RESEARCH ARTICLE

Can an Ecoregion Serve as a Seed Transfer Zone? Evidence from a Common Garden Study with Five Native Species

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Abstract

Prairie restoration is often limited by the availability of appropriate local plant materials. Use of locally adapted seed is a goal in restoration, yet little information to inform seed transfer guidelines is available for native plant species. We established common gardens of five plants (*Eriophyllum lanatum* var. *leucophyllum, Epilobium densiflorum, Potentilla gracilis* var. *gracilis, Lupinus polyphyllus* var. *polyphyllus*, and *Saxifraga oregana*) frequently used in prairie restoration in the Pacific Northwest of North America to determine if populations differed in morphological and phenological traits and whether this variability was structured by geography, climate, or habitat. Ordination techniques were used to summarize the observed variability of multiple traits for each species. Ordination distance was significantly correlated with geographic distance in *L. polyphyllus* var. *polyphyllus*, and populations of this species differed significantly among geographic groups within an ecoregion. Little or no spatial structure was detected in the remaining species, despite correlations between ordination scores and monthly temperatures. We suggest that a single seed zone in the Willamette Valley ecoregion may be appropriate for all species examined except *L. polyphyllus* var. *polyphyllus*. Ecoregions in general may be useful boundaries for seed transfer zones, especially in regions with relatively little topographical or climatic variation.

Key words: common garden, ecoregion, restoration, seed transfer zone.

Introduction

Native seeds are widely used for restoration, reintroduction, and the creation of new populations by government and private organizations (Reinartz 1995). Maximizing establishment of plant materials is an essential goal in restoration efforts, as is protecting the genetic integrity of native plant populations in proximity to restoration sites (Knapp & Rice 1994; Kaye 2001; McKay et al. 2005). Locally adapted plant materials are widely recommended because of their increased chances of establishment success and lower potential for genetic swamping of surrounding populations with maladaptive genes (Lesica & Allendorf 1999; Hufford & Mazer 2003; Rogers & Montalvo 2004; Gustafson et al. 2005). However, substantial uncertainty remains regarding how to select populations within a species as seed sources for restoration efforts (McKay et al. 2005).

Although the use of locally adapted seed is prudent, it is also important to consider the genetic diversity of restored populations to maximize their adaptive capability and long-term sustainability (Moritz 1999). Small relict plant populations near restoration sites, which may be located in areas of considerable habitat loss or degradation, are likely to be genetically depauperate or inbred because of recent isolation or fragmentation (Ellstrand & Elam 1993; Keller & Waller 2002). Restricting the use of plant materials to populations near the restoration site may result in restored populations with low genetic diversity and limited potential to evolve in response to a changing environment (Broadhurst et al. 2008). The costs of producing seeds from small populations for use at a single restoration are higher than seed production at a larger scale for use at multiple sites (Ward et al. 2008).

Seed transfer zones are geographic areas within which plant materials can be moved freely with little disruption of genetic patterns or loss of local adaptation. When developed by relating patterns of genetic variation among populations to environmental factors (McCall 1939; Erickson et al. 2004; Johnson et al. 2004; Doede 2005), they can allow restorationists to achieve a balance between minimizing unintended consequences of moving plant materials and maximizing efficiency and genetic diversity (Kramer and Havens 2009). However,

most land managers are faced with timing and funding constraints that prevent them from conducting the genetic studies required to delineate seed transfer zones (McKay et al. 2005; Smith et al. 2006). In the absence of genetic data, restoration practitioners often use geographic distance to guide seed movement (Lane Council of Governments 1996; Conservation Commission of Western Australia 2004 in Broadhurst et al. 2008; State of Minnesota 2008), or draw on topographic, climatic, and edaphic data to define zones of ecological similarity within which seed may be moved (Knapp & Dyer 1998; Mortlock 2000; McKay et al. 2005). Ecoregions have been suggested as potential seed transfer zones (Jones 2005; Withrow-Robinson & Johnson 2006; Native Seed Network 2009) because they encompass geographic areas with similar geology, climate, vegetation, soils, and hydrology (Bailey 1983; Griffith et al. 2008).

Common gardens are a useful tool for examining genetic differences between populations through variation in phenotype and identifying environmental factors that may cause local adaptation (Knapp & Rice 1994; Gordon & Rice 1998; Montalvo & Ellstrand 2001; Nunez-Farfan & Schlichting 2001; Erickson et al. 2004; Johnson et al. 2004; Doede 2005). Plants grown in a common garden are exposed to a uniform environment, so that in general differences expressed through their phenotype are due to their underlying genetic differences. Common gardens can help identify populations that are associated with a particular environment to help match seed sources to restoration sites, and thus improve the success of restoration plantings. Although local adaptation has been documented in plants at small (Sork et al. 1993) and large (Fenster & Galloway 2000) spatial scales, not all plant populations are locally adapted and populations are often found along a continuum with some more adapted than others (Raabova et al. 2007; Broadhurst et al. 2008). Generally, local adaptation is structured by strong environmental gradients such as climate and topography (among many selective forces) and often varies based on breeding system and habitat (Gordon & Rice 1998).

We established common gardens to determine if genetic variation among populations is associated with geography, climate, or habitat for five vascular plants frequently used in restoration of prairies in the Pacific Northwest of North America. Here we ask (1) do geography or environment explain variation among populations? and (2) is phenotype associated with spatial groups, including ecoregions, that might be useful for delineating seed transfer zones? For each species, we use our results to evaluate the need for a single seed transfer zone or multiple zones within a level III ecoregion (Griffith et al. 2008).

Methods

Species and Population Sampling

Five herbaceous plant species were included in this study: *Lupinus polyphyllus* var. *polyphyllus* (bigleaf lupine, Fabaceae, perennial), *Eriophyllum lanatum* var. *leucophyllum* (woolly sunflower, Asteraceae, perennial), *Epilobium densiflorum*

(denseflower willowherb, Onagraceae, annual), *Potentilla gracilis* var. *gracilis* (slender cinquefoil, Rosaceae, perennial), and *Saxifraga oregana* (Oregon saxifrage, Saxifragaceae, perennial). Each of these species is commonly used for prairie restoration efforts in western Oregon (Ward et al. 2008). Seeds were collected in 2005 and 2006 from populations distributed throughout the Willamette Valley, Oregon (Fig. 1). Three additional populations of *E. lanatum* var. *leucophyllum*, two from high elevations in the Olympic Mountains of Washington and one from the Umpqua Valley in Douglas County, Oregon (not shown on map), were also included. All seeds from each species were pooled by population. Latitude, longitude, and elevation were recorded for each seed source.

Common Garden Design

Seeds were randomly selected from each population and sown into flats of Ray Leach "Cone-tainers" and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center (PMC) in Corvallis, Oregon. For each species, thirty cone-tainers were started for each population. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After 10 weeks in the greenhouse, plants were moved to a shade house and allowed to acclimate prior to field planting.

A separate common garden was planted for each of the five species in farm fields at the PMC. Plants were placed 2 m apart within rows and rows were 1 m apart. Plants were transplanted into plots using a completely randomized design. To buffer against edge effects, each plot was surrounded by a border row of plants. Drip tapes were placed in long rows across the field 3 ft apart. The fields were irrigated once every 2 weeks to aid establishment. Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. The soil was also covered with 3 inches of bark mulch to reduce weeds.

A total of 3–30 samples (individual plants grown from seed) from each of 10–19 populations, depending on the species, were included in the common gardens (Table 1). Uneven sample sizes per population resulted from variable wild population sizes and seedling survival (Table 1). Two populations, #4 and #9, of *P. gracilis* var. *gracilis* were very small in the wild and were represented in the common gardens by only 4 and 3 individuals, respectively.

Plant Trait Measurement

Traits were chosen based on characteristics described in Hitchcock and Cronquist (2001) and/or Hickman (1993). Traits thought to have adaptive significance, association with reproductive success, taxonomic importance, and a high degree of variability between varieties were included, and those selected varied among taxa. For *E. densiflorum*, we measured dates of seedling emergence, flowering and seed set, plant height and diameter, pubescence, flower petal length, and leaf length and width. *E. lanatum* var. *leucophyllum* plants were measured for date of seedling emergence and flowering,
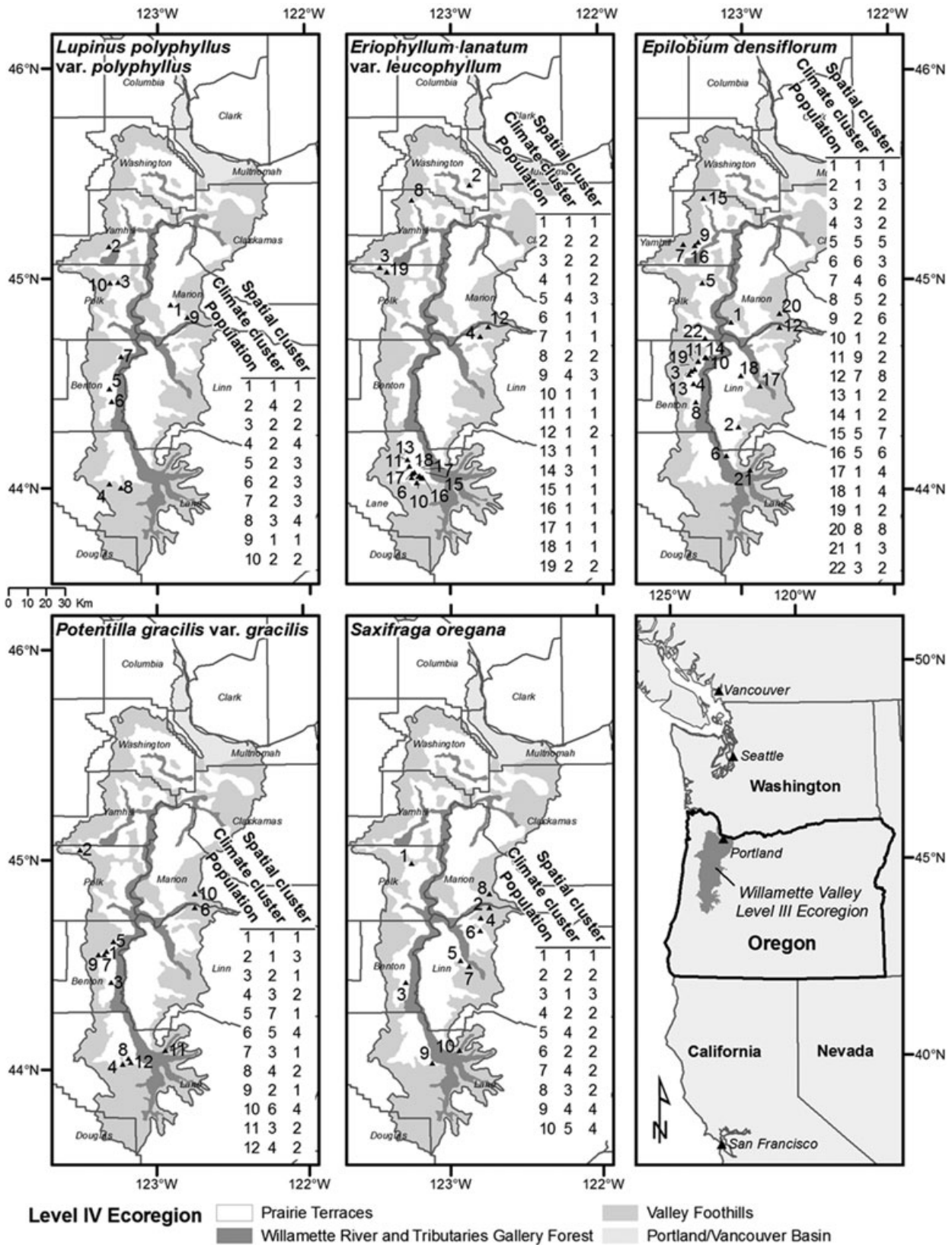
Figure 1. Collection locations within the Willamette Valley ecoregion in Oregon, U.S.A. of each species included in the common gardens. Spatial and climate clusters are listed for each population.

Table 1. Number and size of wild populations sampled, number of individuals per population in the common gardens, mean percentage survival of seedlings germinated, and the total number of individuals grown and measured for each species included in the common garden.

Species	Number of Populations Sampled	Wild Population Size	Number of Individuals per Population in the Common Garden	Mean Seedling Survival ± SE (Range)	Total Individuals Measured
Epilobium densiflorum	22	50–1000	15	99% ± 0.4 (93–100%)	331
Eriophyllum lanatum var. *leucophyllum*	19	3–2000	13–21	56% ± 2 (43–70%)	319
Lupinus polyphyllus var. *polyphyllus*	10	8–200	20–30	98% ± 1 (90–100%)	281
Potentilla gracilis var. *gracilis*	12	3–1000	3–30	50% ± 11 (10–100%)	179
Saxifraga oregana	10	3–1000	18–25	89% ± 2 (83–97%)	222

ray petal length and width, capitulum diameter, number of flowering stalks, plant height and diameter, leaf arrangement and edge type, length of inner and outer color bands on ray flower and color categories (often rays were of two colors), number of rays per flower head, number of flower heads per stalk, peduncle length, leaf color, basal leaf area, basal leaf lobe number, leaf tip shape, and leaf width. Traits measured for *L. polyphyllus* var. *polyphyllus* included date of seedling emergence, flowering and seed set, leaflet length, width and shape, leaflet apex shape, pubescence, banner and keel colors, banner length, width and shape, plant height, inflorescence length, and number of flowers per inflorescence. On *P. gracilis* var. *gracilis,* we measured flowering date, total plant and basal leaf heights, inflorescence diameter, diameter of basal leaf crown, number of flowering stalks, petal length and width, basal leaf length, length of leaf tooth, distance from tooth base to midvein, number of branches per flowering stalk, and number of flowers per branch. Traits measured for *S. oregana* included seedling emergence date, plant height and diameter, inflorescence length, petal length and width, number of stems and branches, leaf length and width, leaf margin type, and leaf surface type. Details of traits and measurement protocols are available in Appendix S1 (Supporting Information).

Phenological and morphological traits were measured for all individual plants during a single year in the summer (either 2007 or 2008). Each growth trait was measured on a single day. Floral traits were measured using only new flowers. When measuring a single trait more than once (typically three times but only one or two for plant parts produced in low numbers) on an individual, no measurements were made from the same organ (e.g. petal length was the mean length of three petals from three separate flowers chosen arbitrarily). Emergence date (date when the first seedling germinated within each container) was monitored daily. Flowering and seed set dates were monitored on transplanted individuals three times per week. Plants with missing values or defined outliers (those greater than 2 SD from the mean) were removed from the analysis.

Population means were calculated for each variable and were used as the main data matrix for subsequent analyses. Mean population values were used because individuals planted in the common gardens originated from bulked seed collections and were not statistically independent (see Horning et al. 2008). As the number of individuals within the common garden sometimes exceeded the number of individuals

in the natural population, and relatedness was not known, analysis of individuals would constitute pseudoreplication (Hurlbert 1984). We therefore assume that variation in population means reflects population differentiation, and our analyses do not account for within population variation.

Spatial Classification and Clustering

Prior to analysis, populations were classified by county, location, and U.S. Environmental Protection Agency level IV ecoregion (Griffith et al. 2008) (Fig. 1). These geographic, administrative, or habitat (i.e. ecoregion) units were logical potential seed transfer zone boundaries. We used hierarchical clustering analysis to define geographically similar groups (information retained at 75% level) of populations using Euclidean distance and nearest neighbor as the linkage method (PCORD 5, McCune & Mefford 1995). This approach grouped populations that are most similar based solely on latitude and longitude of individual populations. Separate ordination and clustering analyses were performed for each species.

Ecoregions in the United States have been delineated as hierarchical polygons of similar vegetation, soils and climate at four spatial scales, from level I through level IV, with increasing resolution (Griffith et al. 2008). Each ecoregion is a unique polygon from level I through III, but level IV ecoregions are noncontiguous polygons that subdivide the level III ecoregion. Level IV ecoregions were used in our analysis to represent habitats. All our populations within the Willamette Valley, Oregon, fell into one of three level IV ecoregions: Prairie Terraces, Valley Foothills, or Willamette Valley and Tributaries Gallery Forest (Thorson et al. 2003). Those populations outside the level III Willamette Valley ecoregion (only in *E. lanatum* var. *leucophyllum*) included two additional ecoregions: High Olympics (in Washington State) and Umpqua Valley Foothills (just south of the Willamette Valley, Oregon).

Climatic Data

Climatic conditions at each location were characterized using digital maps produced in ArcGIS 9.3 and with data generated by Parameter Regression on Independent Slopes Model (PRISM) climate models (PRISM Group 2008). These

models were parameterized to use 1971–2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for each location is no better than half the resolution of the cell. Variables were modeled monthly and an annual value was calculated by averaging the monthly grids. For this study, mean monthly temperature (minimum and maximum °C) and precipitation (mm) were gathered for each population. Again hierarchical clustering analysis (see methods in spatial classification and clustering above) was used to group populations into climatic clusters based on similar climatic conditions.

Statistical Approach and Data Analysis

We used two approaches to evaluate our study questions, one to identify spatial and environmental factors that affect continuous variation in plant traits across the landscape, and one designed to test for differences between groups that might be used to define seed transfer zones. For the first approach, we performed ordinations with morphological and phenotypic data for each species separately and tested for correlations between ordination scores and geographic distance as well as environmental variables including latitude, longitude, elevation, and mean monthly temperature and precipitation.

Ordinations were performed with Nonmetric Multidimensional Scaling (NMS) based on Euclidean distances (Kruskal 1964) in PCORD 5 (McCune & Mefford 1995) using population means for each trait. To account for trait measurement on different scales, data were centered using the standard deviates of each trait. To ensure that ordinations were below an acceptable level of stress, a random seed with 250 runs of real data was used and dimensionality assessed visually by scree plot. The probability that the final stress could have been obtained by chance was assessed using Monte Carlo simulations ($n = 250$ replicates). A stability criterion of 0.0001 was used. The percentage of variation in the ordination was recorded for each axis after a rotation to load the strongest correlate to axis 1. To clarify the distribution of sampling units in ordinations space, additional matrices encapsulating county, spatial and climatic clustering, and ecoregion level IV were overlaid. These overlays allowed us to visually assess the data and provided the matrices need for testing differences between groups (used below in our second approach).

We used Mantel tests (Mantel 1967) to determine if differences between populations of each species were correlated with geographical distances. In these analyses, a pairwise Euclidean distance matrix based on ordination axis scores was constructed for all pairwise combinations of populations. The congruent pairwise geographical distance matrix (in km) was likewise calculated. Analyses were performed using a variant of the computer program Alleles in Space (Miller 2005) modified for use with ordination data as inputs. One thousand randomization replicates were used to evaluate p-values for observed correlations in each analysis. In addition, we used linear regression to test for a relationship between the axis that explained the greatest amount of variability and geographic and climatic variables for each species. The R^2 selection method in SAS (SAS Institute, Inc. 2008) was used for initial model building. The most parsimonious model with the largest regression coefficient was selected. No selected model contained more than two variables.

In our second approach, we used Multi-Response Permutation Procedure (MRPP) to test for differences among populations grouped by county, spatial cluster, ecoregion level IV, and climate cluster, factors that could be used to develop seed transfer guidelines. MRPP provides a measure of within-group homogeneity (A statistic) such that, in ecological data, values of $A > 0.3$ are biologically significant and can be interpreted as providing evidence that observations are more similar within than among these groups (McCune & Grace 2002). A Bonferroni correction was used when multiple comparisons were performed.

Results

Correlation of Spatial and Environmental Variables With Plant Variation

NMS ordinations resulted in two or three dimensional solutions to the data explaining 86.3 to 95.6% of the variation, depending on the species, with a final instability of 0.00001 in each case (Table 2; Fig. 2). The number of iterations to a stable solution varied from 45 to 223, and the final instability ranged from 3.2 to 10.6 (Table 2). Using Clarke's cutoff for acceptable instability, stress values between 1 and 20 represent a visually interpretable picture (McCune & Grace 2002).

Ordination Versus Geographic Distances. Mantel tests suggested significant correlations between genetic similarity (as

Table 2. NMS ordination information for each of five prairie species included in the common garden, including the final number of axes, percentage of variance explained by all axes (and each axis respectively), and final stress of the number of iterations needed.

| Species		Percentage Variance Explained by Axis				
	Total Variance Explained (%)	Axis 1 (%)	Axis 2 (%)	Axis 3 (%)	Final Stress	Number of Iterations
Eriophyllum lanatum var. *leucophyllum*	86.30	27.70	36.20	22.40	10.6	111
Epilobium densiflorum	91.50	63	5.20	23.30	10.1	223
Lupinus polyphyllus var. *polyphyllus*	87.10	77.10	10.70	—	9	62
Potentilla gracilis var. *gracilis*	95.60	78.70	16.90	—	6	45
Saxifraga oregana	91.70	48.80	22.80	20.10	3.2	59
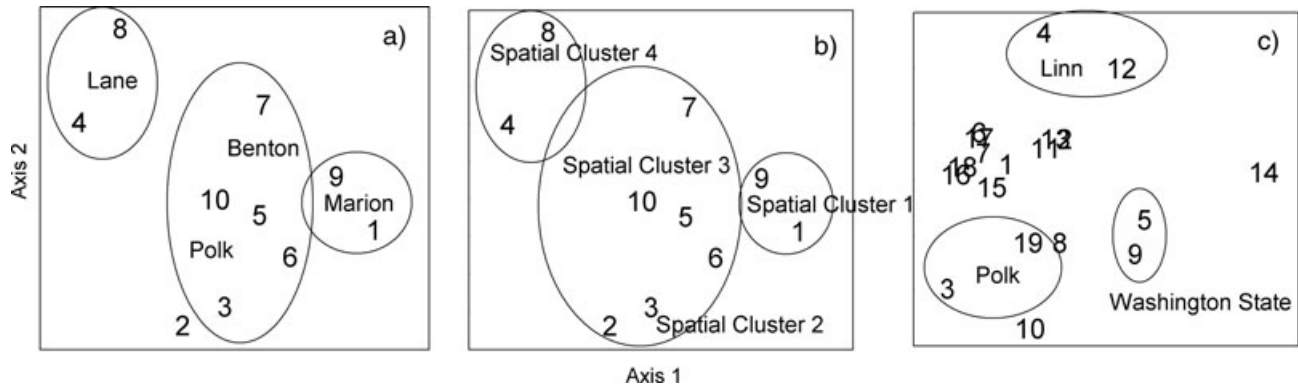
Figure 2. NMS ordinations for (a) *Lupinus polyphyllus* ssp. *polyphyllus* with outlines for significantly different counties, (b) *L. polyphyllus* ssp. *polyphyllus* with significantly different spatial groups, and (c) *Eriophyllum lanatum* var. *lanatum* with significantly different counties and states. The axes that explained the most variance for each species are shown in these figures. Groups of populations contained within outlines differ from other circled groups at the 0.05 level of probability, as determined with MRPP.

measured by ordination distance of phenotype) and geographic distance for populations of *Lupinus polyphyllus* ($r = 0.56$, $p = 0.005$) and *P. gracillis* ($r = 0.34$, $p = 0.02$). Mantel tests were not significant for *Eriophyllum lanatum* ($r = 0.19$, $p = 0.11$), *Epilobium densiflorum* ($r = 0.17$, $p = 0.08$), and *Saxifraga oregana* ($r = -0.11$, $p = 0.32$).

Regression of Ordination Axes With Spatial and Environmental Variables. Model building with linear regression found significant correlations between ordination scores and climatic variables for four species and a spatial variable for one species (Table 3). In all species, a single variable model best fit the data, with little or no increase in predictive power with the addition of more factors and frequent overfit of the model and collinearity of additional variables. October average temperature at the source population was a significant predictor of NMS ordination score in *L. polyphyllus* var. *polyphyllus*. This model explained 65% of the variability in plant traits on axis 1. In *E. lanatum* var. *leucophyllum*, longitude explained 48% of the variability in ordination axis 2. September mean maximum temperature was associated with variation in NMS ordination of *E. densiflorum*, accounting for half the variability of axis 1.

October maximum temperature explained 70% of the variability in *Potentilla gracilis* var. *gracilis* ordination axis 1. In this species, models with more than 2 variables explained an additional 20% of the variability, but variables in these models were strongly multicollinear. Finally, 40% of the variability in *S. oregana* was explained by May maximum temperature.

Tests of Differences Between Groups

Traits of *L. polyphyllus* var. *polyphyllus* differed by county in the MRPP analysis ($A = 0.44$, $p = 0.02$) and pairwise comparisons showed significant dissimilarities between all counties except Polk and Benton. Yamhill County could not be included in the MRPP analysis because only one population was sampled from that area. Northern and central counties were closer to each other in ordination space than to eastern or southern counties (Fig. 2a). Spatial and climatic clusters of this species also showed significant dissimilarity ($A = 0.51$, $p < 0.001$, $A = 0.33$, $p = 0.008$). Pairwise MRPP comparisons found significant differences between all spatial clusters except clusters 1 and 3 (Fig. 2b). Level IV ecoregions ($A = 0.06$, $p = 0.15$) did not differ in *L. polyphyllus* var *polyphyllus*.

MRPP found a significant ($A = 0.38$, $p = 0.001$) effect of county or state of population origin in *E. lanatum* var. *leucophyllum*, excluding Washington, Yamhill, and Douglas Counties because only one population was sampled in each. Pairwise comparisons suggest the largest dissimilarities occurred between populations of *E. lanatum* var. *leucophyllum* in the State of Washington and Polk County ($A = 0.40$, $p < 0.0001$) and Linn County ($A = 0.39$, $p < 0.0001$), and between Polk and Linn counties ($A = 0.39$, $p < 0.0001$) (Fig. 2c). No effects of spatial cluster ($A = 0.02$, $p = 0.35$), climate cluster ($A = 0.20$, $p = 0.10$), or level IV ecoregion ($A = 0.04$, $p = 0.12$) were detected in MRPP for this species.

No significant differences between populations in different counties, spatial clusters, climate clusters, or level IV

Table 3. Regression models for dominant ordination axes for five prairie species included in the common garden.

Species	r^2	F	p	Model
Epilobium densiflorum	0.50	19.8	<0.001	Axis 1 = 37.82 − 1.57 × September mean maximum temperature
Eriophyllum lanatum var. *leucophyllum*	0.48	15.8	<0.01	Axis 2 = 261.0 + 2.12 × longitude
Lupinus polyphyllus var. *polyphyllus*	0.65	14.7	0.005	Axis 1 = 39.178 − 3.33 × October average temperature
Potentilla gracilis var. *gracilis*	0.71	24.1	<0.01	Axis 1 = 50.77 − 2.82 × October maximum temperature
Saxifraga oregana	0.40	5.38	0.05	Axis 1 = −23.72 + 1.22 × May maximum temperature

ecoregions were detected for *E. densiflorum*, *P. gracilis* var. *gracilis*, or *S. oregana*, although the Willamette Valley Gallery Forest ecoregion was represented by only one population of *P. gracilis* var. *gracilis* and could not be included in that analysis.

Discussion

We found potential evidence of local adaptation to climate in four of the five species examined here, but only one species, *Lupinus polyphyllus* var. *polyphyllus*, showed geographic structure to its variability useful for developing seed transfer zones based on mapped spatial patterns. Differences between populations in spatial clusters indicated that three areas delineated by county boundaries (Marion, Lane, and a combination of Polk, Benton, and Yamhill Counties) may serve as seed transfer zones for *L. polyphyllus* var. *polyphyllus* to combine populations of similar genetic type while avoiding mixing of different types.

Similarity between populations declined with increasing geographic distance in *E. lanatum*, but MRPP indicated that this was largely due to differences between populations from high elevations in Washington State and those in Polk and Linn Counties in Oregon. The populations we sampled in Linn County grew on distinct thin soil types and are assumed to be diploid, whereas other populations in the Willamette Valley tend to be tetraploid (Mooring 2008). The association between longitude and population similarity detected in *E. lanatum* reflects the difference between populations in Polk and Linn Counties, which are oriented east–west of one another. Given these results, we suggest the Willamette Valley level III ecoregion as a seed zone for this species, with the caveat that diploid populations or populations on distinct soil types in Linn County should be collected, grown, and planted separately. We also recommend this level III ecoregion as a seed transfer zone for *E. densiflorum* and *Saxifraga oregana* because no geographic structure was detected in Mantel tests or MRPP comparisons of geographic or climate groups. *Potentilla gracilis* var. *gracilis* again showed no obvious geographic pattern, but a significant Mantel test suggests that some level of spatial autocorrelation in genetic variability expressed in the phenotype may be present. Without clear geographic, spatial, or climatic structure we again recommend a level III ecoregion seed zone.

Level IV ecoregions were not efficient mapping units for seed transfer zones in this study; populations within a level IV ecoregion were just as likely to be similar to each other as to populations in a different ecoregion. In contrast, *Elymus glaucus* (Poaceae), a selfing species in western North America, showed strong genetic association with level IV ecoregions and longitude, and these patterns, with supporting evidence from common garden studies, were used to develop seed transfer regions within the Blue Mountains (Erickson et al. 2004). However, this study notes that classification based only on ecoregion would not have properly subdivided the adaptive variation in *E. glaucus* (Erickson et al. 2004).

For many plants, the level III ecoregion may be scaled appropriately to serve as a seed zone. Ecoregions at this scale have been recommended as seed transfer zones, especially in the absence of specific information on genetic variation and local adaptation (Jones 2005; Horning et al. 2008). Horning et al. (2008) found level III ecoregions to be generally useful for identifying zones of genetic variability in *Holodiscus discolor* (Rosaceae), a long-lived shrub, although in some cases, ecoregions sampled variation redundantly or failed to capture unique genetic types. Similarly, variation in *Festuca roemeri* (Poaceae), an outcrossing perennial, clustered into level III ecoregions, and one region was subdivided further to establish seed transfer zones (Wilson et al. 2008). One proposed refinement to the use of ecoregions as areas of plant movement has been to combine them with plant hardiness zones for mapping plant adaptation regions (Vogel et al. 2005).

In general, ecoregions may be most effective as seed movement areas when they lack steep environmental gradients and have a simple floristic past. Elevation and climate gradients appear to contribute significantly to geographic patterns of genetic variation and adaptation in many plants including trees (Campbell 1979; St. Clair et al. 2005), shrubs (Horning et al. 2008), forbs (Doede 2005), and grasses (Wilson et al. 2001). The Willamette Valley ecoregion is relatively small and homogeneous in climate and topography, which may contribute to our observation of poorly developed local ecotypes.

Applications for the Restoration Community

Obtaining seeds from locally adapted and ecologically similar environments has become a common objective for restoration projects (Broadhurst et al. 2008; Erickson 2008). Although restoration practitioners understand that the use of nonlocal seed can be detrimental (Lesica & Allendorf 1999; Krauss & He 2006), acquiring sufficient supplies of locally adapted seeds is often difficult (Boyer 2008). Seed transfer zones must be large enough to be economically practical while preserving the genetic diversity of natural populations (Miller & Hobbs 2007). Generation of genetic data for each species used in restoration, although a noble conservation task, is often logistically and economically unrealistic (McKay et al. 2005). Use of ecoregions as seed transfer zones in place of sound research to document areas of genetic uniformity or differentiation (Parker 1992) poses risks associated with outbreeding depression, poorly adapted genotypes, and inefficient use of resources (Kaye 2001). Whether or not these risks are acceptable will depend on restoration objective, cost, seed availability, and restoration goals (Ehrenfeld 2000; Doede 2005; Wilkinson et al. 2008).

Our results apply to relatively common and widespread species. For rare species, these results may or may not apply, and will depend on the reasons for a species' rarity and its history. For example, ecoregions as seed transfer zones may be appropriate for species that were once widespread but are now rare due to recent habitat loss and fragmentation. Keeping the remaining populations isolated through narrow seed transfer

zones may only contribute to the negative consequences of habitat fragmentation (Kaye 2001). Rare species that have historically disconnected population centers, on the other hand, such as species adapted to isolated habitat types, may tend toward local differentiation. Such species, particularly those on old, stable terrain, may even develop mechanisms to preserve heterozygosity and adapted gene complexes (Hopper 2009). Smaller seed transfer zones may be appropriate in cases like these. Common gardens may be a useful tool for developing seed transfer zones for rare species as well, but these should be evaluated on a case by case basis.

In principle, we agree with Broadhurst et al. (2008) that seed collection and transfer should emphasize the quality and diversity of plant materials to maximize the adaptive potential of species used in restoration for both current conditions and future environments, especially in the face of global climate change. But when genetic information is available that indicates local movement of seeds may be preferable, smaller seed transfer zones should be planned. Further, seed transfer zones may need to be flexible and shift on the landscape as climates change (Kramer & Havens 2009). We have shown that level III ecoregions may be suitable as seed transfer zones for some taxa and that straightforward common garden studies can be used to test their applicability to individual species.

Implications for Practice

- Ecoregions may be appropriate for some species as seed transfer zones in the absence of genetic data to improve the efficiency, economy, and genetic diversity of seeds produced for restoration. This may be especially valid in regions with little variation in topography or climate. At the same time, seed transfer zones of this scale may guard against outbreeding depression and use of poorly adapted genotypes.
- An ecoregion was a suitable seed transfer zone for four of five species investigated in this study.
- Common garden studies can be effective tools for assisting in delineation of seed transfer zones for multiple core restoration species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Phenological and morphological traits measured on *Epilobium densiflorum, Eriophyllum lanatum* var. *leucophyllum, Lupinus polyphyllus* var. *polyphyllus, Potentilla gracilis* var. *gracilis*, and *Saxifraga oregana*.

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