Landscape patterns of phenotypic variation and population structuring in a selfing grass, *Elymus glaucus* (blue wildrye)

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Abstract: Source-related phenotypic variance was investigated in a common garden study of populations of *Elymus glaucus* Buckley (blue wildrye) from the Blue Mountain Ecological Province of northeastern Oregon and adjoining Washington. The primary objective of this study was to assess geographic patterns of potentially adaptive differentiation in this self-fertile allotetraploid grass, and use this information to develop a framework for guiding seed movement and preserving adaptive patterns of genetic variation in ongoing restoration work. Progeny of 188 families were grown for 3 years under two moisture treatments and measured for a wide range of traits involving growth, morphology, fecundity, and phenology. Variation among seed sources was analyzed in relation to physiographic and climatic trends, and to various spatial stratifications such as ecoregions, watersheds, edaphic classifications, etc. Principal component (PC) analysis extracted four primary PCs that together accounted for 67% of the variance in measured traits. Regression and cluster analyses revealed predominantly ecotypic or stepped-clinal distribution of genetic variation. Three distinct geographic groups of locations accounted for over 84% of the variation in PC-1 and PC-2 scores; group differences were best described by longitude and ecoregion. Clinal variation in PC-3 and PC-4 scores was present in the largest geographic group. Four geographic subdivisions were proposed for delimiting *E. glaucus* seed transfer in the Blue Mountains.

Key words: Elymus glaucus, morphological variation, local adaptation, seed transfer, seed zones, polyploid.

Résumé : Les auteurs ont étudié la variance phénotypique reliée à la source, dans un jardin commun, où ils ont observé des populations de l'*Elymus glaucus* Buckley (élyme glauque) provenant de la province écologique de Blue Mountain, dans le nord-ouest de l'Oregon, jouxtant l'état de Washington. Par cette étude, les auteurs cherchent à évaluer les patrons géographiques d'une différenciation potentiellement adaptative chez cette herbacée auto-fertile allotétraploïde, et à utiliser cette information pour développer un cadre de référence afin d'orienter le mouvement des semences et la préservation des patrons adaptatifs de la variation génétique, dans les travaux de restauration en cours. Ils ont cultivé la progéniture de 188 familles pendant 3 ans, sous deux conditions d'humidité, et ils ont mesuré un large ensemble de caractères incluant, la croissance, la morphologie, la fécondité et la phénologie. Ils ont analysé la variation entre les sources de graines en relation avec la physiographie et les tendances climatiques, ainsi qu'avec diverses stratifications spatiales, soient les écorégions, les bassins versants, les classifications édaphiques, etc. L'analyse en composantes principales identifie quatre PC primaires qui couvrent ensemble 67 % de la variance des caractères mesurés. Les analyses par regroupement et par régression montrent une distribution de la variation génétique surtout écotypique ou reliée à la pente. Trois groupes distincts de localisations géographiques expliquent 84 % de la variation indiquée par les PC-1 et PC-2; les différences entre groupes s'expliquent le mieux par la longitude et l'écorégion. La variation clinale indiquée par les PC-3 et PC-4 se retrouve dans les plus grands groupes géographiques. Les auteurs proposent quatre subdivisions géographiques pour délimiter le déplacement des graines, dans les Blue Mountains.

Mots clés : Elymus glaucus, variation morphologique, adaptation locale, déplacement des graines, zones de semence, polyploïdie.

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Introduction

The condition and extent of native forest and grassland communities are declining throughout large portions of the interior western United States (Hann et al. 1997; Hessburg et al. 1999). A number of factors are responsible for this trend, including ungulate herbivory (Hobbs 1996; Belsky and Blumenthal 1997; Augustine and McNaughton 1998), nonnative invasive plants (Mack 1981; Young et al. 1987), and altered fire regimes (Agee 1994; Everett et al. 1994). Land

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management agencies have responded to declines in wildland vegetative conditions by initiating programs to collect and use native plant species in habitat restoration and revegetation projects. Critically lacking in this work is genetic and plant fitness and performance information to help guide decisions regarding the appropriate ecological and geographic distances that seed should be transferred from original source populations.

One of the principal native grass species being collected and propagated for restoration use in the Pacific Northwest is *Elymus glaucus* Buckley (blue wildrye). *Elymus glaucus* is a self-pollinating, allotetraploid species ($2n = 28$), with genomes derived from *Pseudoroegneria* (St genome) and *Hordeum* (H genome) (Dewey 1982; Jensen et al. 1990). This nonrhizomatous, cool-season, perennial bunchgrass occurs in a broad array of ecological settings throughout western North America (Hitchcock et al. 1969). It is most common in riparian areas, along montane meadow edges, and in forest openings under light to moderate shade, but rarely forms pure stands, except in highly disturbed areas such as roadside corridors and sites disturbed by fire or timber harvesting. Although relatively short-lived, *E. glaucus* possesses a number of characteristics that make it well suited for habitat restoration and soil stabilization, including frequent and abundant seed production, rapid germination and early seedling growth, and vigorous and deeply penetrating fibrous root systems. Also, the species provides important forage for wild and domestic animals (USDA 1937; Frischknecht and Plummer 1955).

Owing to a lack of genetic information, conifer seed zones and elevation restrictions are frequently used to help guide seed movement in *E. glaucus*. This framework, which was developed for outcrossing conifers, may be inappropriate for inbreeding graminoids (Knapp and Rice 1996). Thus, large-scale seed transfers based on conifer guidelines could not only adversely affect the mean adaptability and sustainability of introduced populations, but could negatively impact the gene pool of indigenous populations as well through hybridization and introgression (Knapp and Rice 1996; Montalvo et al. 1997; Lesica and Allendorf 1999; Montalvo and Ellstrand 2001; Hufford and Mazer 2003).

Improper seed transfer guidelines also can create management difficulties. An unnecessarily restrictive framework (i.e., many zones each having small seed needs) will have adverse effects on seed cost and supply. In an effort to create seed sources with larger potential markets, some researchers advocate the development of "regional ecotypes" through the mixing of distant gene pools from a wide array of environments (Booth and Jones 2001; Burton and Burton 2002). Information on levels and patterns of genetic variation could help determine the appropriate spatial scale of these practices, and minimize cultural and harvesting complications in commercial seed production operations resulting from wide genetic variation in seed germination rate, plant size, and timing of anthesis and seed maturity. These differences could cause harvests to miss seeds containing valuable genetic variation, resulting in unintentional selection and genetic shifts in plant material germplasm (Campbell and Sorensen 1984).

At present, knowledge of the extent and nature of adaptive genetic variation in *E. glaucus* is very limited. Natural stands of *E. glaucus*, as well as plants in common garden environments are known to exhibit a tremendous array of local phenotypic variation (Snyder 1950; Adams et al. 1999), but obvious geographic clines or patterns of variability have not been documented in this species (Snyder 1950). Conforming to expectations for a selfing species (Hamrick and Godt 1990), isozyme variability among populations is very high with evidence of strong genetic differentiation over relatively small spatial scales (Knapp and Rice 1996; Wilson et al. 2001). In the large and ecologically diverse California Floristic Province, there was no relation between genetic and geographic distance, but there were significant genetic differences based on source elevation and on pubescence (Wilson et al. 2001). However, much evidence indicates that gene markers are rarely good indicators of adaptive response (Giles 1984; Bonnin et al. 1996; Knapp and Rice 1997; McKay and Latta 2002; Volis et al. 2002). Therefore, it is important to study population structure and source-related variance in morphological and phenological traits when devising seed transfer zones for native plant populations used in restoration plantings.

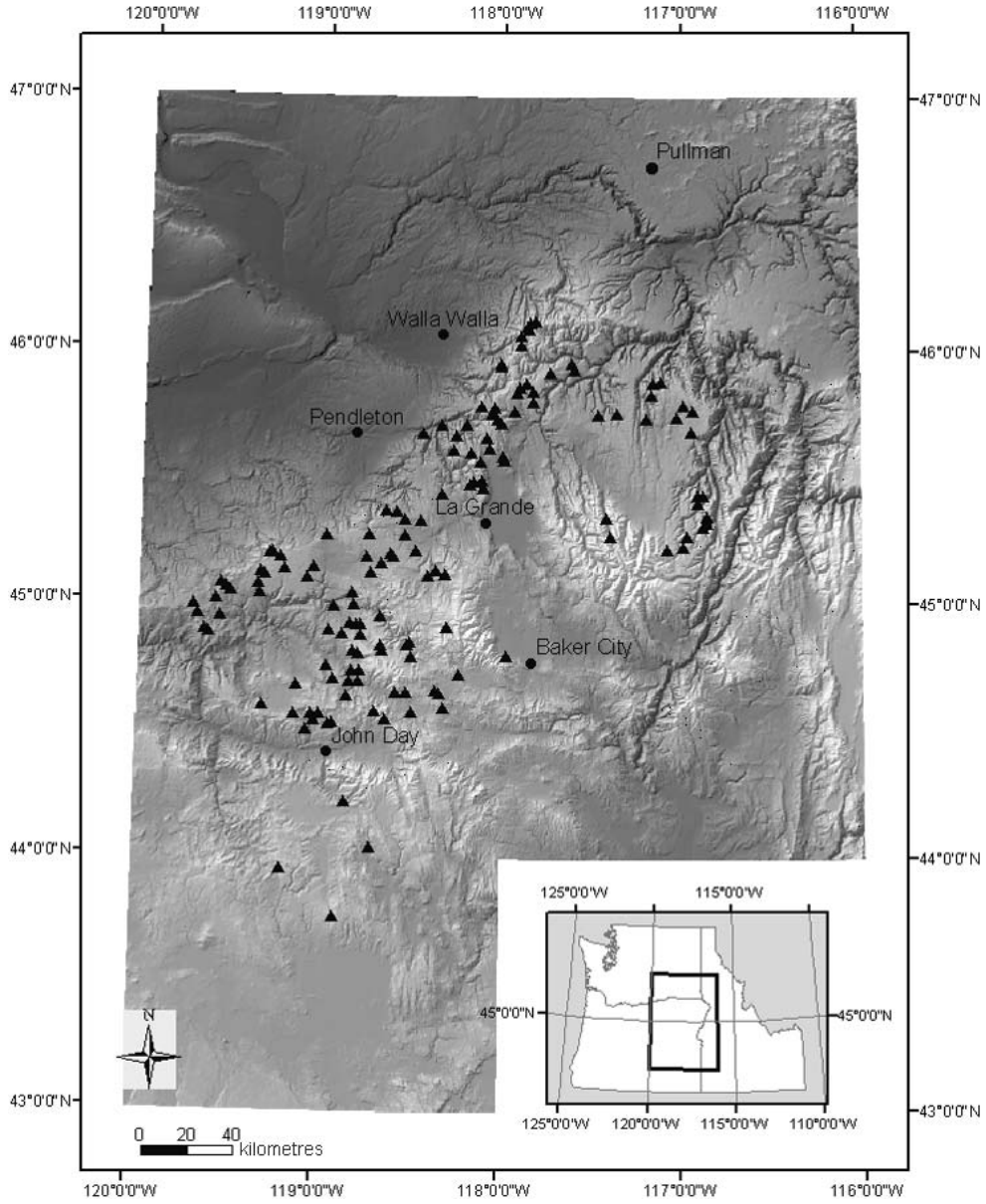
In this paper we describe intraspecific phenotypic variation in *E. glaucus* from a wide range of geographic sources from the Blue Mountains of northeastern Oregon and southeastern Washington. Our purposes were (*i*) to determine levels and patterns of variation in a large number of plant traits measured under common garden conditions, (*ii*) to relate variation among populations, if present, to geographic and climatic trends, and to various environmental stratifications such as ecoregions, watersheds, conifer seed zones, and vegetation and edaphic classifications, and (*iii*) to develop an improved framework for guiding the collection and utilization of *E. glaucus* plant materials in the Blue Mountains Province. Procedures and results are presented in detail to serve as a model for relating natural phenotypic variation to environmental gradients and to environmental classification systems.

Materials and methods

Population sampling

Seed was collected in the summer of 1994 from 153 locations throughout the Blue Mountains Ecological Province (Fig. 1). The selected locations reflected the full range of environmental and climatic conditions over which *E. glaucus* occurs in this area, spanning nearly 2.5 degrees in latitude (range 43°48′N–46°6′N), 3 degrees in longitude (range 116°48′W–119°42′W), and 1260 m in elevation (range 741–1998 m). Sampling included a large number of locations to use regression models to relate trait variation to physiographic and climatic variables (Campbell 1979, 1986). Because of constraints on test size and because the purpose was to describe patterns rather than to estimate values for specific locations, seeds were collected from one (118 locations) or two (35 locations) plants per location. The two-plant collections provided a pooled estimate of the amount of within-population variance across many sites (Hamrick 1976; Podolsky et al. 1997), which could then be used for testing variance among populations. At locations where seeds were collected from two individuals, plants were separated by a minimum of 5 m (range 5–30 m). The purpose of

Fig. 1. Location of *Elymus glaucus* sampling sites in northeastern Oregon and southeastern Washington (USA). The nursery test site (Pullman, Washington) is at the upper right.



the separation was to minimize relatedness within locations (Knapp and Rice 1996), while restricting the two parents to the same environmental site. Seeds from each plant were stored in separate envelopes at room temperature for approximately 5 months until time of sowing.

Two subspecific taxa of *E. glaucus* were included in the samples: *glaucus* and *jepsonii* (Davy) Gould (Hitchcock et al. 1969; Barkworth 1993). Subspecific identity was not retained for three reasons: (*i*) doubt as to the validity of the designation (Wilson et al. 2001; K. Jensen, personal communication), (*ii*) almost completely overlapping distribution in the field, and (*iii*) nearly identical responses to the independent variables in preliminary analyses.

Latitude, longitude, and elevation were recorded for each sample location. Locations were also classified according to conifer seed zones (USDA 1973*a*, 1973*b*), soil types (based on National Forest Soil Resource Inventory maps), eco-region subdivisions (Level IV, Omernik 1987, 1995; Clark and Bryce 1997), US Geological Survey watershed stratifications (Seaber et al. 1987), and plant association vegetation groupings (Johnson and Clausnitzer 1992).

The climatic conditions at each seed collection site were characterized using digital maps and data generated by the PRISM climate model (Daly et al. 1994; http://www.ocs.orst.edu/prism), which provides gridded estimates (4-km resolution) of mean monthly and yearly temperature and precipitation, mean minimum and maximum monthly temperatures, and the mean dates of the last frost in the spring and the first frost in the fall.

Experimental design

In January 1995, seeds were sown in containers in a greenhouse at the Natural Resources Conservation Service Plant Material Center in Pullman, Washington (Fig. 1) (ele-

Fig. 2. Photograph of the *Elymus glaucus* common garden study area in Pullman, Washington. The high degree of morphological uniformity within the two-plant family plot in the foreground was typical.

vation 787 m; mean annual precipitation 571 mm). After 8 weeks, the grass plugs were transplanted to two contrasting test environments (irrigated and nonirrigated). In each test environment, two individuals per family were assigned to row plots within each of four replications (Fig. 2). Plant spacing was 0.3 m between the two individuals in a family plot, 0.9 m between families within rows, and 1.5 m between rows. Each test environment was surrounded by two border rows. Weeds were controlled using mechanical tillage. Irrigated replications were sprinkler irrigated three to four times throughout the summer growing season to maintain field capacity at a depth of 20–30 cm. Plants were measured over three growing seasons. Pooling over treatments, survival at the end of the study was 98%.

Trait measurement

Traits involving growth, phenology, morphology, fecundity, and seed weight/germination were recorded for each individual over a 3-year period. Growth traits, measured on all plants at the end of each of the three growing seasons, included leaf length, leaf width, mature plant height, culm height, inflorescence length, and crown diameter. Indices of plant growth form were derived from ratios of these variables (e.g., total height/culm height, leaf length/leaf width). In year 2, several additional traits were assessed, including leaf, awn, and inflorescence color, and the degree of leaf and stem pubescence and glaucousness. Fecundity was measured in year 1 by counting mature inflorescences and in year 2 by assigning plants to one of four classes representing no, low, medium, or high seed production. Phenological variables included the degree of vegetative senescence in early fall, and the mean Julian date of inflorescence emergence, pollen anthesis, and inflorescence shatter and disarticulation.

Seed weights and germination traits were obtained from seeds harvested from the field test at the end of the second growing season. Ten well-developed inflorescences were clipped from one plant of each family in one replicate of each test environment (irrigated and nonirrigated). Seeds were stored at 1 °C for 2–5 months. Seeds were soaked in 3% H_2O_2 for 24 h, stratified at 1 °C for 5 d, and placed in a germinator at 15 °C. The germination trial consisted of four replications of 25 filled seeds from each family and environment. Observations began after 1% of the seeds had germinated (41 h), and continued up to three times daily for 27 d. Mean germination rates (1/d) and standard deviations of rates were estimated following Campbell and Sorensen (1979).

Statistical models and analyses

Our general statistical methodology followed procedures of Campbell (1979, 1986) for mapping genetic variation across the landscape. Data analyses were performed on family block means using software of SAS, version 8 (SAS Institute Inc. 1999). SAS GLM and SAS VARCOMP procedures (SAS Institute Inc. 1999) were used to determine which traits varied significantly among populations, and to partition the genetic variation into location and family-in-location components (σ^2_L and $\sigma^2_{F/L}$). In the model, families were nested in locations as shown below:

$$Y_{ijk} = \mu + B_i + L_j + F_{k(j)} + E_{ik(,j)}$$

where Y_{ijk} is the mean of the two plants from the *i*th block of the *k*th family in the *j*th location, μ is the grand mean, *B* is the effect of blocking, *L* is the effect of seed source location, *F* is the effect of family, and *E* is the experimental error. Data from the 35 locations where two families were sampled were used to estimate $\sigma^2_{F/L}$, the pooled within-location variance, which was used to test the significance of the among-population variance. Analysis of variance (ANOVA) was also conducted on the total data set, across both irrigated and nonirrigated replications, to determine the effect of test environment on measured traits and the degree of interaction with seed origin. A significant location × test environment interaction would indicate a differential plastic response to the watering treatment among source populations. These effects were tested with irrigation treatment as a fixed effect, and location and families-in-location as random effects.

One hundred twelve traits were recorded, 56 in each environment. Many traits were deleted because of (*i*) highly skewed or kurtotic distributions that could not be normalized, (*ii*) nonsignificant ($P > 0.01$) location effect, or (*iii*) collinearity (Truxillo 2002). If the correlation between two traits was high ($r > 0.80$), the trait with the lesser location effect was deleted. If the location × test environment

Table 1. Vegetative, floral, and seed traits of *Elymus glaucus* and their descriptions.

Trait	Description
LVWI2	Maximum width (mm) of longest flag leaf at maturity, year 2
LVWI3	Maximum width (mm) of longest flag leaf at maturity, year 3
R11W	Leaf length/leaf width ratio, year 1, irrigated. Leaf length (cm) was measured from the stem to the tip of the longest leaf
R13D	Leaf length/leaf width ratio, year 3, nonirrigated. Leaf length (cm) was measured from the stem to the tip of the longest leaf
TOTHT1	Mature height (cm) from ground level to top of inflorescence of tallest culm, year 1
TOTHT2	Mature height (cm) from ground level to top of inflorescence of tallest culm, year 2
TOTHT3	Mature height (cm) from ground level to top of inflorescence of tallest culm, year 3
INFLE2	Mean length (cm) of inflorescence, year 2
INFLE3	Mean length (cm) of inflorescence, year 3
R22	Total height/culm height (ground level to base of inflorescence) ratio, year 2
CRWI1	Width (cm) of widest portion of lower crown leaves, year 1
CRAVG3	Average crown radius (cm), year 3
EMERG2	Date when 50% of inflorescences have emerged from boot, year 2
INFNO1	Number of inflorescences emerged from boot, year 1
FEC2	Amount of seed production (classes = none, low, medium, high), year 2
PUB2	Amount of lower leaf pubescence (classes = none, sparse, dense, long), year 2
INFCOL2	Inflorescence color (classes = green, green with some purple, very purple), year 2
AWNCOL2	Awn color (classes = green, green with some purple, very purple)
GMEANW	Mean germination rate (1/d)
GSTDW	Standard deviation of germination rate (1/d)

Note: Data were pooled across irrigated and nonirrigated test environments unless otherwise noted by a final W or D for irrigated and nonirrigated, respectively.

interaction was nonsignificant for a trait, then either the irrigated trait or the nonirrigated trait or the trait across both environments was used, depending on which exhibited the greatest location effect. Otherwise, the same trait measured in each of the two environments was treated as two separate variables (Campbell and Sorensen 1978). After deletions and combinations, 20 traits were retained for subsequent analysis (Table 1).

Principal component analysis was used to reduce redundancy among the 20 retained traits and capture as much of the location variance as possible in a smaller set of unique, uncorrelated, orthogonal variables (components). A matrix of location level (rather than family level) correlation coefficients was used as input for the principal component analysis, because adaptive variation important in seed transfer is most directly related to variation among source locations. Principal component (PC) scores were calculated for each location and family from the eigenvectors of the first four PCs.

Location PC scores were used in multiple regression and hierarchical classification models to examine continuous and stepped clinal patterns of genetic variation over the entire area and, subsequently, within subgroups. Multiple regression models were built by relating PC scores to physiographic and estimated climatic variables for each location. Physiographic predictor variables included latitude, longitude, and elevation, and their quadratic and interaction terms (Campbell 1979, 1986). Estimated climatic variables included monthly temperature and precipitation (means, minimums, and maximums), and several annual and seasonal summary variables such as the speed of spring warming (May minimum temperature – February minimum temperature). Model building was accomplished using the R^2 selection method to identify the model with the largest R^2 for each number of variables considered (Neter et al. 1983). Lack of fit to the selected equations was tested by using, as replicate observations, the two families sampled at each of 35 locations (Neter et al. 1983).

A number of classification systems commonly used to stratify spatial and environmental variation were evaluated, including conifer seed zone, watershed, ecoregion, plant association, and soil type (Campbell and Franklin 1981; Campbell 1991). For each PC, analysis of variance was performed on family means according to the model shown below for conifer seed zone:

$$Y_{ijk} = \mu + Z_i + L_{j(i)} + F_{k(i,j)}$$

where Y_{ijk} is the mean of the two plants from the kth family in the jth location in the ith conifer seed zone, μ is the grand mean, Z is the effect of conifer seed zone, L is the effect of seed source location within a seed zone, and F is the effect of family within a location. Lack of fit to the classification model was tested by the significance of the P value for location within classes. Components of variance were estimated for location with and without the classification in the model as a fixed effect ($\sigma^2_{L/Z}$ and σ^2_L). The percentage of location variance accounted for by the classification was calculated as follows:

$$(\sigma^2_L - \sigma^2_{L/Z})/\sigma^2_L \times 100$$

Cluster analysis of principal component scores (cluster procedure, Ward's minimum variance method, SAS Institute Inc. 1999) was used to investigate ecotypic structure and to group seed source locations by their relative phenotypic similarity. Discriminant analysis and classification were used to determine which physiographic and climatic variables best

Table 2. Test means, coefficients of variation for location (CV_L (%)) and families within location ($CV_{F/L}$ (%)), and the proportion of total genetic variance attributed to location (σ^2_L (%)) and to families within location ($\sigma^2_{F/L}$ (%)) for selected traits and four principal components.

Trait	Mean	CV_L (%)	$CV_{F/L}$ (%)	σ^2_L (%)	$\sigma^2_{F/L}$ (%)
LVWI2	13.16	16.2	8.4	78.7****	21.3***
LVWI3	9.80	11.8	5.4	82.6****	17.4*
R11W	1.35	20.8	5.2	94.1****	5.9****
R13D	1.37	14.1	7.5	78.2**	21.8*
TOTHT1	66.32	24.7	14.6	74.0****	26.0****
TOTHT2	134.91	7.9	2.9	88.0****	12.0****
TOTHT3	109.04	7.7	3.4	84.0****	16.0***
INFLE2	16.71	9.4	1.9	96.2***	3.8****
INFLE3	13.40	8.9	5.6	71.6**	28.4***
R22	1.14	1.2	0.5	82.2***	17.8**
CRWI1	42.21	12.6	4.6	88.0****	12.0**
CRAVG3	16.24	9.5	6.7	66.5**	33.5***
EMERG2	161.15	1.6	0.8	78.1****	21.9****
INFNO1	34.98	54.3	19.3	88.8****	11.2****
FEC2	2.72	12.1	8.4	67.3***	32.7****
PUB2	1.55	35.7	13.5	87.6****	12.4****
INFCOL2	1.79	23.0	17.1	64.3***	35.7****
AWNCOL2	1.77	13.0	2.8	95.4****	4.6
GMEANW	0.26	24.8	12.0	80.9****	19.1****
GSTDW	0.08	26.3	13.3	79.6****	20.4****
PC-1	0.00	—	—	91.9****	8.1***
PC-2	0.00	—	—	93.5****	6.5**
PC-3	0.00	—	—	67.2***	32.8***
PC-4	0.00	—	—	80.0****	20.0**

Note: Codes representing the traits are described in Table 1. *, statistically significant at $P < 0.05$; **, statistically significant at $P < 0.01$; ***, statistically significant at $P < 0.001$; ****, statistically significant at $P < 0.0001$.

described the differences among the resultant seed source groups. These analyses were conducted using SAS DISCRIM and SAS STEPDISC procedures (SAS Institute Inc. 1999). Qualitative descriptor variables with more than two values, such as ecoregion, conifer seed zone, and soil type, were evaluated with χ^2 analysis of class frequency tables.

Results

Variation in plant traits

The influence of supplemental watering was minimal. There were significant differences between wet and dry treatments for only 17 (30.3%) of the 56 analyzed traits, and seed source location by irrigation interaction was significant for only 11 (19.6%). In all cases, the effect was small and, surprisingly, plants in the dry treatment were significantly taller in the second year. None of the 20 traits selected for the principal component analysis showed significant interaction between water treatment and source population, suggesting little or no difference in phenotypic plasticity of plants from different geographic origins. These results may have been influenced, at least in part, by a lack of strong difference in water availability between the two test environments, especially in year 2 when spring rainfall was unusually high (e.g., April precipitation was 76 mm above normal).

With few exceptions, measured traits exhibited considerable variability across the sampling area, as indicated by their high coefficients of variation for location (mean = 16.8%, Table 2). Partitioning of variance into the two levels of the sampling design (i.e., locations and families within locations) resulted in highly or very highly significant variance components for nearly all plant characters. Of the total genetic variation ($\sigma^2_L + \sigma^2_{F/L}$), most was source ($\sigma^2_L$) related (Table 2). Averaged over all traits, 81.3% of the variation was associated with differences among locations, only 18.7% with difference among families within locations. Visual observations of plants in family plots suggested that within family variability for most traits was strikingly low (Fig. 2).

Principal component analysis

The principal component analysis extracted four primary principal components, which together accounted for 67% of the variance in the 20 original traits (Table 3). Eigenvalues of PC-1 and PC-2 were of substantial and nearly equal importance (5.055 and 4.404), while those of PC-3 and PC-4 were smaller (2.059 and 1.811). Because of the large number of variables, none of the eigenvector loadings were very large, and the principal components were not easily interpreted.

As with individual traits, a large proportion of the total genetic variance in the principal component variables was

Table 3. Principal components (PC) with trait loadings, eigenvalues, and percentage of seed source location variance explained by principal components.

	Loadings			
Trait	PC-1	PC-2	PC-3	PC-4
LVWI2	0.274	–0.244	–0.141	–0.073
LVWI3	0.305	–0.218	–0.015	0.024
R11W	–0.345	0.081	0.110	0.299
R13D	–0.216	0.271	0.143	0.149
TOTHT1	0.280	0.245	0.008	–0.257
TOTHT2	0.237	0.284	0.181	–0.111
TOTHT3	0.231	0.261	0.315	–0.001
INFLE2	0.277	0.185	–0.105	0.421
INFLE3	0.283	0.187	0.008	0.328
R22	0.127	–0.020	–0.292	0.580
CRWI1	0.050	–0.323	0.216	0.095
CRAVG3	0.037	–0.030	0.591	0.138
EMERG2	0.313	–0.030	–0.060	0.118
INFNO1	0.300	0.007	0.117	–0.194
FEC2	0.052	–0.188	0.539	0.140
PUB2	–0.294	–0.087	0.049	0.112
INFCOL2	0.045	–0.261	0.073	0.013
AWNCOL2	0.013	–0.316	0.063	0.236
GMEANW	–0.148	0.324	0.057	0.004
GSTDW	–0.015	0.336	0.004	0.125
Eigenvalue	5.055	4.404	2.059	1.811
Variation explained (%)	25.3	22.0	10.3	9.1
Cumulative %	25.3	47.3	57.6	66.6

Note: Codes representing the traits are described in Table 1.

source related, especially for PC-1 and PC-2 (σ^2_L = 91.9% and 93.5%, Table 2). Patterning of this variation across the landscape was investigated using both classification and cluster analysis, as well as regression analysis with physiographic and climatic predictor variables. Across the entire sampling area, more source-related variation was explained by cluster analysis than by either hierarchical classification or regression models. Consequently, we present results of cluster models first, followed by classification and regression models within groups or subregions where appropriate.

Clustering of location variance

Histograms of PC-1 and PC-2 scores showed a nonnormal frequency distribution, with the values for different seed sources tending to aggregate into distinct classes or groups (data not shown). Cluster analyses of PC-1 and PC-2 scores resulted in locations being separated into three discrete clusters. When clusters where included as a fixed effect in analysis of variance models, they accounted for an amazingly large 84.1% and 89.6% of the total location variance for PC-1 and PC-2, respectively (Table 4, Fig. 3). These results suggest an ecotypic or stepped-clinal partitioning of genetic variation in the traits associated with the first two principal components.

Cluster 1 was separated from the other clusters by low PC-1 scores (Table 5, Fig. 3), which corresponded to locations with short plants that were highly pubescent (TOTHT2 and PUB2 in Table 5), early inflorescence emergence (EMERG2), and low fecundity (INFNO1). Cluster 3 was separated from the other clusters by low PC-2 scores (Table 5, Fig. 3), tall plants (TOTHT2 in Table 5) with narrow crowns (CRWI1) and long inflorescences (INFLE2). Cluster 3 also was exceptionally variable in PC-2 scores, particularly compared with cluster 1 (Fig. 3). Cluster 2 had high mean scores for both PCs and high variability in PC-1 scores (Table 5, Fig. 3). Cluster-2 plants had wide leaves (LVWI2 in Table 5) and crowns (CRWI1), highest first year seed production (INFNO1), and lowest germination rate (GMEANW).

Scatter plots of PC-1 and PC-2 scores revealed that the clusters represented three groups of locations that were quite distinct geographically. Cluster-2 locations generally occurred east of longitude 118°45′W (vertical dotted line, Fig. 4), while cluster-1 and cluster-3 locations were primarily west of longitude 118°45′W. Stepwise discriminant analysis of PC-1 and PC-2 scores showed that longitude was the most important variable contributing to cluster differentiation, explaining over 28% of the variation among the three clusters (Table 6). Two climatic variables, February precipitation and the speed of spring warming (spring heat = May minimum temperature – February minimum temperature), explained an additional 17.8% of the variation contributing to cluster differentiation, for a cumulative total of 46.6% (Table 6). Other PCs had unimodal distributions and did not contribute to cluster differentiation.

Differentiation west of longitude 118°45′W

Two clusters, 1 and 3, occurred west of 118°45′W. These clusters were sharply differentiated from one another in both PC-1 and PC-2 scores (Table 5, Fig. 3); cluster 1 contributing the lower mode to PC-1, and cluster 3 contributing the lower mode to PC-2. Site classification variables were evaluated to determine the influence of soils or vegetative or climatic conditions on cluster differentiation. All classification methods separated the two clusters, suggesting that cluster-1 and cluster-3 sources differed in site preference or habitat requirements. Classifying by ecoregions provided the best discrimination, assigning 20 out of 24 cluster-1 locations to a single ecoregion, 11b (John Day – Clarno Highlands). Ecoregion 11b is distinguished from the other ecoregions west of 118°45′W by greater aridity. Cluster-3 locations, on the other hand, were distributed throughout the area west of 118°45′W, including portions of ecoregion 11b.

Cluster-1 phenotypes, compared with cluster-3 phenotypes, were much more homogenous in the common garden test, particularly for PC-2 scores (Fig. 3). Similarly, cluster-1 habitats were much more restricted to the driest portion of the range. Figure 3 also indicates that cluster-3 phenotypes would at most only rarely overlap cluster-1 phenotypes. Cluster-3 locations do occur in ecoregion 11b, but close examination of Fig. 4 indicates that cluster-3 locations are occurring only marginally in the area where cluster-1 locations are concentrated. This suggested that whatever the origin of the two clusters, their present separation is defined both by habitat limitation of cluster-3 locations and by distinct differences in terms of habitat preference.

Differentiation east of longitude 118°45′W

With few exceptions, locations east of 118°45′W were within cluster 2. As noted earlier with regard to PC-1 and

Table 4. Components of variance for location with ($\sigma^2_{L/C}$) and without (σ^2_L) clusters in the ANOVA model as a fixed effect, and the proportion of location variance attributed to clusters ($(\sigma^2_L - \sigma^2_{L/C})/\sigma^2_L \times 100$) for four principal components.

Trait	σ^2_L	P value for σ^2_L [a]	$\sigma^2_{L/C}$	P value for $\sigma^2_{L/C}$ [b]	$(\sigma^2_L - \sigma^2_{L/C})/\sigma^2_L \times 100$
PC-1	4.409	<0.0001	0.700	0.0064	84.1
PC-2	3.902	<0.0001	0.405	0.0223	89.6
PC-3	1.025	0.0009	0.980	0.0010	4.3
PC-4	1.189	0.0001	1.073	0.0003	9.8

[a]Significance of mean square associated with location variance component when cluster effect is excluded from the ANOVA.
[b]Significance of mean square associated with location variance component when cluster effect is included in the ANOVA.

Fig. 3. Scatterplot of PC-1 versus PC-2 values, showing the presence of three well-separated clusters.

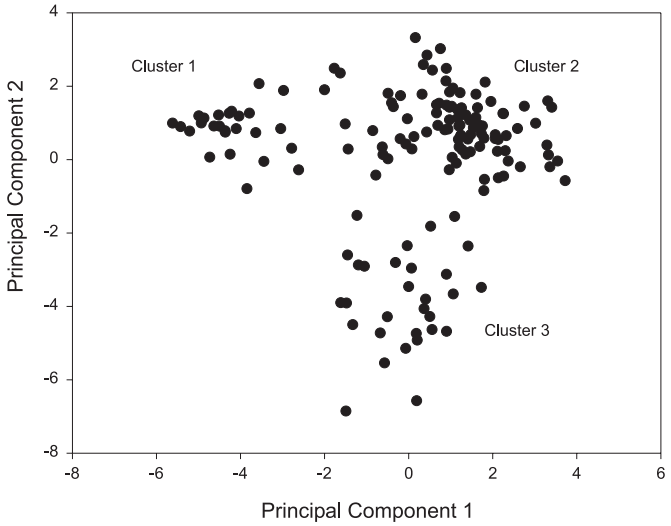


Table 5. Cluster means and standard deviations (in parentheses) for selected individual traits and four principal components.

	Cluster		
Trait	1	2	3
PC-1	–4.26 (0.78)	1.06 (1.36)	–0.10 (0.95)
PC-2	0.72 (0.55)	0.98 (0.85)	–3.80 (1.33)
PC-3	0.34 (1.09)	–0.12 (1.60)	0.13 (1.06)
PC-4	0.66 (1.02)	–0.02 (1.37)	–0.45 (1.33)
LVWI2	10.65 (0.67)	14.56 (1.89)	10.73 (1.29)
TOTHT2	120.39 (5.16)	133.95 (8.62)	147.84 (8.70)
INFLE2	14.54 (1.33)	16.87 (1.92)	17.63 (1.25)
CRWI1	42.93 (2.60)	44.45 (4.82)	35.01 (6.56)
EMERG2	157.07 (1.16)	162.38 (2.32)	160.70 (3.14)
INFNO1	6.74 (7.17)	42.25 (18.90)	36.98 (10.29)
PUB2	2.52 (0.18)	1.39 (0.50)	1.33 (0.42)
GMEANW	0.29 (0.04)	0.21 (0.05)	0.38 (0.12)

Note: Codes representing the traits are described in Table 1.

PC-2 scores, most of the location variance was associated with clusters, less than 15% being among locations within clusters (Table 4). In contrast, PC-3 and PC-4 scores showed little or no variance among clusters, but had highly significant variation among locations within clusters (Table 4).

Multiple regression analysis of PC scores using physiographic factors as independent variables indicated only weak trends among locations east of 118°45′W, although PC-3 and possibly PC-4 indicated some geographic patterning (Table 7). When climatic factors were added as independent variables, all of the regressions were significant (Table 7). This indicated patterned, possibly adaptive, variation among the eastern populations and suggested that the site stratification methods used west of 118°45′W might work for this geographic area as well.

Five site stratification methods were evaluated (Table 8). No method worked for PC-1 and PC-2 scores, as expected; all but one classification (soil type) was significant ($P < 0.05$) for PC-3. In two stratification methods (plant association and ecoregion), classes explained 37% and 53% of the location variance in PC-3 scores (Table 8). Two classification methods (soil type and plant association) accounted for 18% and 22% of the location variance in PC-4 scores. In all cases, remaining variance among locations within classes was significant ($\sigma^2_{L/C}$, Table 8).

Location variation in summary

Although clinal variation was present, it was stepped, and the picture that emerged based on the first four principal components was predominantly one of ecotypic subdivision. Evidence for this was in two forms. First was the large amount of location variance that was accounted for by clusters (over 84% of the variance in PC-1 and PC-2 scores) and by spatial stratification procedures (53% of the variance in PC-3 scores in the large eastern portion of the study area was explained by the two groups of ecoregions). Second, R^2 values for multiple regressions using physiographic and climatic factors as independent variables were, for three of the PCs, larger within the eastern area alone (Table 7) than they were across the entire sample area (entire sample R^2 values = 0.415, 0.319, 0.420, and 0.233 for PCs 1–4, respectively), and in no case did regression coefficients of determination explain as much of the location variance as did clusters or the best stratification method.

Discussion

Distribution of genetic variance

This study provides insights into the genetics of morphometric traits in a self-compatible and frequently selfing species, *E.glaucus*, and into the principal factors contributing to variation patterns and population structuring. The vast majority of total family variance in measured traits was distrib-

Fig. 4. Source locations plotted against latitude and longitude by cluster membership as determined from analysis of PC-1 and PC-2 scores. Vertical dotted line is 118°45′W longitude.

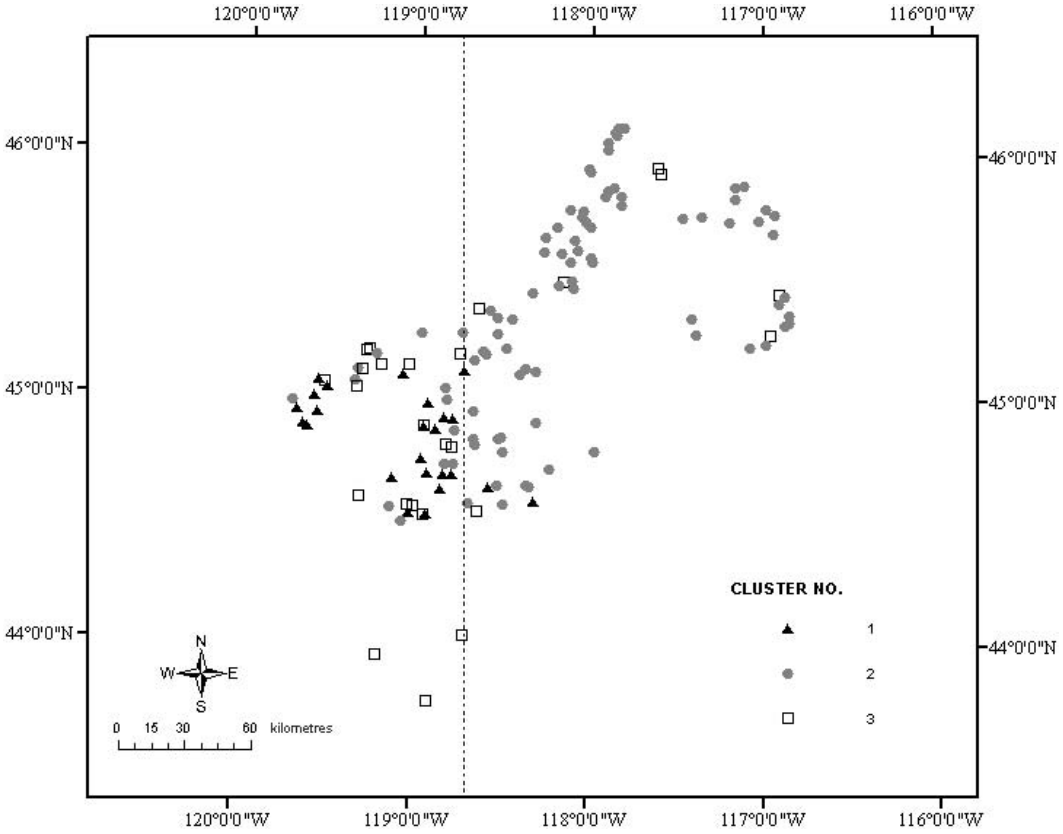


Table 6. Stepwise discriminant analysis of physiographic and climatic variables important for discriminating among three groups of *Elymus glaucus* seed source locations.

Variable	Partial R^2	Cumulative R^2	F
Longitude	0.2888	0.2888	30.45***
February precipitation	0.1230	0.4118	10.45***
Spring heat	0.0545	0.4663	4.26*

Note: *, statistically significant at $P < 0.05$; ***, statistically significant at $P < 0.001$.

Table 7. Coefficients of determination (R^2) for regressions of principal component scores on seed source climate and geography within the eastern zone.

	Geographic variables		Geographic and climatic variables	
	R^2	No. of variables	R^2	No. of variables
PC-1	0.079	8	0.296	15
PC-2	0.040	8	0.510	15
PC-3	0.262	8	0.502	15
PC-4	0.171	8	0.386	15

uted among populations (Table 2, σ^2_L vs. $\sigma^2_{F/L}$). Our mean estimate of the proportion of among-population variance in *E. glaucus* (81.3%) was similar to that reported for other inbreeding species (Bonnin et al. 1996; van Rijn et al. 2000) when the same criterion was used for analysis (deletion of presumably neutral traits; i.e., those with no or very little location variance).

Morphometric versus marker-based traits

Partitioning of genetic variance through the use of ANOVA-based ratios such as $\sigma^2_L/(\sigma^2_L + \sigma^2_{F/L})$ is equivalent to Wright's F_{st} coefficient derived from molecular marker data (Falconer 1981; Berg and Hamrick 1997; McKay and Latta 2002). As a consequence, direct comparisons can be made between the levels of interpopulation differentiation observed in biometric traits versus marker-based estimates (e.g., Spitze 1993; Podolsky and Holtsford 1995; Bonnin et al. 1996). The amount of population differentiation observed in the present study is substantially higher than that detected in allozyme studies of selfing grasses in general (41%, Godt and Hamrick 1998), as well as *E. glaucus* sampled at much broader geographic scales (54%, Knapp and Rice 1996; 42%, Wilson et al. 2001). The significant source-related variance in allozyme markers generally was unrelated to physiographic variables, indicating that a large portion of the location variance can be random in origin. The still considerably larger amount of source-related variation we report for phenotypic traits implies an additional important role of selection in this species. We will discuss the random and adaptive components separately.

Demographic and mating system contributions to location variance

Elymus glaucus is an aggressive colonizer of disturbed areas and has a natural distribution that is extremely patchy

Table 8. Components of variance for location with ($\sigma^2_{L/C}$) and without (σ^2_L) various environmental classifications in the ANOVA model as fixed effects, and the proportion of location variance attributed to the classification ($(\sigma^2_L - \sigma^2_{L/C})/\sigma^2_L \times 100$) for four principal component variables.

Classification	No. of classes	No. of locations[a]	No. of families	Trait	σ^2_L	*P* value for σ^2_L[b]	$\sigma^2_{L/C}$	*P* value for $\sigma^2_{L/C}$[c]	$(\sigma^2_L - \sigma^2_{L/C})/\sigma^2_L \times 100$
Watershed	11	93	114	PC-1	1.672	0.0002	1.446	0.0006	13.5
	11	93	114	PC-2	1.842	<0.0001	1.897	<0.0001	0.0
	11	93	114	PC-3	1.188	0.0096	1.078	0.0177	9.2
	11	93	114	PC-4	1.461	0.0001	1.420	0.0002	2.8
Conifer seed zone	11	93	114	PC-1	1.672	0.0002	1.448	0.0006	13.4
	11	93	114	PC-2	1.842	<0.0001	1.891	<0.0001	0.0
	11	93	114	PC-3	1.188	0.0096	1.077	0.0177	9.3
	11	93	114	PC-4	1.461	0.0001	1.416	0.0002	3.1
Soil	6	48	56	PC-1	2.224	<0.0001	2.291	0.0001	0.0
	6	48	56	PC-2	2.006	0.0050	2.090	0.0048	0.0
	6	48	56	PC-3	2.961	0.0002	3.038	0.0002	0.0
	6	48	56	PC-4	1.043	0.0125	0.857	0.0246	17.8
Plant association	12	82	100	PC-1	1.719	0.0008	1.767	0.0008	0.0
	12	82	100	PC-2	1.222	0.0005	1.332	0.0004	0.0
	12	82	100	PC-3	1.344	0.0110	0.849	0.0376	36.8
	12	82	100	PC-4	1.442	0.0007	1.126	0.0030	21.9
Ecoregion groups	2	91	112	PC-1	1.691	0.0001	1.715	0.0001	0.0
	2	91	112	PC-2	1.884	<0.0001	1.900	<0.0001	0.0
	2	91	112	PC-3	0.970	0.0168	0.459	0.0153	52.7
	2	91	112	PC-4	1.493	<0.0001	1.482	0.0002	0.8

Note: Data are for eastern zone locations only.
[a]Number of locations varied by classification because of missing site data and removal of extreme outliers in classes with few observations.
[b]Significance of mean square associated with location variance component when classification effect is excluded from the ANOVA.
[c]Significance of mean square associated with location variance component when classification effect is included in the ANOVA.

over space and time. This regeneration habit, because it limits gene exchange, may promote population divergence (Smith 1966; Dickenson and Antonovics 1973; Heywood 1991).

High levels of selfing can also result in increased interpopulation variance (Allard et al. 1968; Lande 1977; Govindajaru 1989). *Elymus glaucus* shows a high degree of self-fertility after controlled pollinations (Jensen et al. 1990), and within-family morphological uniformity in the present study was high (Fig. 2). These results, combined with the nearly complete allozyme homozygosity of individuals (99.9%, Knapp and Rice 1996; 98.5%, Wilson et al. 2001), indicate consistently high levels of selfing over many generations.

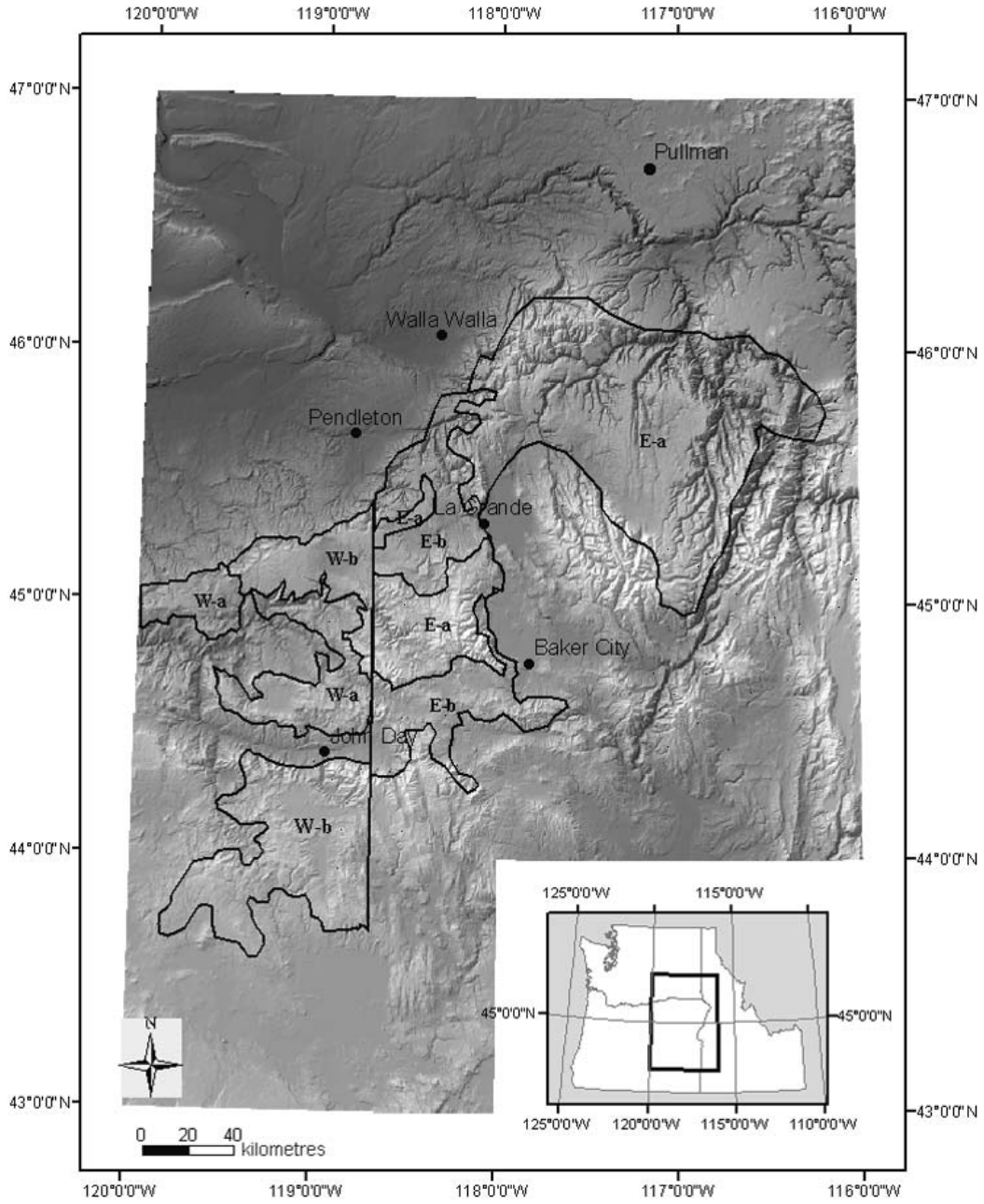
Prolonged inbreeding gives rise to linkage disequilibria and results in the fixation of alternative gene combinations that form tightly integrated segregation blocks or so-called co-adapted gene complexes (Haldane 1949; Allard et al. 1972, 1992; Andersson 1993; Ford-Lloyd et al. 2001). Although the complexes arise through inbreeding and drift, they also can be important in adaptation, because natural selection acts on these loci as a unit (Allard et al. 1992).

Evidence for adaptive variation

In spite of the potential importance of random processes in causing differentiation in selfing species, several findings from our study are consistent with a hypothesis that natural selection played a significant role in the contemporary patterns of genetic variation in *E. glaucus*. First, simulation studies have shown that local differentiation and population substructure develop rapidly under isolation-by-distance models (Turner et al. 1982; Sokal and Wartenburg 1983), and this would seem to be particularly true for highly selfed species such as *E. glaucus*. These studies suggest that if selective pressures were weak or lacking in the study area, we should have observed a large number of random genetic groups. Instead, seed sources were nested into three primary clusters that accounted for over 84% of the location-related variance associated with the PC-1 and PC-2 trait complexes (Table 4). Moreover, the frequency distribution of PC-1 and PC-2 scores, combined with the distinct spatial patterning of the clusters (Fig. 4) and their association with key climate and environmental stratification variables, indicated disruptive selective pressures east and west of longitude 118°45′W and between the habitats that separate clusters 1 and 3 (Lawrence 1984).

West of longitude 118°45′W, the strong ecotypic separation of cluster-1 from cluster-3 locations and the narrow range of cluster-1 phenotypes (Fig. 3) were suggestive of environmental conditions and habitats with intense selective regimes. The rainshadow effect of the Cascade Mountains (200 mi. to the west (1 mi. = 1.609 km)) is strong in this area, and both summer and winter precipitation is markedly less than in adjoining or other sampled areas in the Blue Mountains. Given such conditions, moisture- and temperature-related stresses are likely to limit the length of the effective growing season, especially for cool-season grasses (Heslop-Harrison 1964; Clary 1975). Several features associated with cluster-1 ecotypes, such as rapid seed germination and early flowering (Table 5), can thus be inferred to

Fig. 5. Proposed *Elymus glaucus* seed zones in the Blue Mountains. Western zone-a (W-a) is that portion of ecoregion 11b occurring west of longitude 118°45′W and western zone-b (W-b) occupies the remaining portion of the sampling area west of longitude 118°45′W. The eastern zone-a (E-a) contains ecoregions 10f and 11l east of 118°45′W, and eastern zone-b (E-b) contains ecoregions 11c, 11d, and 11f east of 118°45′W.



be physiological adaptations to arid habitats. Dense leaf pubescence and narrow leaf shape, both characteristics of the cluster-1 phenotype (Table 5), are also known to have a functional significance in arid environments in terms of reducing evapotranspiration and radiation load (van Rijn et al. 2000; Holmes and Keiller 2002). Soil characteristics may also play a role in population differentiation in this area. Cluster-1 locations all occurred within the John Day – Clarno Highlands ecoregion (11b), where soils have limited water-holding capacity (Clark and Bryce 1997). Precipitation differences between cluster-1 and cluster-3 locations are not large, but these differences may be amplified by water-holding characteristics of the soils. Population differentiation is most likely to occur where environmental factors vary in space, but are relatively stable over time (Snaydon and Davis 1972). Edaphic differences fit these criteria.

Cluster-2 locations are east of longitude 118°45′W. This is the wettest part of the sample area because of maritime air systems flowing through the Columbia River Gorge. It is also the coldest part of the sample area, and warms most rapidly in the spring, because mountains reach their highest elevation here. Adaptive differentiation between cluster-2 locations and those west of 118°45′W seems likely, but they are not clear-cut. Cluster-2 phenotypes differ distinctly from

cluster-1 and cluster-3 phenotypes (Fig. 3), but in each comparison by a different trait complex. It appears as if some factor, perhaps historical, has been or is still interacting with the adaptive differentiation.

PC-3 and P-4 scores showed highly significant variation among locations within clusters ($\hat{\sigma}^2_{L/C}$, Table 4). This variation seemed adaptive in that regression analyses of PC-3 and PC-4 scores showed complex clinal associations with seed source climate and physiography in the eastern portion of the study area (Table 7). Classification models, which grouped sites based on similarity in various environmental features, suggested coincidence between morphological types and site classes (Table 8), especially for PC-3 (a component loading heavily for crown radius and fecundity, Table 3). For example, the ecoregion model accounted for over half of the source-related variance in the PC-3 trait complex (Table 8); i.e., plant origins in the low-elevation ecoregion group (11c, 11d, 11f) had large PC-3 scores, with wider crowns and greater fecundity compared with those originating from the high elevation ecoregion group (10f, 11l).

Implications for restoration and management

We have assumed that the location-based variation patterns observed in the common garden tests reflected variation in fitness (i.e., "home-site advantage" hypothesis, Montalvo and Ellstrand 2000, 2001), as detected in reciprocal transplant studies in other grasses and short-lived perennials (Schoen et al. 1986; van Tienderen and van der Toorn 1991; Miller and Fowler 1994; Kindell et al. 1996). Based on these findings, we used the observed patterns of morphological differentiation in *E. glaucus* to construct seed-use zones that greatly reduced location variance within zones.

We propose four geographic subdivisions (seed zones) for managing native germplasm within the Blue Mountains Province (Fig. 5). Specifically, western zone-a (W-a, Fig. 5) is that portion of ecoregion 11b occurring west of longitude 118°45′W, and western zone-b (W-b) occupies the remaining portion of the sampling area west of longitude 118°45′W. The eastern zone-a (E-a) contains ecoregions 10f and 11l east of longitude 118°45′W, and eastern zone-b (E-b) of ecoregions 11c, 11d, and 11f east of longitude 118°45′W. Our results suggest that seed movement between western zone-a and other areas should be especially restrictive because of the strong ecotypic differentiation and low variability of cluster-1 locations. Within a zone, seed collections should sample as many populations as possible, but with only small sample sizes per population (e.g., <50 individuals). We recommend sampling especially broadly in the two eastern zones (E-a and E-b), where within-zone variability was high and not fully explained by ecoregion subdivisions (i.e., lack of fit to the ecoregion model was significant and large for three of the four PCs, Table 8).

The restoration framework and seed management guidelines proposed here are far less restrictive than procedures currently in use by federal agencies in the Blue Mountains and elsewhere, but yet are considerably better at matching seed-use guidelines to the phenotypic variation pattern. Large-scale seed-increase efforts involving *E. glaucus* will benefit from the proposed zoning, as will the adaptability and sustainability of introduced plant materials.

Finally, a cautionary comment on ecoregions, which were quite useful in delineating our proposed seed-use zones. The grouping of ecoregion classes into zones involved observation of bimodal distribution of PC scores and use of discriminant analysis based on plant morphology or performance in the common garden. There was no a priori ecoregion evidence that would have divided the area at 118°45′W, nor that would have grouped, for example, 10f and 11l in one zone and 11c, 11d, and 11f in another. In other words, ecoregions were a helpful classification tool, but without the supporting common garden information, a classification based solely on ecoregions would not have properly subdivided adaptive variation in *E. glaucus*.

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