Genetic Structure and Gene Flow in *Elymus glaucus* (blue wildrye): Implications for Native Grassland Restoration

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Abstract

Interest in using native grass species for restoration is increasing, yet little is known about the ecology and genetics of native grass populations or the spatial scales over which seed can be transferred and successfully grown. The purpose of this study was to investigate the genetic structure within and among populations of *Elymus glaucus* in order to make some preliminary recommendations for the transfer and use of this species in revegetation and restoration projects. Twenty populations from California, Oregon, and Washington were analyzed for allozyme genotype at 20 loci, and patterns of variation within and among populations were determined. Allozyme variation at the species level was high, with 80% of the loci polymorphic and an average expected heterozygosity (an index of genetic diversity) of 0.194. All but two of the populations showed some level of polymorphism. A high degree of population differentiation was found, with 54.9% of the variation at allozyme loci partitioned among populations (F_{st} = 0.549). A lesser degree of genetic differentiation among closely spaced subpopulations within one of the populations was also demonstrated (F_{st} = 0.124). Self-pollination and the patchy natural distribution of the species both likely contribute to the low level of gene flow (Nm = 0.205) that was estimated. Zones developed for the transfer of seed of commercial conifer species may be inappropriate for transfer of *E. glaucus* germplasm because conifer species are characterized by high levels of gene flow. Limited gene flow in *E. glaucus* can facilitate the divergence of populations over relatively small spatial scales. This genetic differentiation can be due to random genetic drift, localized selective pressures, or both. In order to minimize the chances of planting poorly adapted germplasm, seed of *E. glaucus* may need to be collected in close proximity to the proposed restoration site.

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Introduction

The value of grasses for revegetation, soil stabilization, and erosion control has long been recognized. Grasses are commonly planted on forest lands after wildfire, along road cuts, on mine tailings, and in other areas where human or natural processes have reduced or eliminated the existing plant cover. In the majority of cases, the grass species used for revegetation are not native to the site. The high degree of plasticity characteristic of many exotic grasses has made them practical for fulfilling short-term management objectives, but their invasiveness and long-term effect on both the biological and genetic diversity of ecosystems is an increasing concern (D'Antonio & Vitousek 1992). Large-scale use of native grass species for revegetation and ecosystem restoration is a relatively new phenomenon. The current widespread interest in native grasses is due, in part, to the recent availability of plant material as well as recognition of the role of native species in the restoration of biological diversity and the conservation of endangered species and habitats.

Commercial sources of native grass seed are often produced through agricultural increase of a limited number of collections, and the potential therefore exists for genotypes from one source population to be spread over large geographic areas. This has caused increasing concern about local adaptation and maintenance of the genetic integrity of existing native grass populations (Millar & Libby 1989; Knapp & Rice 1994). The latest U.S. Forest Service Region 6 policy on the use of native plants on National Forest lands states that "to the extent practicable, seeds of plants used in erosion control, fire rehabilitation, riparian restoration, forage enhancement, and other vegetation projects shall originate from genetically local sources of native plants" (U.S. Forest Service 1994). This new policy reflects both the shift toward interest in native species and an emphasis on properly matching germplasm to the local environment. Successful implementation of this policy depends on information on the spatial scale over which populations of native plant species are considered to be "genetically local."

The designation of clearly defined tree seed zones (see Kitzmiller 1990) has aided in revegetation efforts

Figure 1. *Elymus glaucus* is commonly found in open patches of mixed hardwood and conifer forest in the western United States. This photograph was taken at the 1000 m elevation in the Eldorado National Forest, California.

with commercial conifer species by providing a framework for how local a seed collection should be to a restoration site. Seed zones are based on known patterns of genetic similarity across the landscape, or on climatic contours when genetic information is lacking. Planting of seed outside of the zone in which it was collected is avoided. Furthermore, within each zone, seed is also generally not planted on sites differing by more than 1000 feet in elevation from the seed collection site (Buck et al. 1970). These regulations were developed to promote the planting of seed stock of similar adaptive capacity to native tree stands within a region. Unfortunately, genetic information on which to base recommendations for seed transfer is available for few species other than trees. The applicability of seed zones for tree species to other groups of plants, such as native grasses, may be limited because these guidelines are based on the genetic structure characteristic of outcrossing conifers.

Genetic differentiation in grasses has been shown over a range of spatial scales, from broad climatic regions (Clary 1975; Rice & Mack 1991) to sharp environmental clines of just a few meters (Bradshaw 1959; Hamrick & Allard 1972). Genetic differentiation can result from localized selection pressures or from stochastic processes such as random genetic drift. The probability that genetic differentiation will occur, whether caused by selection or drift, is dependent on both the magnitude and scale of gene flow.

The purpose of this study was to use allozyme markers to investigate the genetic structure of a native grass, *Elymus glaucus* (blue wildrye), from populations collected over a broad geographic area. A primary goal was to make some preliminary recommendations for the transfer and use of this species in revegetation and restoration projects. *E. glaucus* is a nonrhizomatous, self-fertilizing, perennial grass found in a wide range of habitats throughout the western United States and as far north as Alaska (Cronquist et al. 1977) (Fig. 1). The species is also extremely variable in growth form (Hitchcock 1935; Cronquist et al. 1977). Because it is one of the dominant native grass species in many habitats, interest in its use for restoration and revegetation is widespread and growing. Commercially produced *E. glaucus* seed is increasingly available, and approximately 41,000 pounds were produced in 1995 in California alone (Scott Stewart & John Anderson, personal communication).

Materials and Methods

Seed Collection. Seed from 20 *E. glaucus* populations was collected during the summer of 1993 in cooperation with personnel from the U.S. Forest Service and The Nature Conservancy in California, Oregon, and Washington (Table 1; Fig. 2). Distances between populations ranged from approximately 20 km (populations *R* and *S*) to over 1600 km (populations *A* and *K*). Open-pollinated seed was sampled from a total of 50 plants per population. Wherever possible, a minimum distance of 5 m between sampled plants was maintained in order to avoid collecting seed from close relatives. Seed from each plant was put into a separate envelope.

To investigate the genetic architecture within a population on a very local spatial scale, seed from population *Q* (El Gato Meadows, Umpqua National Forest, Oregon) was collected along a rough linear transect that bisected four meadows or forest openings. Subpopulations of *E. glaucus* were found only in these forest openings and not in the surrounding mixed conifer-hardwood forest. The distance between the midpo

of the subpopulations 1 and 2 as well as subpopulations 3 and 4 was approximately 200 m, and the distance between the midpoints of subpopulations 2 and 3 was less than 100 m.

Electrophoresis. Seed was germinated on moistened, regular-weight germination towels (Anchor Paper Co., 480 Broadway Street, St. Paul, MN 55165–0648). We attempted to use one 10-day-old seedling from each open-pollinated plant sampled in the field for the electrophoretic analysis. This sampling protocol minimized the opportunity for variable germination or selection among lab-germinated seedlings to alter the measures of population genetic diversity. Seedlings were ground separately in depressions drilled into a Plexiglas tray, by means of a flat-bottomed, 1-cm-diameter Plexiglas rod. Five drops of a chilled Tris-HCl extraction buffer described by Gottlieb (1981) were added prior to tissue homogenization. The extract was absorbed onto 2 mm × 12 mm wicks cut from Whatman #3 filter paper that were then placed in 0.5 ml microfuge tubes and stored at −70°C until needed.

A Tris-citrate buffer system (Meizel & Markert 1967) and a sodium-borate buffer system (Poulik 1957) were used to resolve all loci. Buffer systems used for each stain are given in Table 2. Components and molarities of these buffers are listed in Wendel and Weeden (1989). Gels were made with 12% w/v Sigma brand starch. Gels using the tris-citrate buffer system were run at 125 Volts and 50 mA for 5.5 hours, and gels using the sodium borate buffer system were run at 50 mA, with the voltage gradually increased to 200 Volts until the borate front migrated 8 cm from the origin (approximately 6.5 hours). A plastic bag filled with chilled water was placed on top of the gel during electrophoresis to aid in cooling.

Eight stains were used to obtain genotype data at 20 loci (Table 2). All staining recipes were those of Wendel and Weeden (1989), except for ACO, which was stained according to Morden et al. (1987), and IDH, which was stained according to Soltis et al. (1983). The genetic basis of banding patterns was inferred from known enzyme subunit structure and intercellular compartmentalization for these enzymes (Kephart 1990). To further facilitate the determination of genotype from several of the initially more complicated banding patterns, we also investigated segregation patterns of open-pollinated progeny arrays. Loci were numbered sequentially from the fastest- to the slowest-migrating for each stain.

Measures of within- and among-population allozyme diversity were obtained using the Biosys-1 computer program for analysis of allelic variation and other genetic parameters of populations (Swofford & Selander 1989). This program was also used to perform a cluster analysis by the unweighted paired group method using

Table 1. Letter designation, name, location and subspecies of *E. glaucus* populations sampled

Letter	*Population Name*	*State*	*National Forest (where applicable)*	*County*	*Elevation (meters)*	*Subspecies*
A	Descano	CA	Cleveland		1620	
C	Milford	CA	Plumas		1850	*glaucus*
D	High Siskiyou	OR	Rogue River		1710	*glaucus*
E	Grouse Creek	OR	Rogue River		670	*glaucus*
F	Randall Saddle	OR	Suislaw		230	*glaucus*
G	Mallory Flat	OR	Umatilla		1310	*jepsonii*
H	Grasshopper Mtn.	OR	Willamette		1490	*glaucus*
	Clover Creek	OR	Winema		1620	*glaucus*
	Snyder	WA	Gifford Pinchot		585	*glaucus*
K	Klipchuck	WA	Okanogan		910	*glaucus*[2]
L	Upper Chiwawa River	WA	Wenatchee		760	*jepsonii*
M	Stanford	CA		Santa Clara	50	*glaucus*
N	Lake Natomas	CA		Sacramento	60	
O	Horse Mtn.	CA	Six Rivers	Humboldt	1430	*glaucus*
P	Hershberger Mtn.	OR	Rogue River	Jackson	1740	*glaucus*
Q	El Gato Meadows	OR	Umpqua	Douglas	550	
R	Round Top Butte	OR		Jackson	910	
S	Lower Table Rock	OR		Jackson	460	*glaucus*
U	Pi Pi Valley	CA	Eldorado	Eldorado	1220	*glaucus*
V	Scadden Flat	CA		Nevada	730	*glaucus*

[1]No voucher specimen provided.
[2]Subspecies determined by George Wooten, collector of seed.
[3]Appeared intermediate between the two subspecies, with sparse hairs on margins of sheaths.

arithmetic averages (UPGMA) on a matrix of genetic distances (Nei 1978) between populations. All loci were used in the calculations. Loci for which the frequency of the most common allele did not exceed 0.99 were considered polymorphic. The geographic distance between populations was estimated from the distance between points on a map of the western United States. The relationship between the geographic distance matrix and the genetic distance matrix was determined with a Mantel test, which was calculated using the RT computer program (Manley 1994).

Results

Seed germination rates were variable among populations. Enough seedlings germinated for most populations to provide an adequate sample size (Table 3) for accurately assessing within-population variability. Although seed within some of the collections was obviously green and immature, this did not appear to appreciably reduce seed viability. Lowest germination rates were obtained for population *N* and population *U*, both of which were collected late in the growing season. Many of the best-filled seed had dropped by the time these seed were harvested. This suggests that if seed harvest cannot be timed at optimum maturity, harvesting early may be better than harvesting late.

E. glaucus is an allotetraploid ($2n = 28$) that likely arose as a result of a hybridization event between *Hordeum* and *Agropyron* (Dewey 1968; Jensen 1993). More loci were observed for many of the stains (ACO, IDH, MDH, and PGI) than would be expected in a diploid (Weeden & Wendel 1989), which is consistent with gene duplication associated with allotetraploidy. Gene duplication in many cases increased the number of bands observed, making initial interpretation more difficult, but it also increased the amount of information that could be obtained from the gels. Duplicated loci often had identical mobility, with one band the result of staining at two separate loci. In such cases it was not possible to detect the presence of multiple loci unless at least one of the loci was polymorphic. Duplicated loci with enzyme products having the same mobility in a gel were found for IDH1 and IDH2, PDG1 and PGD2, and MDH1 and MDH2. The genetic basis for these observations was elucidated by segregation analysis of open-pollinated progeny arrays from selected plants. Loci visualized with the SKD stain and the fastest-migrating locus for the AAT stain and for the PGI stain were not scored. These loci all appeared polymorphic but could not be clearly or consistently resolved.

A high degree of allozyme variation was found in *E. glaucus*. At the species level, 80% of the allozyme loci scored were polymorphic, and the number of alleles averaged 3.3 per locus. The only loci monomorphic across

Figure 2. Map of Washington, Oregon, and California, showing the locations of the 20 *E. glaucus* populations sampled.

all populations were IDH1, MDH2, MDH3, and PGD2. Average expected heterozygosity (an index of gene diversity) in this species was 0.194.

Considerable variability was also found at the population level (Table 3). An average of 31.4% of loci within populations were polymorphic, with an average of 1.4 alleles per locus. Average expected heterozygosity within populations was 0.085. No detectable allozyme variation was found within populations *M* and *N*. Both of these seed collections were made from small, isolated populations. It is possible that populations *M* and *N* experienced a genetic bottleneck or are the result of a founding event in which the present population arose from one or few individuals. The lack of variation present in population *N* might be due to sampling error; few seed germinated, and allele frequency estimates are based on just 10 individuals.

Amount of heterozygosity was low (Table 3), despite the moderate level of allozyme polymorphism found within most populations. Only 0.1% of the individuals screened were heterozygous at one or more loci. The number of heterozygotes was significantly less ($p \leq 0.05$) at all polymorphic loci than would have been expected if the populations were at Hardy-Weinberg equilibrium (assuming completely random mating, infinitely large population size, no selection, and no migration).

Using Wright's *F* statistics to examine genetic structure, we calculated that 54.9% of the variation at al

Table 2. Enzymes for which gels were stained, number of loci scored, and buffer system with which enzyme was resolved.*

Stain (abbreviation, enzyme commission number)	*Number of Loci Scored*	*Buffer System*
Aconitase (ACO, E.C.4.2.1.3)	4	
Alcohol dehydrogenase (ADH, E.C.1.1.1.1)	2	
Aspartate aminotransferase (AAT, E.C.2.6.1.1)	2	
Isocitrate dehydrogenase (IDH, E.C.1.1.1.42)	2	
Malate dehydrogenase (MDH, E.C.1.1.1.37)	4	
Phosphoglucoisomerase (PGI, E.C.5.3.1.9)	2	
Phosphoglucomutase (PGM, E.C.5.4.2.2)	2	
Phosphogluconate dehydrogenase (PGD, E.C.1.1.1.44)	2	
Shikimate dehydrogenase (SKD, E.C.1.1.1.25)	0	

*Buffer systems are numbered following Wendel and Weeden (1989).

zyme loci was partitioned among populations (F_{st} = 0.549) and that 45.1% of the variation was partitioned within populations. The high level of differentiation among populations indicates that gene flow may be quite limited at the spatial scale over which these populations were sampled. The relationship between F_{st} values and gene flow can be described by the following equation:

$$F_{st} \simeq 1/(1 + 4Nm),$$

where N is the effective size of each local population and m is the immigration rate (Wright 1951). By rearranging terms, an indirect estimate of gene flow (Nm) can then be obtained from the following equation:

$$\mathrm{Nm} \simeq 1/4(1/F_{st} - 1).$$

Slatkin and Barton (1989) demonstrated that this indirect method for estimating the average rate of gene flow in natural populations may be superior to maximum likelihood methods and rare alleles methods. Estimates of gene flow for *E. glaucus*, calculated using the F_{st} statistic, show that gene flow is very low (Nm = 0.205). Low levels of gene flow increase the potential for local population differentiation as a result of either natural selection, genetic drift, or both. At low levels of gene flow ($Nm << 1$), population differentiation may be expected due to random genetic drift alone, whereas at high levels of gene flow ($Nm >> 1$), gene flow will overcome the effect of drift and prevent local population differentiation (Wright 1951; Slatkin 1994).

Although much genetic variation in *E. glaucus* is distributed among populations, a large amount of variation is still contained within most populations. We found that the within-population variability often appears to be distributed in a nonrandom fashion. This was demonstrated in population *Q* (El Gato Meadows, Umpqua National Forest, Oregon), for which the seed was collected from four discrete subpopulations within the collection area. Genetic differentiation among the subpopulations was surprisingly high (F_{st} = 0.124), given their close proximity to each other. Certain alleles at polymorphic loci were found in only some but not all subpopulations (Table 4). Subpopulation 2 was the most genetically diverse for the allozyme loci investigated and was also the largest of the four subpopulations. Gene flow among subpopulations was moderate at this spatial scale (Nm = 1.766) but not so high that random genetic drift could not explain the divergence of these subpopulations.

Cluster analysis of between-population genetic distance values grouped populations by their relative genetic similarity and further demonstrated the distinctness of many populations in the study (Fig. 3). Few clusters of populations with strong genetic similarity were detected at the broad spatial scale over which these populations were sampled. Populations *C* (Plumas National Forest, California) and *H* (Willamette National Forest, Oregon) had similar genotypes at allozyme loci, as did populations *K* (Okanagan National Forest, Washington) and *L* (Wenatchee National Forest, Washington). The latter two populations were also collected in relatively close proximity to each other. However many other populations collected within a comparable distance were much more dissimilar. For example the three populations collected within the Rogue River National Forest, Oregon (*D*, *E*, and *P*) were among the most divergent of populations in the study. Populations *R* (Round Top Butte, Oregon) and *S* (Lower Table Rock, Oregon) were also quite different genetically, even though the sites were in close proximity to each other.

A Mantel test (Mantel 1967) indicated that there was no relationship between geographic distance and the degree of genetic divergence among populations (r = 0.0186, p = 0.84). This nonsignificant result confirmed our observation that genetic similarities among populations in this species rarely seemd to follow predictable geographic boundaries. For example, population *A*, collected from the Cleveland National Forest in San Diego

Table 3. Mean number of plants scored per locus (sample size), mean number of alleles per locus, percentage of loci that were polymorphic, and observed as well as expected heterozygosity for each population.*

Letter	Population Name	Mean Sample Size/Locus	Mean No. Alleles/Locus	Polymorphi Loci (%)	Mean Heterozygosity	
					Direct Count	H-W Expected
A	Descano	43.8 (0.1)	1.6 (0.2)	40.0	0.000 (0.000)	0.090 (0.030)
C	Milford	47.5 (0.3)	1.3 (0.1)	25.0	0.000 (0.000)	0.067 (0.029)
D	High Siskiyou	32.0 (0.1)	1.4 (0.1)	30.0	0.000 (0.000)	0.070 (0.029)
E	Grouse Creek	35.0 (0.1)	1.5 (0.2)	35.0	0.000 (0.000)	0.104 (0.041)
F	Randall Saddle	38.7 (0.7)	1.4 (0.1)	30.0	0.001 (0.001)	0.117 (0.046)
G	Mallory Flat	44.6 (0.2)	1.6 (0.2)	50.0	0.002 (0.002)	0.138 (0.033)
H	Grasshopper Mtn.	43.5 (0.2)	1.4 (0.1)	35.0	0.001 (0.001)	0.052 (0.019)
I	Clover Creek	35.9 (0.1)	1.4 (0.2)	30.0	0.000 (0.000)	0.096 (0.040)
J	Snyder	41.0 (0.0)	1.5 (0.1)	40.0	0.000 (0.000)	0.044 (0.013)
K	Klipchuck	32.0 (0.0)	1.4 (0.1)	30.0	0.000 (0.000)	0.075 (0.036)
L	Upper Chiwawa River	35.2 (0.3)	1.4 (0.1)	30.0	0.002 (0.002)	0.085 (0.036)
M	Stanford	49.0 (0.0)	1.0 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
N	Lake Natomas	9.9 (0.0)	1.0 (0.0)	0.0	0.000 (0.000)	0.000 (0.000)
O	Horse Mtn.	43.0 (0.1)	1.3 (0.1)	25.0	0.000 (0.000)	0.040 (0.024)
P	Hershberger Mtn.	31.1 (0.3)	1.5 (0.1)	50.0	0.003 (0.003)	0.142 (0.044)
Q	El Gato Meadows	50.8 (0.8)	1.5 (0.2)	45.0	0.010 (0.005)	0.090 (0.035)
R	Round Top Butte	44.0 (0.0)	1.5 (0.2)	40.0	0.000 (0.000)	0.120 (0.046)
S	Lower Table Rock	45.8 (0.1)	1.9 (0.2)	55.0	0.000 (0.000)	0.249 (0.058)
U	Pi Pi Valley	27.0 (0.0)	1.2 (0.1)	15.0	0.000 (0.000)	0.062 (0.034)
V	Scadden Flat	36.0 (0.0)	1.2 (0.1)	20.0	0.001 (0.001)	0.070 (0.034)
Mean		38.3	1.4	31.4	0.001	0.085

*Standard errors (where applicable) are in parentheses.

County, California, clustered with populations from Oregon and not with other populations from California. Population *C* from the Plumas National Forest, California, clustered close to population *H* from the Willamette National Forest, Oregon, even though they were collected from widely separate regions.

E. glaucus in North America is often broken down taxonomically into three different subspecies (Hickman 1993) or varieties (Cronquist et al. 1977). Although two subspecies (*glaucus* and *jepsonii*) are represented in this study (Table 1), cluster analysis of allozyme data did not support this intraspecific subdivision. The two populations that keyed to ssp. *jepsonii* (*G* and *L*) were both far more similar to other populations that keyed to ssp. *glaucus* than they were to each other.

Discussion

At the species level, *E. glaucus* contained considerably more allozyme variation than the average plant species. A review of the allozyme literature showed that an average of 50.5% of loci in plant species were polymorphic and that an average of 2.0 alleles were found per locus, while the average expected heterozygosity (genetic diversity index) was 0.149 (Hamrick & Godt 1989). Equivalent calculations for *E. glaucus* showed that 80% of the loci were polymorphic, the number of alleles per locus averaged 3.3, and expected heterozygosity was 0.194.

Conversely, slightly less genetic variation was found within *E. glaucus* populations than is typically found within populations of other plant species. The average *E. glaucus* population contained 31.4% polymorphic loci and 1.4 alleles per locus, and it had an expected heterozygosity of 0.085, whereas the review of the plant allozyme literature by Hamrick and Godt (1989) showed that within the average plant population 34.2% of loci were polymorphic, an average of 1.5 alleles was found per locus, and the average expected heterozygosity was 0.113. Our study sampled populations across a large geographic area, and widespread species are often more variable at allozyme loci than species with more limited distributions (Hamrick & Godt 1989). *E. glaucus* is highly variable in morphology and phenology across different locations within its range (Hitchcock 1935; Cronquist et al. 1977), and the high levels of allozyme variation we found are consistent with this observed variability for other traits. This high degree of allozyme variability contrasts, however, with the limited amount of allozyme variation found in a related species, *Elymus canadensis* (Sanders et al. 1979), which also has a broad geographic range.

The deficit of heterozygotes we observed is consiste

with accounts of the predominantly self-pollinating nature of *E. glaucus* (Cronquist et al. 1977). Plants that produce seed primarily through self-pollination are often highly homozygous because half of all heterozygosity is lost with each generation of complete selfing. It is interesting that many of the individuals with at least one heterozygous locus (10 out of the 18) were found in one population (Q—El Gato Meadows, Umpqua National Forest, Oregon). The differences in levels of heterozygosity among populations could be due to among-population variation in the breeding system, a phenomenon that has been reported in other species (Schoen 1982; Glover and Barrett 1986). This observation might also be explained by differences in the spatial arrangement or density of plants within populations, although the plants in population Q did not appear to be growing any closer together than plants in other populations.

The strong differentiation found among *E. glaucus* populations is characterized of self-pollinating species. Hamrick and Godt (1989) calculated that, on average, in plants where seed production is primarily by self-pollination, 51.0% of allozyme variation is found among populations, and the rest (49.0%) is found within populations. These average values are similar to our calculations for *E. glaucus* (54.9% among populations and 45.1% within populations). In contrast, an average of only 14.8% of variation is distributed among populations, while the majority (85.2%) is distributed within populations in predominantly outcrossing species (Hamrick & Godt 1989).

Gene flow among populations was estimated to be limited, as would be predicted for a species with a mating system that promotes high levels of self-pollination (Heywood 1991). The low levels of gene flow indicate that populations of *E. glaucus* may be able to differentiate at local scales. Rates of gene flow can determine the extent to which local populations of a species act as independent evolutionary units (Slatkin 1994). Gene flow among populations can buffer against genetic divergence, whereas populations lacking a flow of genetic material among them tend to evolve separately.

Gene flow is affected by both pollen and seed dispersal. Biological reasons for limited gene flow in this species include a mating system that appears to be predominantly self-pollinating and a lack of specialized seed-dispersal structures. As a result, effective pollen transfer is limited, and most seed likely falls in close proximity to the parent plant. Landscape-level processes may also contribute to the lack of gene flow. Distribution of *E. glaucus* populations is often patchy; plants are most commonly found at the margins of meadows and in forest clearings. The distance to the next such suitable habitat may be quite large relative to the potential for pollen and seed dispersal. The result of these factors is that populations of *E. glaucus* can develop and maintain genetic distinctness, even if they are not widely separated geographically. For example, the three populations collected within the Rogue River National Forest, Oregon, were very dissimilar despite their relative proximity to each other (collections *D* and *E* were made approximately 30 km apart, whereas collection *P* was made approximately 110 km from both *D* and *E*). This national forest contains lands in separate mountain ranges, with part in the Siskiyou Mountains and part in the Cascade Range. Very different geologic and evolutionary processes may have operated in these two regions. In addition, gene flow between populations may have been impeded by the valley that divides the ranges. These allozyme data thus indicate that using national forest boundaries as "seed zones" would not be a good strategy for *E. glaucus*. A better option might be to develop seed zones from patterns of observed or predicted gene flow. These patterns could be inferred, as was done in this study, by examining the spatial structure of neutral genetic markers or, less directly, by

Table 4. Allele frequency at polymorphic loci for four subpopulations of *E. glaucus* within population *Q* (El Gato Meadows, Umpqua National Forest, Oregon).

		Subpopulation			
Locus	*Allele*	*1*	*2*	*3*	*4*
PGM1	1	1.000	0.804	1.000	0.929
	4	—	0.196	—	0.071
MDH1	1	1.000	0.913	1.000	1.000
	2	—	0.087	—	—
AAT1	1	0.883	0.739	0.444	0.615
	2	0.167	0.261	0.556	0.385
AAT2	1	1.000	0.804	1.000	1.000
	2	—	0.196	—	—
ACO1	1	0.667	0.913	0.889	1.000
	5	0.333	0.087	—	—
	7	—	—	0.111	—
ACO3	1	1.000	0.957	1.000	1.000
	2	—	0.043	—	—
ACO4	1	0.583	0.913	0.667	1.000
	2	0.417	0.087	0.333	—
PGI1	1	0.083	0.391	0.333	0.543
	2	0.167	0.043	0.111	—
	6	0.750	0.565	0.556	0.357
PGI2	1	1.000	1.000	1.000	0.857
	8	—	—	—	0.143
Sample size/locus		6	23	9	14
No. alleles/locus		1.3	1.5	1.3	1.2
% Polymorphic loci		20.0	40.0	20.0	20.0

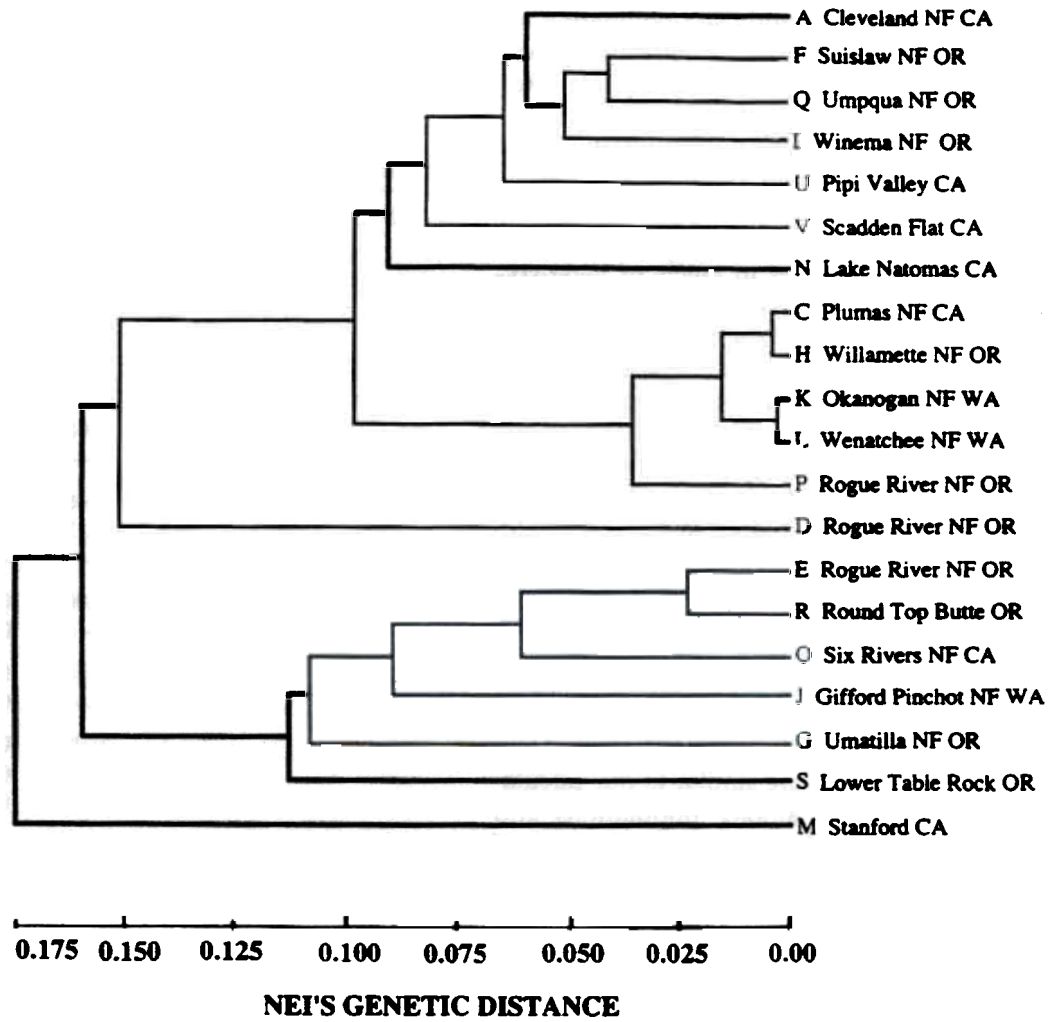

Figure 3. Cluster analysis of Nei's (1978) genetic distances among populations, based on allozyme genotype at 20 loci.

geographic analysis of potential physical impediments to gene flow. It should be emphasized that further research (such as common garden studies) is needed to determine whether the observed genetic differences for allozyme markers correspond to genetic differences for morphological and phenological traits with potentially greater adaptive importance.

Genetic structuring was evident among subpopulations within population Q (El Gato Meadows, Umpqua National Forest, Oregon) that were only 100–200 m apart. Narrow bands of forest dividing more-open *E. glaucus* habitat are apparently sufficient for restricting gene flow to the point that genetic distinctness could be maintained in these subpopulations. It remains to be seen whether local adaptation will also be found at this spatial scale. Genetic differentiation resulting from highly localized selective pressures is conceivable because the products of selection (locally adapted populations) are protected from the homogenizing effects of gene migration. This potential for localized adaptation suggests that seed of *E. glaucus* should not be transferred over large distances and that a close matching of environment of the restoration site to the environment of the seed collection site would be desired in many cases. But limited gene flow can also increase the importance of genetic drift in shaping the genetic structure of *E. glaucus* and thus increase the probability of genetic differentiation that is unrelated to selection. Therefore, it should not be automatically assumed that local collections are the best adapted germplasm available. Chance colonization events can result in the formation of populations that may not represent the best adaptive "solution" to selection within a particular site. The genetic composition of the individuals colonizing a site from a nearby source population usually represents only a small subsample of the overall genetic potential of a species. A newly founded population may establish, persist, and even flourish. But as a result of this limited sampling

the species' overall genetic variation during colonization, the genotypes in the newly established population may be less fit than genotypes available in other populations that, by chance alone, were not introduced into the site.

Several cases of presumed maladaptation have been demonstrated by reciprocal transplant studies designed to examine local adaptation. A study by Rice and Mack (1991) on ecotypic variation in *Bromus tectorum* L. (cheatgrass) along an elevational and precipitation gradient in eastern Washington and northern Idaho indicated that colonization dynamics in this selfing weed may have reduced the potential for local adaptation. For example, within an *Abies grandis* (grand fir) clearcut, the population growth rate of the local *B. tectorum* population was significantly lower than that of experimentally introduced non-local populations. Except for two sites that were on the opposite extremes of the elevational gradient, there was no indication of local adaptation among any of the *B. tectorum* populations examined during three years of demographic monitoring. Similar results were found for *Agrostis capillaris* L. in a study examining regional adaptation in this perennial grass in southern New Zealand (Rapson & Wilson 1988). In a number of sites that ranged from coastal pastures to high-altitude meadows, there was evidence for local maladaptation at the population level in terms of growth, tiller dynamics, or floral phenology.

It is likely that seed zones developed for commercial conifer species (Kitzmiller 1990) will not be appropriate for *E. glaucus* because of the considerable differences in genetic structure between the two life forms. In contrast to *E. glaucus*, conifers produce seed primarily through cross-pollination, and much of the genetic variation is distributed within instead of among populations (Loveless & Hamrick 1984). Greater seed and pollen dispersal is generally found in conifers than in herbaceous species (Levin 1981). In addition, the spatial distribution of many of the commercially important conifer tree species is often relatively continuous, with less disjunction among populations, thus increasing the likelihood for uninterrupted gene flow. Because the potential for genetic differentiation at smaller spatial scales is greater in *E. glaucus* than in commercially important conifer species, seed zones for germplasm transfer in this grass species will likely need to be more spatially restricted.

Efforts to define seed zones emphasize genetic variation among populations resulting from past evolutionary processes and events. But the potential for populations to evolve in response to future environmental challenges depends on the existence of genetic variation at the within-population level as well. The presence of genetic variation is especially critical for germplasm used in restoration and revegetation, because this seed may be planted across an array of local habitats where selection may favor different combinations of genes. A population containing genetic variation will enhance the likelihood that some plants in the existing collection will possess the best combination of genes for that particular habitat. Given that a seed collection for a particular seed zone may represent a mixture that is only "coarsely" adapted to regional climatic conditions, genetic variation within the mixture will allow for selective forces to fine-tune populations for adaptation to local conditions. The numerous allelic variants found within *E. glaucus* populations emphasize the importance of collecting seed from many plants in order to obtain a seed source containing as much genetic variation of the original population as possible. Indications of considerable genetic structuring of allozyme variation within subpopulations of *E. glaucus* further demonstrate that seed collection should not be limited to small areas within a population. Collecting seed from different subpopulations within a region is advised in order to maximize the potential for evolutionary response and thus the sustainability of restored populations.

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