according to a model of random effects

[1] $Y_{ijk} = \mu + P_i + B_j + E_{ij} + W_{ijk}$

where Y_{ijk} is an observation on seedling k in block j from population i; μ is the mean; P_i and B_j are the effects of populations and blocks, respectively; E_{ij} is the experimental error, the interaction of blocks

with populations; and W_{ijk} is the sampling error. Under the assumption that blocks and populations are random variates, the experimental error becomes the variance appropriate for testing differences among populations. Results are presented as variance components for which σ_P^2, σ_B^2, σ_E^2, and σ_W^2 are the variance components for the effects listed above, and the total phenotypic variance is the sum of σ_P^2, σ_E^2, and σ_W^2. For these analyses, the mean number of observations per plot was 8.3 at Benton Flat, 9.6 at Tarlac, 9.80 at Moscow, and 8.6 for the greenhouse.

The third step in the statistical analyses was to describe patterns of genetic variation across the landscape with multiple regression models that use physiographic predictors (latitude, longitude, elevation, and various transformations thereof). These analyses used procedures that have been detailed earlier (Rehfeldt 1989) and have been used frequently for these purposes (e.g., Rehfeldt 1990, 1991, 1994).

Rates of differentiation along the clines described by the regression models were interpreted relative to the least significant difference (LSD; Steel and Torrie 1960) among populations at the 20% significance level (LSD 0.2). Values of LSD were used because models developed from numerous independent variables are subject to overfitting (Draper and Smith 1981). The use of LSD guarded against accepting fallacious results. The 20% significance level was used to reduce the possibility of accepting no differences among populations when differences actually exist (type II errors); such errors provide potential for faulty interpretations when models are applied. Values of LSD were calculated from the experimental error mean square.

Using LSD to assess rates of differentiation intuitively suggests that interpretations are dependent on sample sizes and experimental errors (uniformity of cultural conditions). However, in this study, experimental error mean squares are composed not only of microenvironmental effects at the planting site but also of genetic variances within populations. This means that even if microenvironmental effects were zero, LSD would approximate the genetic variances within populations, an ideal error for studying population differentiation.

Results

Preliminary analyses indicated that the three populations of Engelmann spruce from the Southwest (Arizona and New Mexico) performed so differently from the northern populations that the primary objectives best would be served by considering the populations under study as representatives of three taxonomic groups: Engelmann, blue, and Southwest spruce. Genetic variation in the spruce of the Southwest will be considered in detail in a subsequent series of studies.

Interspecific relationships

Genetic differences in the growth and development of Engelmann, Southwest, and blue spruce were pronounced (Table 3). Compared with Engelmann, blue spruce was generally larger (GEL, GHT, and BDEV4), had a longer duration (GDUR) and later cessation (GEND) of shoot elongation, suffered more winter damage at the most severe sites (BWI2 and TWI2) but less winter damage at the mildest site (MDB2 and MDT3), and had a lower proportion of trees with a blue hue (TCL). The greater susceptibility of blue spruce to winter damages at Tarlac is also reflected in the deviation from regression of 4-year height on 2-year height (TDEV4); Engelmann spruce seedlings grew more from a common 2-year height than those of blue spruce. Table 3 also shows Southwest spruce to be intermediate between Engelmann and blue spruce for eight characters, similar to blue spruce for others (GEND, GDUR, MDT3, and BWI2), similar to Engelmann for others (TDEV4 and MDEV3), and similar to neither for still others (TCL and MSF3).

Patterns of shoot elongation (Figs. 2 and 3) further illustrate genetic differences among these groups. While illustrating the accuracy with which the logistic function can be fit to disparate
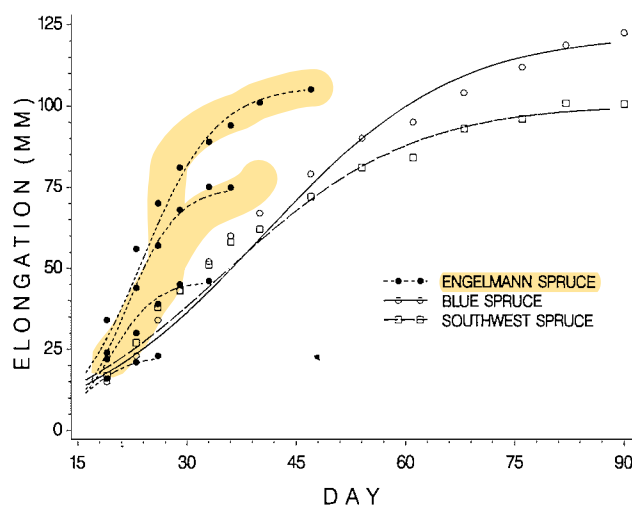

FIG. 2. Observed (symbols) and predicted (lines) cumulative shoot elongation for individual seedlings of four Engelmann, one blue, and one Southwest spruce. Days are numbered after greenhouses were warmed, and elongation is plotted after day 15 because no measurements were made until after bud burst was completed on all trees.

growth curves, these figures also illustrate two different mechanisms controlling shoot growth in spruce seedlings. The pronounced sigmoid shape and early completion of shoot elongation in seedlings of Engelmann spruce imply determinant control (see Rhomberger 1963). By contrast, in young blue spruce, indeterminate growth customarily follows the completion of predetermined growth (Bongarten 1986). The curves of the blue and Southwest spruce (Fig. 2) thus suggest that determinant growth ceased at about day 50, after which indeterminate growth ensued for another 30 days. Because determinant growth occurs at a faster rate than indeterminate growth, the modeled curve tended to underestimate the observed elongation during the early portion of the curve and overestimate elongation during the latter portion.

The capacity for indeterminate shoot growth allowed seedlings of blue and Southwest spruce to grow for a longer period and achieve a greater height than those of Engelmann spruce (Table 3). Indeed, still-elongating on day 47 were 6% of the Engelmann spruce, 88% of the Southwest spruce, and 95% of the blue spruce. On day 54, comparable values were 2% for Engelmann spruce, 56% for Southwest spruce, and 62% for blue spruce. Of the Engelmann spruce elongating on either of these dates, nearly all were from either the San Juan Mountains or the Canyonlands (Fig. 1) where a transition in the control of juvenile shoot growth may be occurring.

The monoterpene assay also showed pronounced differences between the Engelmann and blue spruce. Compared with Engelmann, blue spruce had a lower percentage of α-pinene (25 vs. 38), a lower percentage of β-pinene (5 vs. 16), a lower percentage of myrcene (3 vs. 12), and a higher percentage of 3-carene (42 vs. 14). Southwest spruce, however, had monoterpene complements that were quite similar to that of Engelmann spruce; for all terpenes assayed, mean values for the three Southwest populations fell within the range of mean values for the Engelmann populations.

The large mean differences among Engelmann, Southwest, and blue spruce allowed canonical discriminant analyses to readily separate populations into three groups (Fig. 4). Also apparent, however, was the canonical classification of one
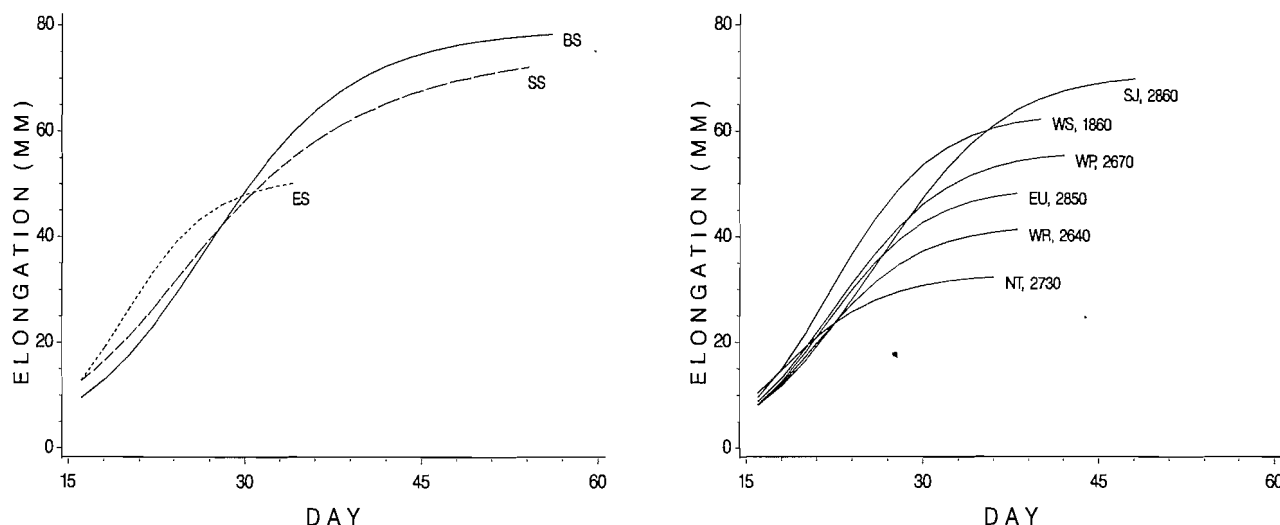
FIG. 3. Predicted mean cumulative shoot elongation for Engelmann (ES), blue (BS), and Southwest (SS) spruce (left) and for individual populations of Engelmann spruce (right) from the indicated physiographic province (Fig. 1) and elevation. SJ, San Juan Mountains; WS, west Salmon River Mountains; WP, Wasatch Plateau; EU, east Uinta Range; WR, Wasatch Range; NT, north Teton Basin.
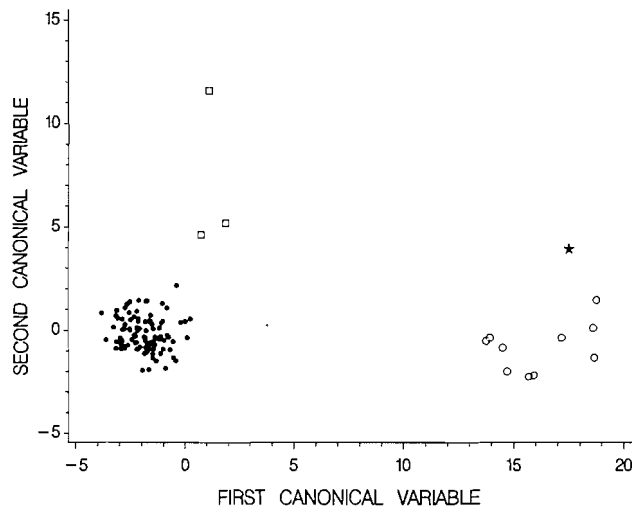

FIG. 4. Population means plotted according to the first two canonical variables developed from a discriminant analysis. ●, Engelmann spruce; ○, blue spruce; □, Southwest spruce; ★, misidentified blue spruce.

TABLE 4. Results of multiple regression analyses and the proportion of the total variance accounted by regression models

Dependent variable	R^2	Independent variable	Proportion of total variance[a]
BDEV4[b]	0.61	4	0.15
BWI2	0.28	4	0.02
THT4[b]	0.65	6	0.18
TDEV4[b]	0.70	5	0.15
TCL[b]	0.64	5	0.11
MDEV3	0.56	6	0.08
MDB2	0.42	4	0.03
MSF3	0.58	5	0.08
MDT3	0.26	3	0.01
GEL[b]	0.55	4	0.10
GBB	0.14	2	0.01
GEND[b]	0.65	4	0.12
GDUR[b]	0.65	4	0.11
GRT	0.36	5	0.04
GHT	0.41	4	0.09

[a]Product of R^2 and the intraclass correlation for the effects of populations in Table 3.
[b]Key variable. Variables are defined in Table 2.

population of blue spruce that had been incorrectly identified as Engelmann spruce in the field. This population was from east-central Arizona (Fig. 1) and is hereafter considered to be blue spruce rather than Engelmann. (The mean values cited above and in Table 3 reflect the classification of this population as blue spruce.) With this correction, the canonical discriminant analysis (Fig. 4) readily separated blue spruce from the Engelmann and Southwest populations. The same analysis closely aligned populations of Southwest spruce with the Engelmann populations to the north but nevertheless suggested separation between them. Regardless, pronounced and discrete separation of the Engelmann and blue spruce populations suggests that none of the Engelmann populations were introgressed.

Intraspecific variation in Engelmann spruce

Genetic variation in Engelmann spruce was assessed from the performance of seedlings from the 100 populations that

remained after eliminating the three Southwest populations and the misidentified population.

Genetic differentiation

Large mean differences contributed to the statistically significant effects of populations (Table 3) that were detected ($p < 0.05$) for all variables analyzed except winter injuries at Tarlac (TWI2). At Benton Flat, for instance, populations differed by as much as 63% for the trees injured by winter freezing (BWI2), at Tarlac by 97% in the proportion of trees that were of blue hue (TCL), and in the greenhouse by 6 days in the mean date of bud burst (GBB) and 12 days in both the cessation (GHEND) and duration (GDUR) of shoot elongation.

Population differentiation is aptly illustrated by patterns of shoot elongation (Figs. 2 and 3). In the greenhouse, bud burst of individual trees occurred between 4 and 15 days after the
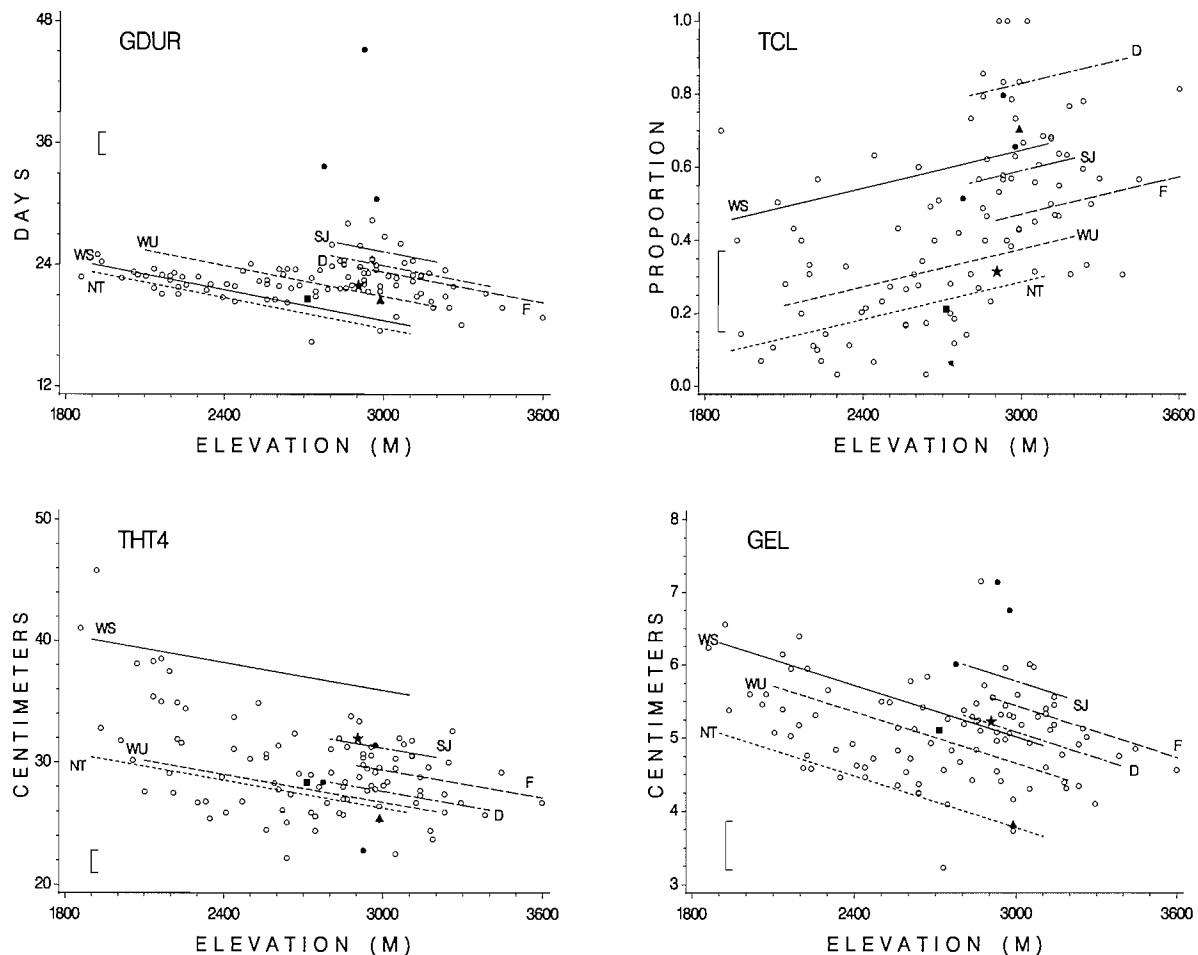
FIG. 5. Population means for four variables (see Table 2) plotted by the elevation of the seed source. The vertical bracket near the origin quantifies LSD 0.2. Regression lines represent different physiographic provinces. D, Dixie; WU, west Uinta Range; NT, north Teton Basin; SJ, San Juan Mountains; WS, west Salmon River Mountains; F, Colorado Front Range. Observed values for three isolated populations: ★, Tavaputs Plateau; ■, Raft River Mountains; ▲, Great Basin.

greenhouse was warmed, and shoot elongation was completed between days 29 and 68. This provided between 5 and 12 observations for the logistic regressions that described shoot elongation of individual trees nearly perfectly (Fig. 2): values of R^2 ranged from 0.94 to essentially 1.0, averaging 0.994.

As shown in Fig. 3, populations differed considerably in the rate, cessation, and amount of elongation. Noteworthy in this figure are (i) the high growth potential and long duration of shoot elongation of trees from 2850 m in the San Juan Mountains where a transition seems to be occurring in the control of juvenile shoot growth; (ii) the performance of a population from a low elevation (1850 m) in the western Salmon River Mountains that combined the most rapid rate of elongation with a short duration to achieve a large amount of elongation; and (iii) the remaining graphs that compare populations from about the same elevation (2650−2850 m) and combine with the San Juan population to illustrate genetic differences among populations from similar elevations but widely different geographic localities.

Despite the large mean differences, effects of populations accounted for more that 20% of the total phenotypic variance (Table 4) for only four variables (BDEV4, THT4, TDEV4, and GHT). Most of the remaining variance occurred among trees within plots, an error variance that accounted for at least 64% of the phenotypic variance. This error variance reflects micro-

environmental effects at the planting site and genetic differences among trees within populations. Because (i) the tests were intensively cultured to reduce extraneous environmental effects and (ii) genetic variances in the characters under study tend to be large (Rehfeldt 1992a, 1992b), one can assume that genetic variances within populations strongly contributed to the sampling error. These results imply, therefore, that genetic variability is abundant both within and among populations.

Patterns of genetic variation

Multiple regression analyses were used to assess patterns of genetic variation for the 15 variables for which effects of populations were detected (Table 3). Values of R^2 for these variables were as high as 0.70 while averaging 0.50 (Table 4). The results demonstrate that much of the variation among populations follows systematic patterns that can be described nicely by regression models. The regression equations are available from the author.

Multiplying R^2 from Table 4 by the intraclass correlation (Table 3) provides an estimate of the proportion of the total phenotypic variance in a variable that has been accounted for by the regression model. Such estimates were as high as 18% for height at Tarlac (THT4) but were less than 10% for eight of the variables (Table 4). Despite statistical significance, therefore, the models left unexplained more than 82% of the

GEND   BDEV4

FIG. 6. Geographic patterns of genetic variation for two variables (see Table 2). Patterns are depicted as isopleths as predicted by regression models for a constant elevation. The interval between isopleths equals ½LSD 0.2. The mean isopleth ($\bar{x}$) along with the positive (+) and negative (−) deviations from the mean are marked. Shading represents the distribution (after Little 1971) of Engelmann spruce.

phenotypic variance. Not surprising, patterns of variation for variables for which regression models accounted for less than 10% of the total variance tended to be weak, redundant, and spurious. Patterns, therefore, will be interpreted according to variation in seven of the original variables, hereafter designated as the key variables (Table 4).

The regressions described genetic variation as occurring along geographic clines with strong elevational and latitudinal components. Figures 5 and 6 together illustrate the clines for six of the key variables. Patterns of variation for TDEV4 that are not presented paralleled those of THT4 (Fig. 5) and BDEV4 (Fig. 6).

Elevational clines were described for all key variables, and in each case the best fitting cline was linear. As the elevation of the seed source increased, growth potential (reflected in BDEV4, THT4, TDEV4, and GEL) decreased, the cessation (GEND) and duration (GDUR) of shoot growth decreased, and the proportion of trees with a blue hue (TCL) increased. Elevational clines with intercepts for six physiographic provinces are presented in Fig. 5 for four variables. Intercepts were positioned according to the average latitude and longitude of the populations located within the province. Data on the duration of shoot elongation additionally emphasize differentiation of Southwest spruce from Engelmann.

Rates of differentiation along linear clines are readily interpreted by the ratio of LSD 0.2 to the regression coefficient. For the duration of shoot elongation (GDUR), the variable with the steepest elevational cline, populations within the same locality need to be separated by at least 420 m in elevation before one can be reasonably certain that the populations differ genetically. This interval approached 500 m for the amount (GEL) and duration (GDUR) of shoot elongation and was over 700 m for the remainder of the key variables.

The models thus suggest that elevation and therefore length of the frost-free period are related to genetic differentiation. The relationships, however, do not necessarily suggest that populations are closely attuned physiologically to their environment: across 420 m of elevation the average frost-free period varies by about 38 days (Baker 1944). This implies that popu-

lations are capable of inhabiting environments that vary by more than 1 month in the average frost-free season. Still, for localities such as the western Salmon River Mountains and the Teton Basin, where the elevational distribution of Engelmann spruce is broad, genetic differentiation across the landscape can still be pronounced (Fig. 5). However, for localities in the southern portion of the region (e.g., Dixie, Fishlake Plateau, Great Basin, Canyonlands, and the San Juan Mountains), where populations are distributed across a few hundred metres of elevation on only the tallest mountains, little differentiation can be expected.

A geographic component to genetic variation was detected in all of the key variables and is illustrated in Fig. 5 by regression lines of different intercept. This component is shown in detail for two variables in Fig. 6 where isopleths represent genetic variation among populations predicted for an elevation of 2716 m, the mean elevation of all populations. In this figure, the interval between isopleths equals ½LSD 0.2. This means that populations from the same elevation that are separated by a geographic distance equaling two intervals are expected to differ at about the 20% probability level. This also means that the amount of differentiation expected between populations occupying adjacent isopleths is equivalent to that expected between populations on the same isopleth that are separated by 210 m of elevation.

Geographic patterns for the key variables followed one of only two variations on a latitudinal cline; both variations are presented in Fig. 6. Regardless of whether the variables were obtained in the field or greenhouse, populations from the Teton Basin expressed the lowest growth potential, which developed from the shortest duration of shoot elongation (Fig. 6). From the Teton Basin, growth potential increased toward both the west and south. For the variables measured under benign greenhouse conditions (GDUR, GEL, GEND), however, the clines were steeper toward the south than toward the west. As a result, southern populations (e.g., San Juan Mountains) expressed the highest growth potentials, which developed from a long duration of shoot elongation. In northern field environments, however, southern populations evidently were incapable of

expressing their innate potential. Consequently, as shown for BDEV4 (Fig. 6) and THT4 (Fig. 5), populations from the western Salmon River Mountains became the tallest. Geographic variation in foliage color (TCL) paralleled that illustrated for BDEV4 (Fig. 4); trees from the Teton Basin had the greenest foliage while those from the western Salmon River Mountains and Dixie were the bluest (see also Fig. 5).

Even though variation in all key variables was described by clines for which effects of elevation and latitude were prominent, intercorrelations among the variables were not necessarily strong. Despite statistical significance ($p < 0.05$) of 16 of the 21 coefficients, only six of the relationships accounted for over one-half of the covariance ($r > 0.7$). The strongest associations involved (i) TDEV4, THT4, BDEV4, and GEL, and (ii) GEL, GEND, and GDUR.

Discussion

By readily separating seedling populations of blue and Engelmann spruce according to morphometric characters and monoterpenes, the canonical discriminant analyses corroborated the work of Schaefer and Hanover (1985, 1986) who used similar variables to distinguish seedlings of these species. While detecting one population that had been misidentified, the canonical discriminant analysis suggested that none of the Intermountain populations of Engelmann spruce sampled in this study were introgressed, a conclusion typical of those studying hybridization in natural populations (Fechner and Clark 1969; Daubenmire 1972; Mitton and Andalora 1981; Ernst et al. 1990). Only Taylor et al. (1975) reached the conclusion that introgression was occurring to only a limited extent in sympatric populations. It seems reasonable to assume, therefore, that introgression from blue spruce has not influenced the results of these studies with Engelmann spruce.

Adding intrigue into the interrelationships among species of spruce in the Intermountain West was the performance of three populations from the Southwest. These populations had the terpene composition of Engelmann spruce while having many seedling shoot growth characteristics that were similar to blue spruce. While the canonical discriminant analysis suggested that Southwest populations were discrete from but closely aligned with the populations of Engelmann spruce to the north, additional evidence is required to determine whether these populations represent a southward continuation of the Engelmann spruce clines or a taxonomic entity separate from Engelmann spruce. It is worthwhile noting, moreover, that (i) Taylor and Patterson (1980) could detect no differences in either terpene composition or morphological characters between Picea mexicana and Southwest populations; (ii) Dallimore and Jackson (1967) noted that Picea mexicana seems to be intermediate between Engelmann and blue spruce for many characters; (iii) one of the populations Taylor et al. (1975) believe to reflect hybridization between Engelmann and blue spruce was in southern Arizona; and (iv) the population that was misidentified in the present study originated from south-central Arizona. Epitomized by references to a Picea engelmannii complex (Taylor et al. 1994), Southwestern populations of spruce indeed deserve greater attention.

Analyses of variance detected genetic differences among Engelmann spruce populations for 15 of the 16 characters measured. Skeptics, however, might argue that the observed differences were induced by testing populations as much as

$10°$ of latitude north of their origin. For Engelmann spruce to be of bluer hue than the average blue spruce population would intuitively support their argument. Yet in studying populations of Pinus ponderosa from the Southwest, patterns of genetic variation developed from variables measured in the greenhouses at Moscow were essentially the same as those generated from the field at two locations in Arizona as well as at Benton Flat (Rehfeldt 1991). While inappropriate for predicting responses in native habitats, tests in non-native environments still allow detection of the genetic differences that are expressed in differential phenotypic responses.

Mathematical models described genetic variation in Engelmann spruce as occurring along geographic clines. While accounting for as much as 70% of the variance among populations, the models accounted for less than 10% of the total phenotypic variance in half of the variables analyzed, and for no variables did the models account for more than 18% of the phenotypic variance. Because most of the unexplained variance was likely due to genetic variances within populations, the high values of R^2 associated with the regression models must primarily reflect the broad scope of this study. Genetic differentiation should be expected among populations dispersed across $7°$ of latitude and 1700 m of elevation.

Geographic patterns of genetic variation described by the models were related to elevation and latitude, two variables with direct environmental relationships. The patterns, therefore, have direct microevolutionary interpretations. As either elevation or latitude increase, temperatures decrease, with a reduction in mean annual temperature of $1°C$ leaving 15 fewer frost-free days (Baker 1944). Consequently, populations distributed along either elevational or latitudinal gradients display adaptations to growing seasons of different length. When compared in common environments, populations from either low elevations or low latitudes ordinarily express a high growth potential, grow for a relatively long period, and become large; populations adapted to short growing seasons cease development early and tend to be small.

In the Intermountain region, an elevational interval of 1000 m tends to be associated with a change of 90 frost-free days (Baker 1944). For differentiation of Engelmann spruce populations to be associated with 420 m of elevation suggests that populations occupying environments that differ by about 38 frost-free days tend to differ genetically. This environmental interval across which single populations are adapted is intermediate between those of other western conifers on which similar data are available. In Pseudotsuga menziesii var. glauca, populations inhabiting environments that differ by at least 18 days in the mean frost-free period tend to be differentiated (Rehfeldt 1989), while in Pinus monticola differentiation has not been detected among populations dispersed across environments differing by as much as 80 frost-free days (Rehfeldt et al. 1984). These results, therefore, add evidence that species of wind-pollinated conifers have adjusted to similar environmental gradients in much different ways.

Clines with a strong latitudinal component also seem to reflect adaptation to a variable frost-free period. As shown in Fig. 4, populations from the San Juan Mountains are expected to have a greater growth potential and longer duration of shoot elongation than those from the Salmon River Mountains at the same elevation. For instance, Baker (1944) shows the frost-free period at 2100 m to be about 145 days in the San Juan Mountains and about 45 days in the Salmon River Mountains.

TABLE 5. Elevational intervals listed according to geographic region in which genotypes similar to the targeted population are expected to occur

	Target populations							
Geographic region	East Uinta Range (2800 m)	South Teton Basin (2500 m)	Fishlake Plateau (3000 m)	San Juan Mountains (3100 m)	West Salmon River Mountains (2200 m)	Great Basin (2900 m)	Raft River Mountains (2800 m)	Tavaputs Plateau (2950 m)
West Salmon River Mountains	—	—	—	—	2000–2400	—	—	—
East Salmon River Mountains	—	—	—	—	2100–2200	—	—	—
North Teton Basin	—	2250–2600	—	—	—	—	—	—
South Teton Basin	—	2250–2750	—	—	—	—	—	—
Wasatch Range	2550–2750	2500–2800	—	—	—	—	—	—
Raft River Mountains	—	—	—	—	—	—	2700–2900	—
West Uinta Range	2550–3000	—	—	—	—	—	—	2550–2950
East Uinta Range	2600–3000	—	—	—	—	—	—	2800–2900
Great Basin	—	—	—	—	—	2900–3100	—	—
Wasatch Plateau	—	—	2900–3000	—	—	3200–3250	—	2700–3150
Tavaputs Plateau	2900–2950	—	—	—	—	—	—	2900–3000
Fishlake Plateau	—	—	2800–3200	—	—	3000–3400	—	—
Dixie	—	—	2900–3300	—	—	—	—	—
Canyonlands	—	—	3000–3200	2800–2900	—	3250–3300	—	—
San Juan Mountains	—	—	—	2900–3200	—	—	—	—
Front Range	—	—	—	—	—	—	—	—

NOTE: Predicted recurrence is based on the seven key variables; see Table 4.

Because each isopleth in Fig. 4 roughly indicates the amount of differentiation associated with an environmental difference of about 19 frost-free days, one could expect about six isopleths to separate the two regions if a variable frost-free period is controlling differentiation. The plots for GDUR and BDEV4 concur nicely.

Not concurring, however, are patterns involving the Teton Basin, the province from which populations had the shortest duration of shoot elongation and the lowest growth potential. For constant elevations, Baker (1944) shows the mean frost-free period in the Teton Basin to be about 65 days longer than in the Salmon River Mountains and therefore 35 days shorter than in the San Juan Mountains. This means that growth potential should increase from the Salmon River Mountains to the Teton Basin and that two isopleths should separate the Teton Basin from the San Juan Mountains. However, Fig. 4 shows that both the duration of shoot elongation (GDUR) and growth potential (BDEV4) decrease between the Salmon River Mountains and the Teton Basin, a trend that was also documented for an associated conifer, Pinus contorta (Rehfeldt 1988). The same figure also shows the Teton Basin to be separated from the San Juan Mountains by five isopleths. It seems, therefore, that the Teton Basin is colder than documented by Baker (1944), the populations that inhabit the Teton Basin are not optimally adapted, or Engelmann is responding to the climate in the Teton Basin as if it was colder than indicated by the weather records.

Despite relatively gentle slopes, the clines describe complex patterns across the landscape. As described for Pinus ponderosa (Rehfeldt 1991), visualizing these patterns is facilitated by using the regression models to examine the frequency by which genotypes capable of similar phenotypic responses recur with respect to several variables. Each regression equation can be used to generate a data base containing predicted values for the geographic and elevational distribution of Engelmann spruce within each of the physiographic regions of Fig. 1. Then, by surrounding each observation in the data base with a confidence interval of $\pm$ ½LSD 0.2, the amount of recurrence readily can be assessed.[1]

The expected recurrence of populations capable of similar responses is illustrated in Table 5 for eight targeted populations. For large populations in the center of the region of study, recurrence is expected to be widespread. A targeted population at 2500 m in the southern Teton Basin, for example, is expected to perform similarly to populations dispersed across an elevational interval of 450 m within the region itself and also to be similar to populations in the northern Teton Basin and Wasatch Range. Widespread recurrence is similarly expected for large centralized populations in the eastern Uinta Range and the Fishlake Plateau. While Table 5 suggests that the degree of recurrence may be limited for large populations on the periphery of the region of study (e.g., western Salmon River Mountains, San Juan Mountains, or Colorado Front Range), the prediction is undoubtedly influenced by the sampling; similar genotypes are probably widespread in adjacent regions that were not sampled.

Recurrence of the genotypes contained in small isolated populations (e.g., Tavaputs Plateau, Great Basin, or the Raft River Mountains) can also be examined with the models. Table 5 suggests widespread recurrence for genotypes on the Tavaputs Plateau, a region that is centrally located despite its isolation (Fig. 1). Widespread recurrence is likewise expected for genotypes in the Great Basin, despite being on the western periphery of the study area. Yet the models do suggest that the isolated population from the Raft River Mountains may be genetically unique. That the widespread recurrence predicted for the Tavaputs and Great Basin populations is not an artifact

[1]For this use, a confidence interval of $\pm t_{0.2} s_{\bar{x}}$ would be more appropriate. LSD is used to allow ready comparison to previous work and can be converted to $2 s_{\bar{x}}$ by multiplying by $\sqrt{2}$. Confidence intervals based on $\pm$½LSD 0.2 are essentially the same as those of $\pm t_{0.35} s_{\bar{x}}$ and therefore are conservative.

induced by modeling procedures that considered only continuous variables is suggested by the placement of the observed values for these populations in Fig. 5. Quite similarly, the positioning of the Raft River population suggests that it is not necessarily composed of rare genes but that characters are occurring in relatively atypical combinations.

Practical uses of models of genetic variation are numerous and diverse (see Rehfeldt 1991). For artificial reforestation, one can assume that the targeted locations represent either planting sites or seed production areas. The model can then be used (*i*) to locate sources of seeds that should be genetically compatible with the environment at the target or (*ii*) to select planting sites for which seeds gathered from the targeted population should be adapted. The model might also be used to locate populations, such as that in the Raft River Mountains, that seem to be composed of genotypes sufficiently unique to deserve conservation measures. Additional uses might include assessing the impact of climate change on the adaptedness of populations, understanding phenotypic variation, and assembling adaptively similar populations for tree breeding. Nevertheless, until verified, models should be used with discretion.

Genetic variation among populations was described by relatively flat clines, low rates of differentiation, and widespread recurrence. Considering (*i*) the disjunct geographic distribution of Engelmann spruce in the region of study (Fig. 1), (*ii*) the limited elevational distributions within many of the physiographic provinces (Table 1), and (*iii*) that Engelmann spruce has occupied much of the region of study since mid-Wisconsin times (Betancourt 1990), it is surprising perhaps that microevolution has not produced steeper clines.

In accounting for the relatively flat clines, one explanation might be that equilibrium has not yet been reached between gene frequencies and environmental gradients. Yet, for spruce throughout much of the Intermountain region, post-Wisconsin migrations have merely occurred upward 700–1000 m (Betancourt 1990). A lack of equilibrium, therefore, seems untenable. Another explanation might be that high levels of genetic variability have been retained within these populations because selection has not yet had the opportunity to act. However populations tested in this study come from environments that differ by as much as 85 days in the mean frost-free period (Baker 1944), and nearly all populations are older than 100 years. As well, natural even-aged reproduction of many thousands of seedlings per hectare is customarily reduced to a few hundred per hectare at maturity (Alexander 1987). Selection, therefore, has been afforded ample opportunity.

Perhaps the most plausible explanation of the flat clines is that temporal environmental heterogeneity has precluded differentiation such that plasticity becomes the predominant evolutionary mechanism for adapting to a heterogeneous environment (see Sultan 1992). The widespread distribution of this species would attest to the power of a strategy that combines high levels of plasticity with low rates of differentiation.

Acknowledgments

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