Genetic variation in Broadleaf Lupine

on the Mt Hood National Forest and implications for seed collection and deployment

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ABSTRACT

Analysis of a common-garden study of broadleaf lupine (*Lupinus latifolius* Lindl. ex J.G. Agardh ssp. *latifolius* [Fabaceae]) indicates that use of watershed delineations is better than use of plant association series for determining seed zones on the Mt Hood National Forest. Risk analysis further confirmed that only 4 seed zones are required, providing a reasonable compromise between managing costs and maintaining local adaptation. Overall, moderate amounts of genetic variation were found in 84 seed sources. Two principal components (PCs) summarized 58% of the variation in 24 measured traits, and variation in PC scores was significantly correlated with topographic, geographic, and climatic variables. Regression analyses showed that these variables accounted for 47% of the variation in the first PC and 34% of the variation in the second PC.

KEY WORDS
adaptation, seed transfer risk, seed transfer zones, common-garden

NOMENCLATURE
USDA NRCS (2004)

Photo by Caitlin Cray

The goal of most revegetation projects is to establish plant populations that thrive, fulfill project objectives (such as erosion control or providing wildlife habitat), and successfully reproduce. Outplanting failure is costly; natural resources may be irrevocably damaged or lost, and funds or time may not be available to replant the project. Plants must be adapted to the environment in which they are planted if they are to thrive and reproduce, thus understanding patterns of adaptive genetic variation can be crucial to the success of these projects (Campbell 1975; Millar and Libby 1989; Knapp and Rice 1994).

Transferring seeds too far from their place of origin incurs a risk that some portion of the seedlot will not be adapted to the outplanting site. Relative transfer risk is defined as the adaptive genetic mismatch between a population of seedlings being transferred and a population of seedlings native to the outplanting site (Sorensen and Weber 1994). The proportion of non-adapted seeds (or amount of risk) depends on the mode of adaptation for the species (specialist versus generalist), the environmental distance between seed origin and outplanting site, and the amount of genetic variability at the seed origin (Rehfeldt 1984; Campbell 1986).

One way to ensure seeds are adapted to the outplanting environment is to delineate geographic areas within which seeds can be safely moved about, or seed transfer zones, based on conservative assumptions about patterns of adaptive variation (that is, plants are specialists and narrowly adapted to their growing environment). Overly conservative assumptions about patterns of adaptive variation, however, can lead to maintaining more seedlots than necessary, resulting in an unnecessarily complicated system and in excessive costs for seedlot maintenance and seed increase operations. Current USDA Forest Service Region 6 (Oregon and Washington) policy for native species seed collection and usage is to use fifth field watersheds (a unit corresponding to one level below the USGS Hydrologic Cataloguing Unit [USDI 1976]) and to use stock within 305-m (1000-ft) elevation bands.

Common-garden experiments that describe patterns of adaptive variation in conifers have been used to develop guidelines to limit the distance seeds are moved for reforestation activities (Campbell and Sorensen 1978; Rehfeldt 1978; Campbell and Sugano 1979; Rehfeldt 1984). These studies have demonstrated that individual plant species have unique patterns of adaptation, and it is very difficult to determine *a priori* how far seeds can be moved and still be adapted to their outplanting environment. The methods developed for conifer species provide a model for investigating adaptive variation and ways to limit seed transfer in native forbs and grasses used for revegetation purposes.

Broadleaf lupine (*Lupinus latifolius* Lindl. ex J.G. Agardh ssp. *latifolius* [Fabaceae]) is widespread throughout the Cascade Range from British Columbia to California (USDA NRCS 2004). It is an insect-pollinated, short-term perennial plant with diploid and tetraploid forms occurring in some populations (Phillips 1957; Wilson and Hipkins 2002). It is a good candidate for erosion control seed mixes because it grows well in droughty and low-fertility sites, colonizes disturbed areas, has a deep root system for stabilizing soil, and forms associations with nitrogen-fixing bacteria. Other taxa in the genus possess similar characteristics, and 'Hederma' (*Lupinus albicaulis* Dougl. var. *albicaulis*) and 'Armex' (*Lupinus elegans* Kunth) cultivars have been developed for roadside erosion control (USDA NRCS 2004).

There is evidence for adaptive genetic variation in perennial lupines. Kittelson and Maron (2001) found strong evidence for local adaptation and spatial structure at 3 source locations of bush lupine (*L. arboreus* Simms.) in coastal southern California. In another perennial legume, *Lotus scoparius* (Nutt.) Ottley, Montalvo and Ellstrand (2000) found significant source variation and a "home-site" advantage among 12 populations, also from coastal southern California. These studies suggest that adaptive genetic variation probably exists in broadleaf lupine, however, no studies have been undertaken to determine just how far seeds can be moved and still be adapted to local conditions.

My study objectives were to determine how closely patterns of genetic variation in broadleaf lupine populations match patterns of environmental variation in their source locations, and to quantify the risk associated with transferring seeds beyond their local range of genetic variability.

MATERIALS AND METHODS

Sampling

Seeds from 152 mother plants growing at 84 different source locations throughout the Mt Hood National Forest were collected during the summers of 1995 and 1996 (Figure 1). The entire collection spanned 1372 m (4501 ft) of elevation, 37° of longitude (47.2 km [29.3 mi]) and 40° of latitude (74.7 km [46.4 mi]). All seeds from a single mother plant comprised a family, and family identity of all seeds and progeny was maintained throughout the experiment. Pods were stored and allowed to dry and shatter inside kraft paper bags. Seeds were then cleaned, sealed in plastic bags, and stored in a freezer until sowing in spring of 1997.

Source locations were entered into a GIS system and the following geographic, topographic, and climatic variables were determined for each source location: UTM latitude and departure (in meters), elevation (feet), aspect (degrees), plant association series (Hall 1998), and fourth, fifth, and sixth field watersheds. Fifth and sixth field watersheds are local extensions of the Hydrologic Cataloguing Unit (USDI 1976) or the fourth field watershed. In this system size decreases as order increases (for example, fourth field is larger than fifth field). Climate vari-

ables obtained from the PRISM model (Daly and others 1994) included: average monthly temperature (Jan–Dec, °C), average monthly precipitation (Jan–Dec, cm), average monthly snow (Jan–Dec, cm), average monthly growing degree-days (Jan–Dec, days above 10 °C [50 °F]), average date of last spring frost (Julian date), and average date of first fall frost (Julian date). Plant association series, defined by Hall (1998), used for classifying sites were: Pacific silver fir, grand fir, western hemlock, mountain hemlock, eastside Douglas-fir, and ponderosa pine.

Greenhouse and Field Sites

Seeds were scarified and sown into 5-cm (2-in) peat pots in early February 1997 at the Natural Resources Conservation Service Plant Materials Center (PMC) greenhouse in Corvallis, Oregon. Plants were arranged in 2 replications of a 16-plant plot of each family for greenhouse growth. By May 1 seedlings had developed extensive root systems and were ready for outplanting.

Seedlings were outplanted to 2 field sites, one at the PMC on May 8 and 9, and the other at the USDA Forest Service Wind River Nursery (WRN) in Carson, Washington, on May 21 and 22 (Figure 1). Plants were spaced 46 x 91 cm (18 x 36 in) in a design where family row plots (4 plants of the same family) were randomized in 2 replications at each location (1216 plants at each site). Experimental plots were surrounded by a two-row buffer to minimize edge effects. Weeds were controlled by covering the plot with woven plastic mulch material, and test plants were planted in 10-cm (4-in) holes cut in the mulch fabric (Figure 2). Outplanting survival was 95%. Plants were grown and observed for 2 y.

Traits

Generally, traits could be grouped into 5 broad categories: greenhouse traits (such as time to emergence), plant size (crown height or diameter), morphology (height-to-diameter ratios, erectness), phenology (flowering date or date of bud burst), and pest resistance (mildew susceptibility) (Figure 3). Measurements were taken at each site and evaluated independently, for example, first year crown height at PMC and first year crown height at WRN are 2 separate traits. I evaluated 74 traits over the course of the study.

Statistical Analysis

Analysis generally follows the methods outlined in Campbell (1986) and Sorensen and Weber (1994). SAS software for personal computers (SAS 1999) was used for all statistical computations. To determine significance of effects and estimate variance components for individual traits, analyses of variance (PROC MIXED) were performed on plot means using the nested model:

$$Y_{ijkl} = \mu + B_i + S_j + F_{k(j)} + E_{l(ijk)}$$

where μ is the grand mean, B is the effect of blocking, S is the effect of source location, F is the family within source effect,

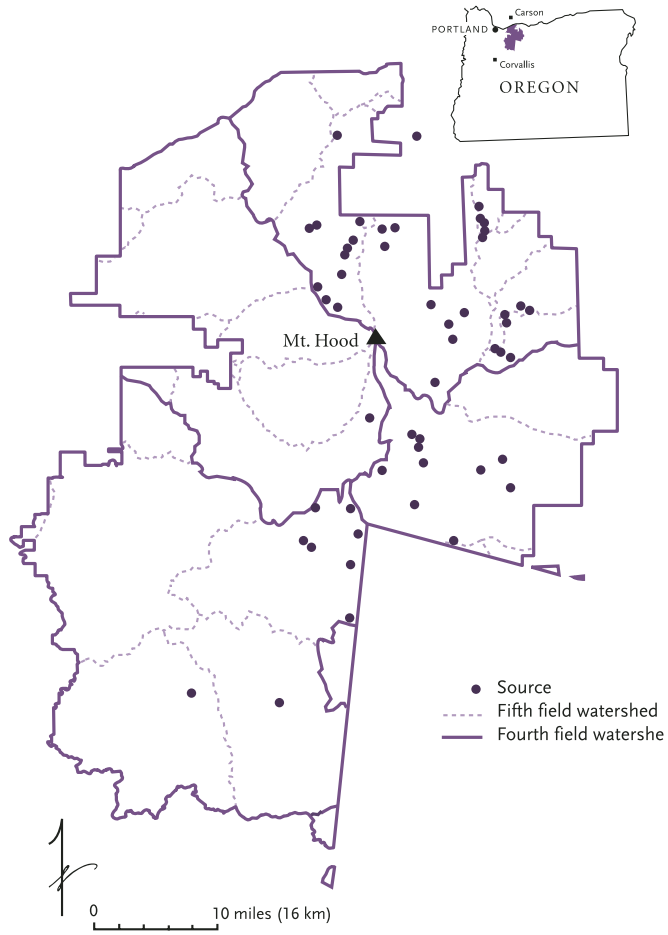

Figure 1. Location of the 2 field plot sites used in the common-garden study with a detail of the *Lupinus latifolius* sampling sites on the Mt Hood National Forest.

Photo by Joan Trindle

Figure 2. The field site at Corvallis, Oregon, showing plot layout.

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Photo by Dale Darris

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Photos by Joan Trindle

Figure 3. Variation in traits in *Lupinus latifolius*. (A) Emergence and early growth in the greenhouse. (B) Differences in plant size and phenology. (C) Differences in plant form, prostrate versus erect. (D) Differences in flower color.

and *E* is plot error (based on 4 plants per plot). Total phenotypic variance was calculated as the sum of the source, family-within-source, and plot error variance components.

Traits were dropped from further analysis if: 1) location variance was non-significant ($P > 0.05$) when tested against the families-within-locations variance; 2) traits occurring early in the life cycle had a high correlation with traits occurring later in the life cycle ($r > 0.90$); or 3) the source variance component was less than 25% of the phenotypic variance.

Principal component analysis was used to summarize location-related information (PROC PRINCOMP), reduce redundancies in the data set, and extract a few unrelated components for further analyses. Source means were used in the analysis. Component scores were computed for source means and family means for use in further analyses. To compute component scores for family means, the family means were first standardized to the overall source mean. Source means were used in regression analyses, while family means were used in classification analyses.

The extent to which variation among sources was related to geographic, topographic, and climatic variables was examined using multiple regression techniques (PROC REG). Independent variables that were highly correlated ($r^2 > 0.90$) with other independent variables were dropped from consideration. Models were selected based on R-square values, ease of interpretation, and overall parsimony. Geographic variables considered were latitude, departure, elevation, and aspect. Aspect was transformed into 2 variables: east–west aspect (sine of aspect in degrees) and north–south aspect (cosine of aspect in degrees). Climate variables calculated from PRISM data and entered were average annual precipitation (cm); frost-free days (days); amount of snow in winter (Dec–Feb); growing degree-days in spring, summer, and fall (Mar–Nov); summer precipitation (Jun–Aug); and an interaction term, annual precipitation x east–west aspect.

Analyses of variance were used to investigate the effectiveness of classification models in explaining genetic variation among sources (PROC MIXED). Sources were classified by fourth, fifth, and sixth field watersheds; plant association series; and 305-m (1000-ft) elevation band. Mixed model analyses of variance were performed on family mean component scores with classifications (such as watersheds or plant association series) as fixed effects and source locations random. For example, the watershed model was:

$$Y_{ijklmn} = \mu + B_i + W_{i(j)} + EL_{l(ij)} + V_{k(ijk)} + S_{m(ijkl)} + F_{n(ijklm)}$$

where μ is the grand mean, *B* is the subbasin (fourth field watershed effect), *W* is the watershed (fifth field) effect, *EL* is the effect of elevation bands, *V* is the sub-watershed (sixth field and smallest watershed division) effect, *S* is the effect of source location, and *F* is the family-within-source effect (error).

Models were compared and lack of fit was evaluated by examining the size of the source location effect—the smaller

the source effect, the better the model explained genetic variation among sources.

Transfer risk among locations within a zone was estimated as the average proportion of genotypes that differ between 2 populations within the zone. This was calculated as the proportion of non-overlap between 2 normal curves (one representing the transferred population and one representing the local, native population) with variances equal to the additive genetic variance within locations and separation equal to the average distance between location means in the zone (Campbell 1986).

Additive genetic variance was calculated as 3X the family-within-source component of variance. A factor of 3 was used because there is some level of inbreeding in these populations (Wilson and Hipkins 2002) that would increase the correlation among progeny of open-pollinated plants. Also, the estimate of variation among location means within a zone includes sampling errors associated with sampling 1 to 4 families per location and genetic effects such as drift and/or migration from non-local pollen sources. An overall risk value of 0.51 implies that 50% of the seedling population is outside the genetic distribution of the local population.

Variation in chromosome number was revealed in isozyme analysis of a subset of the sources used in this study (Wilson and Hipkins 2002). This trait was not quantified directly in this study, however, an attempt was made to classify sources as to tetraploid and diploid cytology indirectly. A discriminant function was developed to classify the cytology of the subset of sources with known cytology (identified by isozyme analysis). A stepwise selection procedure was used to select traits for the discriminant function. This function was then applied to all the sources in this study.

RESULTS

Moderate amounts of genetic variation (source plus family-within-source) were found for broadleaf lupine in this study. Of the 74 traits analyzed, 64 had significant ($P > 0.05$) source or within-source variation. For the majority of traits, the source component was greater than the within-source variance component. In 59 traits where genetic variation was more than 25% of the total phenotypic variation, source variation averaged 28% of the phenotypic variation while within-source variation was 13% of the total. There is also abundant within-source variation and several traits displayed much more variation among families than among sources. For example, genetic variation in emergence was 70% of the phenotypic variance, yet the magnitude of the within-source component was 2.8X

the source component. Other traits displaying this trend were traits relating to flowering date, number of flower spikes, and senescence. Traits in which the majority of genetic variation is exhibited as within-source variation do not provide information about geographic patterns of variation, therefore these traits were dropped from consideration, as were traits highly correlated with other traits occurring later in the life cycle.

I retained 24 traits quantifying size, growth rate, phenology, flower color, and disease resistance for further analysis (Tables 1 and 2). In principal component analysis of 24 traits, 4 components retained more than 72% of the original variability (Table 3). The first component was by far the largest, and summarized 44% of the variability in the dataset. Most of the traits contributed to the variation in this component. The positive coefficients for size, growth rate, and phenology traits indicate that PC1 score increases as these traits increase, and suggest that this component could be a usable surrogate for plant size and vigor. PC2 accounted for 14% of the total variation, and although the patterning of coefficients is not as easily interpreted, there was an emphasis on flower color traits and size at WRN. The remaining 2 components accounted for much less variation (combined about 14%), and there is no clear patterning to the coefficients. Consequently, PC3 and PC4 were dropped from further consideration.

Variation in component scores among sources was significantly correlated with geographic variables (Table 4), with latitude having the strongest correlation with PC1 and departure having the strongest correlation with PC2. Scatter plots of principal component scores plotted by latitude and departure also show trends across the landscape (Figure 4). Regression

TABLE 1

Description of traits used to examine geographic patterns of genetic variation in Lupinus latifolius.

Trait	Description, location,[z] date	Measurement units
CV1H1D1	Crown height-to-diameter ratio, PMC, 7 Jul 1997	cm/mm
CV1HT1	Crown height, PMC, 7 Jul 1997	cm
CV1HT2	Crown height, PMC, 27 Jul 1997	cm
CV1MLD	Mildew score, PMC, 3 Sep 1997	0 = none to 3 = heavy
CV1VIG	Vigor rating, PMC, 3 Sep 1997	1 = unthrifty to 5 = vigorous
CV1VRATE	Rate of crown volume increase, PMC, 1997	cm^3/day
CV1WHC	Amount of white color in flowers, PMC, 15 Jun 1997	0 = light to 3 = strong
CV2BB	Bud burst class, PMC, 17 Mar 1998	1 = dormant to 6 = advanced
CV2DI1	Crown diameter, PMC, 20 Apr 1998	mm
CV2DI2	Crown diameter, PMC, 15 Jun 1998	mm
CV2SH2	Stalk height, PMC, 15 Jun 1998	cm
WR1DI2	Crown diameter, WRN, 1997	mm
WR1GRS	Growth stage, WRN, 15 Sep 1997	1 = green to 5 = senesced
WR1H1D1	Crown height-to-diameter ratio, WRN, 20 Jul 1997	cm/mm
WR1H2D2	Crown height-to-diameter ratio, WRN, 11 Aug 1997	cm/mm
WR1HT2	Crown height, WRN, 11 Aug 1997	cm
WR1RDC	Amount of red color in flowers, WRN, 23 Jun 1997	0 = light to 3 = strong
WR1WHC	Amount of white color in flowers, WRN, 23 Jun 1997	0 = light to 3 = strong
WR1V2	Crown volume, WRN, 20 Jul 1997	cm^3
WR2BB	Bud burst class, WRN, 1 Apr 1998	1 = dormant to 6 = advanced
WR2FORM	Plant form, WRN, 9 Jul 1998	1 = upright to 5 = prostrate
WR2HGT	Crown height, WRN, 27 Jul 1997	cm
WR2LFW	Leaflet width, WRN, 9 Jul 1998	mm
FRSTLEAF	First true leaf, GRN, 1997	Julian date

[z] PMC = Plant Materials Center, Corvallis, Oregon; WRN = Wind River Nursery, Carson, Washington; GRN = Greenhouse at PMC

TABLE 2

Overall means, coefficients of variation, and relative variances (as percentage) for traits measured in the common-garden study of Lupinus latifolius *from Mt Hood National Forest, and 4 principal components.*

Trait [z]	Mean	Coefficient of variation	Total phenotypic variation [y] (%)		
			Sources	Families (Sources)	Plot error
CV1H1D1	0.91	18.8	43.1 ***[x]	11.7 *	45.1
CV1HT1	23.8	27.0	27.7 ***	24.4 **	47.9
CV1HT2	31.6	22.6	40.5 ***	16.9 **	42.6
CV1MLD	1.1	53.3	25.2 ***	21.7 **	53.0
CV1VIG	3.1	11.5	34.7 ***	0.0	65.3
CV1VRATE	542.4	55.2	30.1 ***	8.9	60.1
CV1WHC	1.7	24.3	40.0 ***	7.3	52.6
CV2BB	3.9	28.2	43.8 ***	2.2	54.0
CV2DI1	23.7	35.3	37.9 ***	4.1	58.0
CV2DI2	58.0	27.3	39.4 ***	0.0	60.6
CV2SH2	40.5	29.1	40.6 ***	0.0	59.4
WR1DI2	21.7	19.7	34.6 ***	4.2	61.1
WR1GRS	3.7	27.3	34.3 ***	10.9 *	54.8
WR1H1D1	1.1	24.6	41.0 ***	15.2 **	43.8
WR1H2D2	1.1	21.9	36.0 ***	10.6 *	53.3
WR1HT2	22.9	25.9	45.2 ***	12.2 **	42.6
WR1RDC	1.4	33.6	44.3 ***	10.8 *	44.9
WR1WHC	1.4	31.2	34.4 ***	6.2	59.4
WR1V2	1717.7	43.9	30.5 ***	5.4	64.1
WR2BB	4.3	17.2	35.4 ***	5.8	58.8
WR2FORM	2.6	18.8	50.3 ***	0.8	49.0
WR2HGT	19.5	27.2	47.3 ***	9.5 *	43.2
WR2LFW	10.6	16.6	35.6 ***	4.0 ns	60.4
FRSTLEAF	92.1	4.2	35.8 ***	28.0 ***	36.2
PC1	0.0		79.7 ***	20.3	
PC2	0.0		62.5 ***	37.5	
PC3	0.0		53.3 ***	46.7	
PC4	0.0		17.0 *	83.0	

[z] See Table 1 for trait definitions.
[y] Calculated as $\Sigma^{2TP} = \Sigma^{2S} + \Sigma^{2F(S)} + \Sigma^{2P}$ where Σ^{2S} = variation due to source location, $\Sigma^{2F(S)}$ = variation due to families-within-sources, and Σ^{2P} = variation due to plot error.
[x] *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; ns = not significant

TABLE 3

Principal component analysis of the 24 traits used in this study, with factor loadings and proportions of location variation explained.

Trait [z]	Factor loadings			
	PC1	**PC2**	**PC3**	**PC4**
CV1H1D1	0.18877	0.12923	-0.35632	-0.08614
CV1HT1	0.25167	0.14654	-0.00565	-0.10971
CV1HT2	0.27070	-0.03515	-0.02776	-0.02253
CV1MLD	-0.01777	-0.14292	0.06120	0.63134
CV1VIG	0.24935	-0.05391	0.07771	-0.13662
CV1VRATE	0.26867	0.07128	0.03361	-0.13339
CV1WHC	0.12761	-0.26531	0.16264	0.11600
CV2BB	0.22636	-0.17566	0.15862	0.01430
CV2DI1	0.25080	-0.08058	0.02488	-0.12305
CV2DI2	0.25931	-0.02032	0.06226	-0.07851
CV2SH2	0.25831	-0.09111	-0.01800	-0.06485
WR1DI2	0.07896	0.29605	0.46914	0.22022
WR1GRS	0.05462	0.44274	0.06836	0.13490
WR1H1D1	0.23998	0.20432	-0.23075	0.04779
WR1H2D2	0.21106	0.14553	-0.34572	0.10030
WR1HT2	0.24715	0.27679	-0.01242	0.13539
WR1RDC	-0.15620	0.27836	-0.01425	-0.36505
WR1V2	0.16398	0.33652	0.25854	0.23421
WR1WHC	0.17327	-0.30272	0.03854	0.21040
WR2BB	0.20490	-0.13906	0.02446	-0.04242
WR2FORM	-0.16720	0.16398	0.41640	-0.13454
WR2HGT	0.26901	-0.00349	0.02168	0.03431
WR2LFW	0.11292	-0.24272	0.21026	-0.09674
FRSTLEAF	-0.14104	0.07065	-0.34641	0.38160
Proportion of total variation explained	0.4397	0.1384	0.0908	0.0533
Cumulative proportion	0.4397	0.5781	0.6689	0.7222

[z] See Table 1 for trait definitions.

TABLE 4

Correlation coefficients (r) between factor scores for 2 principal components and 5 geographic and topographic variables.

Geographic or topographic variable	PC1	PC2
Departure	-0.04	-0.55•••
Latitude	0.52 •••	-0.15
Elevation	-0.28 ••	-0.33••
North–south aspect	0.10	0.10
East–west aspect	0.09	-0.08

••• = $P < 0.001$; •• = $P < 0.01$; • = $P < 0.05$

TABLE 5

Amounts of variation among locations (percentage of total sums-of-squares) explained by geographic, topographic, and climatic variables for 2 principal components.

PC	Independent variable (sign of coefficient)	(%)
1	**Intercept**	**2.2**
	Annual precipitation (+)	14.4
	Growing degree-days (+)	12.8
	Frost-free days (-)	5.1
	Annual precip x east–west aspect (-)	2.4
	Latitude (+)	2.3
	East–west aspect (+)	2.0
	Full model (adjusted for number of variables)	46.7
2	**Intercept**	**2.9**
	Growing degree-days (-)	5.7
	Elevation (-)	5.0
	Frost-free days (+)	4.6
	Departure (-)	4.6
	Full model (adjusted for number of variables)	34.0

analysis revealed that moderate amounts of the variation in PC1 could be explained by climatic, geographic, and topographic variables (Table 5). Total precipitation and growing degree-days were the most important independent variables, accounting for approximately 28% and 25% of the model sums-of-squares, respectively. As one would expect, PC1 score (or plant size and vigor) increased as precipitation and growing days increased, and as aspect became more favorable (east or northeast). Of the source variation in PC2, 34% could be explained by frost-free period, growing degree-days, departure, and elevation, each accounting for approximately 25% of the model sums-of-squares.

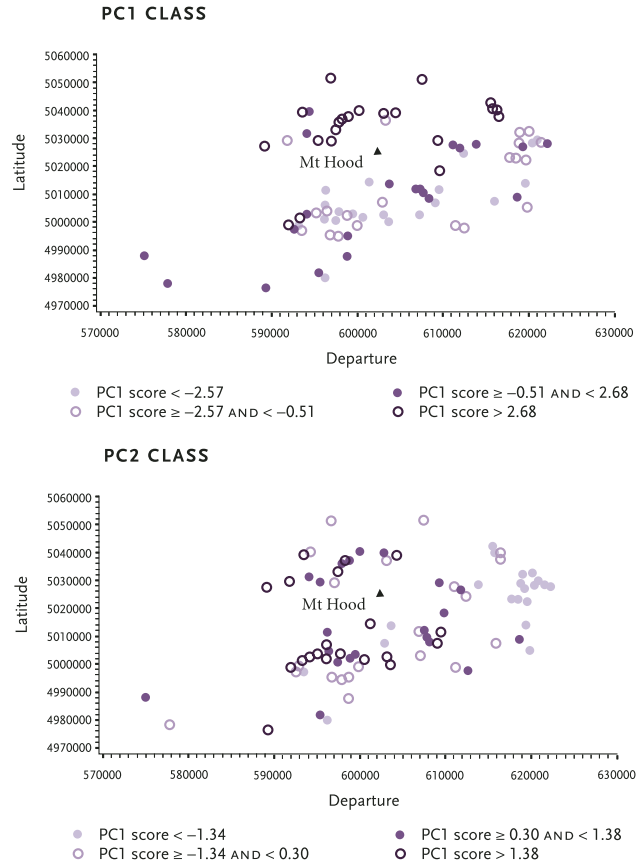

Figure 4. Principal component scores (PC), divided into 4 classes, plotted by latitude and departure. Principal component 1 *(top)*. Principal component 2 *(bottom)*.

Results of analyses on watershed and plant association series classification models are shown in Table 6. All levels of watershed subdivision were significant effects for PC1 and PC2 accounting for 61% and 57% of the sums of squares for sources, respectively. The remaining source variation, or lack of fit, was significant for both components, signifying there were other important variables, such as aspect or soil type, not included in the model. Interestingly, elevation was not a significant effect in classification models, nor in the regression model for PC1. Source location effects were consistently smaller in the watershed model, indicating it explained genetic variation better than the plant association model.

In the plant association models, associations were significant only for PC2, as were elevation bands. Source effects were significant and large for both PCs, and because the watershed models had consistently less lack-of-fit, plant association models were not explored any further.

The stepwise selection procedure selected 8 traits to use in the discriminant function: 1) the amount of red color in flowers at WRN; 2) the amount of red color in flowers at PMC; 3) the

TABLE 6

Comparison of watershed and plant association classification models used to explain variation among sources in 2 principal components.

Effect	Number classes	Proportion of source variation (%) [z]	
		PC1	PC2
Watershed Model			
Fourth field watershed	4	12.3 ***	12.5 ***
Fifth field watershed	13	28.6 ***	23.0 **
Elevation	5	2.0	6.7
Sixth field watershed	24	20.1 *	21.9**
Source	84	37.0 ***	35.9 **
Plant Association Model			
Plant Association	6	2.8	20.9 **
Elevation	5	25.4 **	22.3 **
Source	84	71.8 ***	56.8 ***

[z] Percentage of sums of squares for sources.
*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$

amount of blue color at PMC; 4) ratios of first-year crown height and diameter at WRN; 5) the same ratios at PMC; 6) second-year crown height at PMC; 7) second-year crown diameter at PMC; and 8) the amount of fall regrowth at PMC. Probabilities of correct classification were very high (probabilities were 100% for 74 of 84 sources), however the discriminant analysis was not able to reflect the same variability in cytotype within sources as was seen in the isozyme analysis. Consequently this was not pursued further. If variability in cytotype is noted for a species, any future studies in genetic variation for that species should quantify or score the cytotype of individual plants, as studies in other herbaceous species have shown chromosome number to be of some adaptive importance (Burton and Husband 1999, 2000; Cronn 2004).

DISCUSSION

This study found significant amounts of source-related genetic variation in broadleaf lupine, and furthermore, that patterns of genetic variation among sources correspond to patterns in environmental and geographic variation.

The patterning of source variation, particularly in PC1, along moisture and precipitation gradients suggests that plant performance is related to local conditions at the seed source, and that nonlocal seed sources may or may not perform as well as the local seed source. This means that seeds should not be moved indiscriminately, and some level of control should be exercised on seed transfers. At the same time, the levels of within family and family variation within each source show there is abundant genetic variation within sources, and some degree of transfer should be acceptable.

Under natural regeneration, many seeds will drop to the ground and sprout for each mature plant that develops. For example, Campbell (1979) estimated that a Douglas-fir in an old-growth forest is probably selected from more than 2000 seedlings. A certain amount of natural reproduction will be maladapted due to inbreeding, migration, drift, and recombination during meiosis. Some reproduction, possibly more than half, will die by chance, buried by falling debris, moving soil, predation, and so on. Nevertheless, natural thinning between seedling and mature plant results in a large amount of natural selection acting to produce variation among and within sources

Implications for Seed Transfer

Relative seed transfer risk was quantified by comparing the phenotypic variation within a defined seed zone to the average genetic variation within a source location. What constitutes an acceptable level of risk will probably vary depending on the objectives for seedling outplanting, availability of seeds and resources, and the philosophy of the organization. Sorensen and Weber (1994) suggested an upper limit for risk of 0.51 for reforestation practices, based on final desired crop density adjusted for mortality occurring throughout the life span of the planting. Following a similar methodology, I arrived at an upper level for risk of 0.61. Assumptions included a final crop density of 47 840 plants/ha (19 360/ac) on a 46-cm (18-in) spacing; 76 289 seeds/kg (34 699/lb); broadcast sowing at 22.4 kg/ha (20 lb/ac); 80% germination rate; and 90% fall-down (10% success) because of incorrect planting depth, desiccation, predation, and fungal pathogens (Darris 2004). These assumptions resulted in a need for 35% of the seeds sown to become established ($47\,840/[7620 \cdot 22.4 \cdot 0.8 \cdot 0.1] = 0.349$), and 65% expendable. The difference between an R of 0.61 and the 65% that can be lost provides an additional small cushion for the unexpected.

Transfer risk was estimated for several different seed zone strategies (Table 7). The strategies were chosen to display a range of options for limiting seed transfer effects and were suggested by results of regression and classification analyses. While climatic variables, such as growing degree-days and annual precipitation, were important explanatory variables in regression analyses, this data is not commonly available to field personnel. Consequently geographic land divisions, such as the watershed delineations, were used as a convenient, readily available method for constructing zones. It should be stressed that the calculated risks are relative, have not been field-tested, and the utility of these estimates is primarily in comparing the effectiveness of different strategies.

When seeds are collected from and freely moved about within the entire Mt Hood National Forest, the proportion of

TABLE 7

Relative risk (R) estimates for seed transfer zones for Lupinus latifolius *on the Mt Hood National Forest.*

Effect	R for PC1	R for PC2	Combined R
No subdivision	0.048	0.035	0.66
Fourth field watersheds	0.040	0.031	0.059
Fifth field watersheds	0.033	0.024	0.049
Sixth field watersheds	0.027	0.020	0.042

a seedlot outside the genetic distribution at the planting location is 66%. Looking at it another way, 34% of the seed population is still within the genetic distribution at the planting location, attesting to the significant amounts of genetic variation within local populations of this species.

The most conservative strategy, collecting and deploying seeds within a sixth field watershed (the smallest watershed division), had a relative risk of 0.42. This strategy would be relatively expensive, and necessitate using 87 seed zones for the entire Mt Hood National Forest. However, if the objective for outplanting was to mimic existing patterns of genetic variation, this gives an idea of the amount of mismatch there would be in a collection area this size.

Using fifth field watersheds is very similar to the existing seed collection and deployment policy of the USDA Forest Service. Risk associated with moving seeds within these delineations is 0.49, fairly conservative when compared to the 0.61 allowable risk calculated using the procedure outlined above. It would result in 31 seed zones to cover the entire forest.

Fourth field watersheds are recommended for seed zones for this species on the Mt Hood National Forest (Figure 1). Risk for this level of geographic subdivision is 0.59, below the upper allowable transfer risk of 0.61. This strategy would require only 4 seed zones, and provides a reasonable compromise between maintaining many locally adapted seedlots and keeping management costs for collection and maintenance low. Seeds should be collected from several sites (10 to 16) to sample within-zone variation, but a large number of sites should not be necessary because of moderate amounts of within-source variation found in this study and in allozymes (Wilson and Hipkins 2002). Within a source, many plants, perhaps as many as 30 to 50, should be collected from to adequately sample within-source genetic variation.

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