# Isozyme variation and its environmental correlates in *Elymus glaucus* from the California Floristic Province

**Barbara L. Wilson, Jay Kitzmiller, Wayne Rolle, and Valerie D. Hipkins**

**Abstract**: Genetic variation in the self-fertile, allotetraploid grass *Elymus glaucus* Buckley was assessed using isozymes in 133 populations from southwestern Oregon and from the San Francisco Bay area and central Sierra Nevada mountains in California. *Elymus glaucus* was highly (98.5%) homozygous but also highly variable; 77% of loci were polymorphic, and the mean number of alleles per locus was 2.96. Populations were highly differentiated, with 40% of variation among populations. Geographic and genetic distances among populations were not correlated, except that populations collected within 5 km were generally more similar than average. Genetic distance among populations could not be predicted from geographic distance, geographic location, foliage pubescence, serpentine substrate, or habitat moisture. However, two genetic clusters, associated with elevation, did emerge. The taxonomic status of *Elymus glaucus* ssp. *jepsonii* (Burtt Davy) Gould, based on leaf pubescence, was not supported.

*Key words*: *Elymus glaucus*, isozyme, genetic variation, Poaceae, polyploid, seed transfer.

**Résumé** : À l'aide des isoenzymes, les auteurs ont évalué la variation génétique chez une graminée auto-fertile allotétraploïde, l'*Elymus glaucus*, dans 133 populations du sud ouest de l'Oregon, de la région de la baie de San Fransisco, et des montagnes du centre des Sierra Nevada, en Californie. L'*Elymus glaucus* est fortement homozygote (98,5 %) mais également très variable; 77 % des loci sont polymorphes et le nombre moyen d'allèles par locus est de 2,96. Les population sont fortement différenciées, avec 40 % de variation entre les populations. Les distances géographiques et génétiques entre les population ne montrent pas de corrélations, sauf que les populations récoltées à moins de 5 km sont généralement plus semblables que la moyenne. La distance génétique entre les populations ne peuvent être prédites à partir de la distance géographique, de la localité, de la pubescence du feuillage, du substrat à serpentine, ou de l'humidité de l'habitat. Cependant, on distingue deux regroupements génétiques associés avec l'altitude. Le statut taxonomique de l'*Elymus glaucus* ssp. *jepsonii* (Burtt Davy) Gould, basé sur la pubescence des feuilles, n'a pu être confirmé.

*Mots clés* : *Elymus glaucus*, isozyme, variation génétique, Poaceae, polyploïde, transfert de graines.

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## Introduction

Self-pollinating plants are often viewed as genetically less variable than outcrossed relatives (Richards 1986). However, selfing populations are not necessarily genetically invariable. Selfing species may be highly variable (e.g., Adams and Allard 1977), especially in taxa that cross-pollinate at low rates (Adams and Allard 1982). Although predominantly selfing species have less genetic diversity overall, they display greater differentiation among populations compared with outcrossing taxa (Hamrick and Godt 1996).

Selfers are more often polyploids than outbreeders, because it is thought that polyploidy may circumvent the chemical barriers that prevent self-pollination (Richards 1986; Lewis 1980). Polyploidy tends to slow down the process of homozygous fixation of polymorphic loci and to maintain relatively high levels of variability and heterozygosity compared with diploids. Methods for assessing genetic variation in polyploids are not standardized and depend on both the preferences of researchers and the degree of complexity in the markers used. Approaches appropriately vary from reporting the variation with little statistical analysis (Angelov 1992, 1993) to analyzing the variation as phenotypes (Chung et al. 1991) or genotypes (Knapp and Rice 1996). This study of *Elymus glaucus* Buckley (blue wild rye) provides a thorough characterization of the population genetics of a self-fertilizing, perennial, allotetraploid grass sampled intensively in its native range.

*Elymus glaucus* is a common perennial grass of western North America. It is often a community dominant in meadows, forest edges, thickets, and open forests from near sea

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level to over 2400 m elevation. It is highly self-fertile (Jensen et al. 1990) yet exhibits much phenotypic variation. *Elymus glaucus* individuals often retain their diverse morphological and physiological characteristics when transplanted into a common garden (Snyder 1950; Adams et al. 1999). A previous study of isozyme variability in *E. glaucus* revealed high genetic diversity that was not correlated with geographic distance at the scale of the entire Pacific coast (Knapp and Rice 1996).

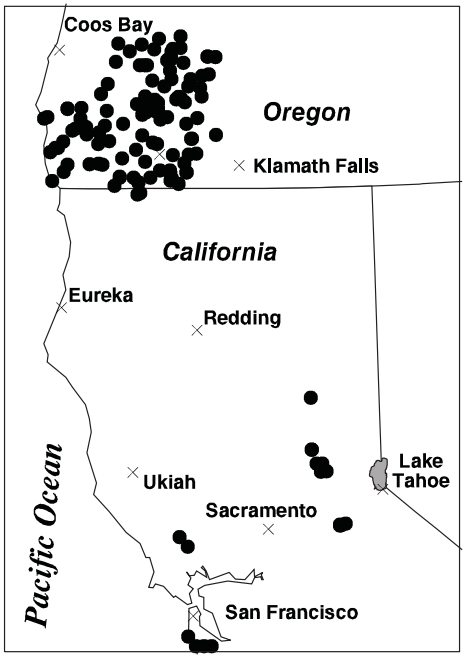
*Elymus glaucus* is an allotetraploid with $4X = 28$ chromosomes and contains the H genome of the genus *Hordeum* and the S genome of *Pseudoroegneria* (Dewey 1982). *Elymus glaucus* has produced viable offspring in experimental crosses with several species in the genera *Agropyron*, *Elymus*, and *Hordeum* (Dewey 1982; Jensen 1993; Salomon et al. 1991). Some hybrids, such as *Eymus* ×*hanseni* Scribner (*Elymus elymoides* (Raf.) Sweezy × *E. glaucus*), occur frequently in nature. When individuals from different *E. glaucus* populations are artificially crossed, however, they may either produce abundant seed or be completely intersterile (Snyder 1950, 1951).

Three intraspecific taxa of *E. glaucus* have been recognized (Barkworth 1993; Hitchcock et al. 1969). The most widespread is *E. glaucus* ssp. *glaucus*, which has 1–3 cm long lemma awns and glabrous to scabrous leaves and leaf sheaths. *Elymus glaucus* ssp. *virescens* (Piper) Gould has unawned to short-awned (<5 mm) lemmas and varies in pubescence of its leaf sheaths. *Elymus glaucus* ssp. *jepsonii* (Burtt Davy) Gould has awns similar in length to those of *E. glaucus* ssp. *glaucus* but differs from that subspecies in having hirsute to pilose leaf sheaths (and often leaves). Both *E. glaucus* ssp. *jepsonii* and *E. glaucus* ssp. *virescens* occur primarily west of the crest of the Cascade Range and Sierra Nevadas, but both are also reported to occur at scattered inland locations. *Elymus glaucus* ssp. *jepsonii* has been reported from Montana, and *E. glaucus* ssp. *virescens* has been reported from Colorado (M. Barkworth, personal communication).

The taxonomic validity of taxa such as *E. glaucus* ssp. *jepsonii* that differ from the widespread variety in only one character is often problematic. If the difference in pubescence is correlated with variation in other morphological or physiological traits, recognition of the pubescent and glabrous morphs as distinct taxa is supported. If there is no correlation between the presence or absence of pubescence and any other character, treating the two morphs as individual variation occurring within a taxon would seem a better option. An intensive but geographically restricted study of *E. glaucus* indicated that various morphologic traits, including foliage pubescence, vary independently (Snyder 1950). We included one population of *E. glaucus* ssp. *virescens* and several populations of *E. glaucus* ssp. *jepsonii* in this study to evaluate the status of these taxa. If the *E. glaucus* ssp. *jepsonii* populations had similar allele frequencies, and therefore clustered together in dendrograms based on genetic similarity among populations, then formal recognition of *E. glaucus* ssp. *jepsonii* would be supported.

Demand for *E. glaucus* for habitat revegetation and restoration is high and has led to the use of nonlocal seeds from both cultivated and wild sources. However, using such seed may conflict with the goal of conserving local genetic diversity (Knapp and Rice 1996). Restoration efforts using nonlocal plants may be less successful because of maladaptation (Templeton et al. 1986). Moreover, successful restoration efforts might have negative effects on nearby native populations through spread of genes associated with poor adaptation to local conditions, the breakup of coadapted gene complexes (Templeton et al. 1986), or more plausibly, through the replacement of diverse local genotypes with a few commercially successful nonlocal strains. To avoid these genetic problems, local adaptation and maintenance of genetic integrity are often managed in terms of seed transfer zones. Seed transfer zones are regions within which plants of a given species can be moved with the expectation that they will grow and will produce no harmful ecological or genetic effects. To aid in the identification and application of such management units, the following questions must be considered. How genetically diverse is *E. glaucus*? How differentiated are populations? Are populations more similar to other nearby populations than they are to distant ones? Is isozyme diversity (a measure of genetic diversity) related to obvious qualitative differences in habitat? Once these basic population genetic question are answered, perhaps practical guidelines for seed transfer can be established. We report an attempt to answer these questions by isozyme analysis of over 3000 *E. glaucus* individuals from 133 populations in three regions (southwestern Oregon, the San Francisco Bay area, and the central Sierra Nevada Mountains) within the California Floristic Province (Hickman 1993).

**Fig. 1.** Collection sites for *Elymus glaucus*.




## Materials and methods

### Seed collection

In southwestern Oregon, *E. glaucus* seeds were collected from populations chosen to represent a wide range of habitats and environmental conditions. A total of 115 populations (hereafter re-

ferred to as Oregon populations) were sampled; 113 populations were sampled from Oregon and two from extreme northern California (Fig. 1). In each population, 50 plants were selected for collection at approximately 5-m intervals along informal transects through each population. All mature spikes (to a maximum of five) were cut from each selected plant, placed in a paper envelope, and labeled by population and individual. Some populations were collected in pairs near one another but in contrasting habitats (e.g., wet vs. dry or serpentine vs. nonserpentine habitats). Field workers classified each site as wet or dry, serpentine or nonserpentine, based on the plant communities present at the site. They classified each population as containing pubescent or glabrous *E. glaucus* plants. They also noted the elevation and the presence of *E. glaucus* hybrid individuals in or near the site. Voucher specimens for five representative populations were deposited in the herbarium of University of California at Davis.

Eleven *E. glaucus* populations (hereafter referred to as Sierra Nevada populations) were sampled from the Eldorado and Tahoe National Forests in the Sierra Nevada Mountains of central California Sierra Nevada (Fig. 1). In the Sierra Nevadas, one seed head per plant was collected, at intervals along transects. Seven populations (hereafter referred to as Bay Area populations) were sampled from the San Francisco area (Fig. 1). Bay area populations were selected by C. Dremann to represent the extremes of habitat (as defined by the plant community present, elevation, and proximity to the coast) and included three taxonomic varieties (*E. glaucus* ssp. *glaucus*, *E. glaucus* ssp. *jepsonii*, and *E. glaucus* ssp. *virescens*). Because all populations sampled in the San Francisco Bay area were small (as small as 20 fertile individuals), seeds were collected from most plants in each population.

Oregon and Bay Area seeds were collected in 1994. Sierra Nevada seeds were collected in 1993 (Tahoe National Forest) and 1994 (Eldorado National Forest). Dry seed heads were stored at room temperature until processed in 1994 and 1995.

A table of the locations and characteristics (names, latitude and longitude, soil, moisture, and leaf pubescence) of the 133 populations is available from the authors upon request.

## Tissue preparation

For the Oregon populations, seeds were germinated from approximately 25 individuals/population. One 8–11 cm long (10- to 21-day-old) seedling per individual was processed for isozyme analysis. Individual seedling shoots (excluding roots) were ground in eight drops of a 0.1 M Tris-HCl (pH 8.0) buffer, containing 10% (w/v) polyvinylpyrrolidone-40, 10% sucrose, 0.17% EDTA ($Na_2$ salt), 0.15% dithiothreitol, 0.02% ascorbic acid, 0.10% bovine albumin, 0.05% NAD, 0.035% NADP, and 0.005% pyridoxal-5-phosphate (USDA Forest Service 1995). Enzyme extracts were frozen at –70°C.

Processing of Bay Area and Sierra Nevada populations was similar, except that 50 individuals/population were macerated for the Bay Area populations, entire seedlings (including roots) were processed, and the extracts were absorbed onto 3 mm wide wicks prepared from Whatman 3-mm chromatography paper, which were stored at –70°C. Initial comparisons revealed that the only difference resulting from the variation in preparation was that seedlings processed as rootless shoots stained so faintly for aconitase and malate dehydrogenase that those enzymes could not be scored consistently (National Forest Genetic Electrophoresis Laboratory (NFGEL)). Therefore, these enzymes were not included in this study.

## Enzyme electrophoresis

Methods of sample preparation and electrophoresis follow the general methodology of Conkle et al. (1982), with some modifications (USDA Forest Service 1995). The following enzymes were examined: diaphorase (DIA), glucose-6-phosphate dehydrogenase (G6PDH), glutamate-oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). All enzymes were resolved on 11% starch gels. A lithium borate electrode buffer (pH 8.3) was used with a Tris-citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve DIA, IDH, LAP, ME, and PGM. A sodium borate electrode buffer (pH 8.0) was used with a Tris-citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve GOT, G6PDH, 6PGD, PGI, TPI, and UGPP. Enzyme stain recipes follow USDA Forest Service (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989).

Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For further quality control, 10% of the individuals were run and scored twice. Genetic interpretations were inferred directly from isozyme phenotypes based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1989) and following a previous study of *E. glaucus* isozymes (Knapp and Rice 1996). *Elymus glaucus* is an allotetraploid (Dewey 1982); between two and four loci were scored for each enzyme. An isozyme band pattern consisting of a single band was considered to consist of two homeologous loci, one in the H genome and one in the S genome (Dewey 1982), producing enzymes with identical mobilities. The data were analyzed as 26 diploid loci, rather than 13 tetraploid loci with polysomic inheritance (Soltis and Rieseberg 1986). Where possible, we followed the assignments of alleles to loci used by Knapp and Rice (1996). All alleles detected in this study but not in Knapp and Rice (1996) were assigned to only one of the two homeologous loci, unless the observed band combinations or intensities suggested that two alleles belonged to different loci.

## Data analysis

Results were analyzed using POPGENE, version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred even once. We calculated unbiased genetic distances (Nei 1978), expected heterozygosity (Nei 1973), and gene flow (*Nm* (the effective number of migrants per year) = $0.25(1 - F_{ST})/F_{ST}$; Slatkin and Barton 1989). The fixation indices for populations (*F*) were calculated in POPGENE (Yeh et al. 1997) following Hartl and Clark (1989), but *F* statistics for the hierarchy of regions within the species ($F_{PT}$); populations within the species ($F_{ST}$); populations within regions ($F_{SP}$); and individuals within the species ($F_{IT}$), regions ($F_{IP}$), and populations ($F_{IS}$) were calculated by the method of Weir (1990). The two methods produced slightly different values for *F.* Dendrograms based on unbiased genetic distances (Nei 1978) were generated using UPGMA and neighbor-joining using PHYLIP (Felsenstein 1989). Information on habitat, foliage pubescence, geographic region, and elevation was mapped on the dendrograms.

Populations located within 5 km of each other were considered pairs. Genetic distances between pairs were compared with distances between unpaired collections (except the Bernal Hill population of *E. glaucus* ssp. *virescens*) using Student's *t* tests calculated with the statistics package in Microsoft Excel (Microsoft Corp. 1997).

Pairwise genetic and geographic distances between all populations (except the Bernal Hill population of *E. glaucus* ssp. *virescens*) were compared with a Mantel test using NTSYS-pc version 1.80 (Rohlf 1994). The matrix of pairwise geographic distances was generated using ARCINFO (Environmental Systems Research Institute 1997) from latitudes and longitudes. These data were provided for Bay Area collection localities. Oregon and Si-

erra Nevada collection localities were reported as township, range, and section. These data were converted to latitude and longitude using the program TRS2LL (Wefald 1998), which reports the latitude and longitude for the center point of each section. To separate populations collected within the same section, correction factors were developed by comparison of reported latitudes and longitudes for sections within the same township or range square. These corrections were more precise than accurate; TRS2LL reports latitudes and longitudes with an estimated error of 0.25 mile (1 mile = 1.609 km) in western states (Wefald 1998), but 1/16 sections are 0.125 × 0.125 mile squares.

We used two approaches to determine if overall allele frequencies were associated with geographic regions of interest to managers. These regions included vegetation zones (coast, Siskiyou Mountains, the Klamath region, western Oregon interior valleys, the western Cascade Range, and the high Cascades; Franklin and Dyrness 1969), arbitrary areas (east and west of Interstate Highway 5), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed transfer zones (USDA Forest Service 1973). First, we mapped these regions on a dendrogram based on genetic distances among populations. Also, we used chi-square tests to test for heterogeneity in allele frequencies among regions.

## Results

Poor seed germination reduced the number of *E. glaucus* seedlings available for analysis from some sites. A total of 3342 seedlings were analyzed: 2744 seedlings (23.9/population) from the Oregon sites; 296 (26.9/population) from the Sierra Nevada sites; and 302 (43.1/population) from the Bay Area sites.

Seventy-seven alleles were identified among the 26 loci scored, and all alleles observed in the Bay Area and Sierra Nevada populations were also observed in the Oregon populations (Table 1). A table of allele frequencies in the 133 populations is available on request from the NFGEL. Despite the low inferred gene flow ($Nm$ = 0.344), only one (1.3%) of the 78 alleles was unique to a single population. In only 21 (0.7%) cases in 17 (13%) of the populations did we fail to observe the most common allele for a locus. Ten of these failures involved G6PDH locus 1.

The most common isozyme band pattern observed consisted of a single band in eight (62%) pairs of homeologous loci (DIA, IDH, ME, LAP, 6PGD, PGI-1 – PGI-2, TPI, and UGPP). That is, either the enzymes produced by both homeologous loci migrated identically, producing one band, or one of the two loci was homozygous for an allele producing a polypeptide that was inactive under experimental conditions. In the remaining five (38%) pairs of homeologous loci (G6PD, GOT-1 – GOT-2, GOT-3 – GOT-4, PGI-3 – PGI-4, and PGM), the isozyme band pattern consisted of two or three bands (depending on whether the enzyme was a monomer or dimer). This could be considered fixed heterozygosity, but some variation was observed, and for two of these locus pairs (G6PD and GOT-3 – GOT-4) the band pattern sometimes consisted of only a single band.

At the species level, *E. glaucus* had high levels of genetic variability (Table 2). A total of 20 (77%) of the 26 loci were polymorphic, with an average of 2.96 alleles/locus. In this study, genetic variation was structured hierarchically; regions within the species, populations within regions, and individuals within populations. After the divergent *E. glaucus* ssp. *virescens* was removed from analysis, $F_{PT}$ (differentiation among regions within the species) was 0.086; $F_{ST}$ (populations within the species) was 0.4158; $F_{SP}$ (populations within regions) was 0.3608; and $F_{IT}$, $F_{IP}$, and $F_{IS}$ (individuals within the species, within regions, and within populations, respectively) exceeded 0.99 in all cases. In other words, regions were moderately differentiated genetically and populations were highly differentiated. For the species as a whole (including the one population of *E. glaucus* ssp. *virescens*), about 40% of the variation was between populations and 60% within populations ($F_{ST}$ = 0.4010). The high $F_{ST}$ for the Bay Area populations (Table 3) resulted from the inclusion of one population of the genetically divergent taxon *E. glaucus* ssp. *virescens*.

Only 52 (1.6%) of the 3342 individuals sampled were heterozygous at any locus, with one of them heterozygous at two loci. These 52 heterozygous individuals were scattered among 34 (25%) of the populations. Twenty-two populations contained a single heterozygote, while 12 additional populations contained between two and four heterozygous individuals. In 8 (67%) of the 12 populations with more than one heterozygote, all heterozygotes were heterozygous for the same pair of alleles. This might suggest that the heterozygosity was produced by segregation in the same lineage but, in only three populations, did all the heterozygotes have the same multilocus genotype.

Despite the high level of homozygosity suggestive of selfing, 504 different multilocus genotypes were detected among the 3151 individuals for which complete 26-locus genotypes were obtained (Table 4). On average, each genotype occurred in 6.26 individuals and 2.88 populations, but as expected (Barrett and Shore 1990), the frequency distribution of the multilocus genotypes was strongly skewed. A total of 275 (55%) of the multilocus genotypes were restricted to a single individual. The most common genotype in the study occurred in 280 (8%) individuals in 69 (52%) populations in all three regions. This genotype was homozygous for the most common allele at every locus. On average, the most common genotype in a population occurred in 34% of sampled individuals of that population, although in some populations as few as 8% or as many as 92% of individuals exhibited the population's most common genotype (Table 4).

Mean genetic distance between populations (Nei 1978) was low, averaging 0.0566 (or 0.0548, if the one population of *E. glaucus* ssp. *virescens* was omitted), and varied from 0.0001 to 0.2742 (or 0.2130, if the one population of *E. glaucus* ssp. *virescens* was omitted). Over all, there was no relationship between geographic and genetic distances between populations (Table 5). However, genetic distances between populations collected within 5 km of one another were significantly smaller than the genetic distances between nonpaired populations ($t$ = 6.91, $p < 1 \times 10^{-7}$).

The Oregon populations located within 5 km of each other were considered pairs. (The two Flanagan Prairie populations were collected from one large grassland and, therefore, were omitted from analysis of paired populations.) Some paired populations had genetic distances that exceeded the average for all populations; Illinois Valley populations had a genetic distance of 0.0651, and two from the Bear Camp area had a genetic distance of 0.0687. Genetic distances between wet–dry pairs averaged significantly smaller than those of nonpaired populations ($t$ = 6.77, $p < 0.001$). Popula-

**Table 1.** Allele frequencies for 26 isozyme loci in *Elymus glaucus*.

| Locus | Allele | Migration | Entire study | Oregon | Sierra Nevada | Bay Area | Bernal Hill |
|---|---|---|---|---|---|---|---|
| DIA-1 | 1 | 27 | 0.9539 | 0.9684 | 0.9524 | 0.9453 | 0.1136 |
| DIA-1 | 2 | 30 | 0.0252 | 0.0124 | 0.0204 | 0.0195 | 0.8864 |
| DIA-1 | 3 | 23 | 0.0209 | 0.0192 | 0.0272 | 0.0352 | |
| DIA-2 | 1 | 27 | 0.9595 | 0.9642 | 0.9830 | 0.9844 | 0.3636 |
| DIA-2 | 2 | N | 0.0147 | 0.0051 | 0.0102 | 0.0156 | 0.6364 |
| DIA-2 | 3 | 17 | 0.0173 | 0.0203 | 0.0068 | | |
| DIA-2 | 4 | 6 | 0.0086 | 0.0104 | | | |
| G6PDH-1 | 1 | 37 | 0.3594 | 0.3174 | 0.7669 | 0.2332 | 0.9333 |
| G6PDH-1 | 2 | 41 | 0.6406 | 0.6826 | 0.2331 | 0.7668 | 0.0667 |
| G6PDH-2 | 1 | 37 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| GOT-1 | 1 | 41 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| GOT-2 | 1 | 37 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| GOT-3 | 1 | 30 | 0.8781 | 0.8936 | 0.7017 | 0.8960 | 1.0000 |
| GOT-3 | 2 | 33 | 0.0480 | 0.0301 | 0.2576 | 0.0040 | |
| GOT-3 | 3 | N | 0.0712 | 0.0730 | 0.0407 | 0.1000 | |
| GOT-3 | 4 | 35 | 0.0021 | 0.0026 | | | |
| GOT-3 | 5 | 28 | 0.0006 | 0.0007 | | | |
| GOT-4 | 1 | 28 | 0.7208 | 0.7172 | 0.9051 | 0.6640 | 0.0444 |
| GOT-4 | 2 | 24 | 0.1768 | 0.1945 | 0.0373 | 0.0120 | 0.9333 |
| GOT-4 | 3 | N | 0.0646 | 0.0510 | 0.0237 | 0.2680 | 0.0222 |
| GOT-4 | 4 | 26 | 0.0370 | 0.0361 | 0.0339 | 0.0560 | |
| GOT-4 | 5 | 21 | 0.0009 | 0.0011 | | | |
| IDH-1 | 1 | 32 | 0.8257 | 0.7969 | 0.9155 | 1.0000 | 1.0000 |
| IDH-1 | 2 | 28 | 0.0078 | 0.0004 | 0.0845 | | |
| IDH-1 | 3 | 35 | 0.1638 | 0.1994 | | | |
| IDH-1 | 4 | 29 | 0.0027 | 0.0033 | | | |
| IDH-2 | 1 | 32 | 0.9991 | 0.9989 | 1.0000 | 1.0000 | 1.0000 |
| IDH-2 | 2 | N | 0.0009 | 0.0011 | | | |
| LAP-1 | 1 | 35 | 0.9906 | 0.9956 | 0.9488 | 0.9882 | 0.9778 |
| LAP-1 | 2 | 43 | 0.0006 | 0.0007 | | | |
| LAP-1 | 3 | 36 | 0.0088 | 0.0037 | 0.0512 | 0.0118 | 0.0222 |
| LAP-2 | 1 | 35 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| ME-1 | 1 | 24 | 0.9790 | 0.9745 | 1.0000 | 1.0000 | 1.0000 |
| ME-1 | 2 | 18 | 0.0018 | 0.0022 | | | |
| ME-1 | 3 | 20 | 0.0192 | 0.0233 | | | |
| ME-2 | 1 | 24 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 6PGD-1 | 1 | 37 | 0.9898 | 0.9876 | 1.0000 | 1.0000 | 1.0000 |
| 6PGD-1 | 2 | 36 | 0.0093 | 0.0113 | | | |
| 6PGD-1 | 3 | 29 | 0.0009 | 0.0011 | | | |
| 6PGD-2 | 1 | 37 | 0.9982 | 0.9978 | 1.0000 | 1.0000 | 1.0000 |
| 6PGD-2 | 2 | N | 0.0018 | 0.0022 | | | |
| PGI-1 | 1 | 46 | 0.9985 | 0.9989 | 0.9932 | 1.0000 | 1.0000 |
| PGI-1 | 2 | 42 | 0.0009 | 0.0004 | 0.0068 | | |
| PGI-1 | 3 | 50 | 0.0006 | 0.0007 | | | |
| PGI-2 | 1 | 46 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| PGI-3 | 1 | 28 | 0.6348 | 0.6148 | 0.7196 | 0.8541 | 0.0444 |
| PGI-3 | 2 | 32 | 0.1403 | 0.1288 | 0.1655 | 0.1070 | 0.8667 |
| PGI-3 | 3 | 30 | 0.1866 | 0.2192 | 0.0270 | 0.0389 | 0.0889 |
| PGI-3 | 4 | N | 0.0168 | 0.0142 | 0.0574 | | |
| PGI-3 | 5 | 27 | 0.0215 | 0.0230 | 0.0304 | | |
| PGI-4 | 1 | 21 | 0.9737 | 0.9887 | 0.9189 | 0.9883 | 0.3333 |
| PGI-4 | 2 | 15 | 0.0127 | 0.0056 | 0.0811 | 0.0117 | |
| PGI-4 | 3 | 27 | 0.0106 | 0.0020 | | | 0.6667 |
| PGI-4 | 4 | 10 | 0.0030 | 0.0036 | | | |
| PGM-1 | 1 | 43 | 0.6162 | 0.5715 | 0.9249 | 0.8100 | 0.1860 |
| PGM-1 | 2 | 40 | 0.3347 | 0.3712 | 0.0546 | 0.1900 | 0.7907 |
| PGM-1 | 3 | N | 0.0228 | 0.0256 | 0.0205 | | |

**Table 1** (*concluded*).

| Locus | Allele | Migration | Entire study | Oregon | Sierra Nevada | Bay Area | Bernal Hill |
|---|---|---|---|---|---|---|---|
| PGM-1 | 4 | 44 | 0.0263 | 0.0317 | | | 0.0233 |
| PGM-2 | 1 | 38 | 0.9221 | 0.9346 | 0.7356 | 0.9920 | 1.0000 |
| PGM-2 | 2 | 34 | 0.0508 | 0.0391 | 0.2068 | 0.0040 | |
| PGM-2 | 3 | 28 | 0.0063 | 0.0015 | 0.0576 | | |
| PGM-2 | 4 | N | 0.0036 | 0.0040 | | 0.0040 | |
| PGM-2 | 5 | 40 | 0.0150 | 0.0183 | | | |
| PGM-2 | 6 | 41 | 0.0021 | 0.0026 | | | |
| TPI-1 | 1 | 56 | 0.9604 | 0.9639 | 0.8908 | 0.9961 | 1.0000 |
| TPI-1 | 2 | 59 | 0.0261 | 0.0313 | 0.0034 | | |
| TPI-1 | 3 | 51 | 0.0135 | 0.0047 | 0.1058 | 0.0039 | |
| TPI-2 | 1 | 56 | 0.9997 | 0.9996 | 1.0000 | 1.0000 | 1.0000 |
| TPI-2 | 2 | N | 0.0003 | 0.0004 | | | |
| UGPP-1 | 1 | 54 | 0.8501 | 0.8207 | 0.9899 | 0.9767 | 1.0000 |
| UGPP-1 | 2 | 64 | 0.1439 | 0.1720 | 0.0101 | 0.0233 | |
| UGPP-1 | 3 | 51 | 0.0060 | 0.0073 | | | |
| UGPP-2 | 1 | 54 | 0.9770 | 0.9723 | 0.9966 | 1.0000 | 1.0000 |
| UGPP-2 | 2 | N | 0.0045 | 0.0051 | 0.0034 | | |
| UGPP-2 | 3 | 52 | 0.0093 | 0.0113 | | | |
| UGPP-2 | 4 | 55 | 0.0087 | 0.0106 | | | |
| UGPP-2 | 5 | 48 | 0.0006 | 0.0007 | | | |

**Note:** The Bernal Hill population of *E. glaucus* ssp. *virescens* is not included among the Bay Area populations. Migration distances are actual distances (mm) from the origin at which the enzyme band produced by this allele was observed, under the electrophoretic conditions used in this study. Alleles were numbered in the order in which they were observed, not in the order of migration speed or frequency. N, null allele.

**Table 2.** Summary of genetic variability in *Elymus glaucus* populations.

| | *N* | *P* (%) | *A* | $A_e$ | $A_p$ | $H_o$ | $H_e$ | *F* |
|---|---|---|---|---|---|---|---|---|
| **Species level** | | | | | | | | |
| All populations | 25 | 77 | 2.96 | 1.21 | 3.60 | 0.0006 | 0.1269 | 0.995 |
| Oregon | 24 | 77 | 2.96 | 1.22 | 3.60 | 0.0007 | 0.1262 | 0.994 |
| Bay Area | 43 | 46 | 1.77 | 1.12 | 2.67 | 0.0005 | 0.1158 | 0.996 |
| Sierra Nevada | 27 | 58 | 2.00 | 1.16 | 2.73 | 0.0000 | 0.1056 | 1.000 |
| **Population level** | | | | | | | | |
| Entire study | | | | | | | | |
| Mean | 25 | 28 | 1.37 | 1.13 | 2.33 | 0.0007 | 0.0774 | 0.99 |
| Maximum | 58 | 54 | 1.73 | 1.27 | 3.00 | 0.0055 | 0.1467 | 1.00 |
| Minimum | 7 | 8 | 1.07 | 1.01 | 2.00 | 0.0000 | 0.0058 | 0.88 |
| Oregon | | | | | | | | |
| Mean | 24 | 28 | 1.37 | 1.14 | 2.35 | 0.0007 | 0.0804 | 0.98 |
| Maximum | 31 | 54 | 1.73 | 1.27 | 3.00 | 0.0055 | 0.1467 | 1.00 |
| Minimum | 7 | 8 | 1.07 | 1.01 | 2.00 | 0.0000 | 0.0058 | 0.88 |
| Bay Area | | | | | | | | |
| Mean | 43 | 22 | 1.26 | 1.06 | 2.17 | 0.0013 | 0.0400 | 0.96 |
| Maximum | 58 | 38 | 1.50 | 1.12 | 2.50 | 0.0045 | 0.0771 | 1.00 |
| Minimum | 20 | 12 | 1.11 | 1.01 | 2.00 | 0.0000 | 0.0138 | 0.89 |
| Sierra Nevada | | | | | | | | |
| Mean | 27 | 29 | 1.35 | 1.11 | 2.27 | 0.0000 | 0.0673 | 1.00 |
| Maximum | 30 | 38 | 1.46 | 1.18 | 2.67 | 0.0000 | 0.0949 | 1.00 |
| Minimum | 17 | 23 | 1.26 | 1.05 | 2.00 | 0.0000 | 0.0427 | 1.00 |

**Note:** *N*, mean number of individuals sampled per locus, per population; *P*, percentage of all loci that are polymorphic; *A*, average number of alleles at all loci; $A_e$, effective number of alleles at all loci; $A_p$, number of alleles at the average polymorphic locus; $H_o$, observed frequency of heterozygotes; $H_e$, frequency of heterozygotes expected under Hardy–Weinberg equilibrium conditions; *F*, fixation index ($F = (H_e - H_o)/H_e$). All values of *F* deviate significantly from the expected 0.5).

tions from contrasting serpentine and nonserpentine substrate had somewhat larger genetic distances than average for paired populations, but the difference was not statistically significant.

A dendrogram based on genetic distances between populations revealed two large groups, plus one very divergent population (Fig. 2). The only population of *E. glaucus* ssp. *virescens* included in the study was genetically distinct from

**Table 3.** Genetic differentiation among populations and genetic groups (Fig. 2) of *Elymus glaucus*.

| | $F_{ST}$ | *Nm* |
|---|---|---|
| All populations | 0.4010 | 0.3735 |
| Oregon populations | 0.3734 | 0.4195 |
| Bay Area | 0.5081 | 0.2420 |
| Bay area excluding Bernal Hill | 0.4873 | 0.2630 |
| Sierra Nevada | 0.3758 | 0.4152 |
| Genetic group 1 (all populations) | 0.2607 | 0.7091 |
| Genetic group 1 (Oregon only) | 0.2607 | 0.7091 |
| Genetic group 2 (all populations) | 0.3418 | 0.4813 |
| Genetic group 2 (Oregon only) | 0.3062 | 0.5666 |

**Table 4.** Distribution of the 504 multilocus genotypes among 133 populations (for the 3151 individuals of *Elymus glaucus* for which complete 26-locus genotypes were available).

| | Mean | SD | Maximum | Minimum |
|---|---|---|---|---|
| **Genotypes/population** | | | | |
| Species level | 10.92 | 3.48 | 22 | 3 |
| Oregon | 11.19 | 3.51 | 22 | 3 |
| Sierra Nevada | 9.54 | 2.42 | 14 | 7 |
| Bay Area | 8.54 | 3.05 | 13 | 4 |
| **Dominance of a single genotype (%)** | | | | |
| Species level | 34 | 17 | 92 | 8 |
| Oregon | 32 | 16 | 92 | 8 |
| Sierra Nevada | 45 | 17 | 72 | 18 |
| Bay Area | 55 | 23 | 86 | 28 |

**Note:** Dominance of a genotype within a population is the percent of sampled individuals with the genotype that is most common within the population.

the remaining populations (Fig. 2). This population has unusual allele frequencies at six loci (DIA-1, DIA-2, GOT-4, PGI-3, PGI-4, and PGM-1), although no unique alleles were detected (Table 1). The remaining 132 populations were divided into two genetic groups (Fig. 2). These groups were stable in various UPGMA-based dendrograms; as Bay Area and Sierra populations were included or removed from the analysis, only 4 (3%) of the populations shifted between positions near the base of group 1 and divergent positions within group 2. The same two genetic groups were also detected in a dendrogram produced with a neighbor-joining algorithm (not shown). Genetic and geographic distances were not correlated within either of the two genetic groups (Table 5).

When various aspects of habitat, range, and morphology were mapped onto a dendrogram based on Nei's genetic distances (Fig. 2), the most striking result was a lack of pattern. Populations from serpentine (vs. nonserpentine) substrates, from moist (vs. dry) habitats, or with pubescent (vs. glabrous) foliage did not cluster together on the dendrogram. In general, populations from the same geographic area did not cluster together, whether at a large scale (Oregon, Bay Area, and Sierra Nevada populations), moderate scale (coastal Oregon, Siskiyous, interior Oregon valley, western Cascades, or high Cascades, in any combination; Franklin and Dyrness 1969), or local scale (Douglas-fir seed collection zones; USDA Forest Service 1973) (data not shown). However, at the regional scale, six of the Sierra Nevada populations formed 75% of a single cluster in group 2 (Fig. 2).

**Table 5.** Results of Mantel tests of the hypothesis that geographic and genetic distance between populations of *Elymus glaucus* are correlated.

| Groups tested | Correlation (*r*) | *t* test | *p* |
|---|---|---|---|
| All populations | 0.13043 | 2.575 | >0.99 |
| All Oregon populations | 0.07938 | 2.465 | >0.99 |
| **Genetic group 1** | | | |
| All populations | 0.33852 | 4.143 | 1.000 |
| Oregon populations | 0.19428 | 4.365 | 1.000 |
| **Genetic group 2** | | | |
| All populations | 0.32782 | 4.174 | 1.000 |
| Oregon populations | 0.03450 | 0.389 | >0.65 |

Distribution of various characteristics among the two genetic groups identified in the dendrogram was examined in detail for Oregon populations. For 39 (95%) of the 41 paired populations, both members of the pair were in the same genetic group. The distribution of most traits examined did not differ significantly between the two groups, but two (elevation and foliage pubescence) showed statistically significant differences (Table 6). Group 2 included 43 populations, most collected at high altitudes, in wet sites, or near the coast. Group 2 contained a higher proportion of populations from above 1500 m elevation than group 1 (37 vs. 1%), and a lower proportion of populations with pubescent foliage (3 vs. 97%). However, 66% of the 89 populations in group 1 had glabrous foliage, and the populations with pubescent foliage did not cluster together on the basis of isozyme frequencies (Fig. 2). The group 1 populations were less genetically differentiated from one another than were the group 2 populations (Table 3).

Distribution of the Sierra Nevada and Bay Area populations among genetic groups paralleled that of the Oregon populations. Most (82% of 11) Sierra Nevada populations were in group 2, and most Bay Area populations (collected at lower elevations) were in group 1.

## Discussion

### Genetic interpretation of band patterns

Researchers have used several approaches to analyzing population genetics of polyploid plants. Polyploids often exhibit isozyme band patterns so complex that they defy genetic interpretation. Such patterns may be treated statistically as phenotypes (Chung et al. 1991; Strefeler et al. 1996). A genetic interpretation of isozyme band pattern data is preferred over a phenotypic analysis, because a genetic analysis provides more precise information about genetic variation (Gottlieb 1977) and because results can be compared directly with compilations of plant isozyme genetics (e.g., Hamrick and Godt 1990). However, statistical analysis of isozyme phenotypes usefully summarizes isozyme data when the complexity of isozyme band patterns in polyploids makes genetic analysis impossible.

Ideally, the loci used to describe genetic variation are a random sample of the taxon's loci. In practice, that usually means that all enzymes that can be detected easily on two or three buffer systems and scored consistently are used.

**Fig. 2.** UPGMA dendrogram derived from Nei's genetic distance values, showing relationships of 133 populations of *Elymus glaucus* based on isozyme data. The definitions of the symbols in the columns to the right of the dendrogram are as follows. Column 1 (region): –, Oregon; B, Bay Area; S, Sierra Nevada. Column 2 (variety): –, ssp. *glaucus*; GV, mixed ssp. *glaucus* and ssp. *virescens*; J, ssp. *jepsonii*; V, ssp. *virescens*. Column 3 (habitat): –, upland; 0, unspecified; P, serpentine substrate; W, moist. Column 4 (elevation): –, low (below 1500 m); H, high (above 1500 m).

**Table 6.** Comparison of habitat and morphology characteristics between Oregon populations of genetic groups 1 and 2 of *E. glaucus* (Fig. 2).

| Characteristics of populations | $\chi^2$ | *P* |
|---|---|---|
| Pubescent vs. glabrous leaves | 12.08 | <0.01 |
| Wet vs. dry habitats | 8.85 | —[a] |
| Serpentine vs. nonserpentine soils | 2.28 | — |
| Geography | | |
| Coast and Siskiyous vs. Cascades | 1.46 | >0.1 |
| Coast vs. inland | 2.20 | >0.1 |
| Coast vs. Siskiyous vs. south Cascades vs. north Cascades vs. interior valleys | 4.60 | >0.1 |
| Coast vs. Siskiyous vs. south Cascades vs. north Cascades (omit valleys) | 5.11 | — |
| Coast and Siskiyous vs. interior valleys vs. Cascades | 1.46 | — |
| West of Interstate 5 (I-5) vs. east of I-5 (north) vs. east of I-5 (south) | 4.60 | >0.1 |
| Klamath region and western Oregon interior valleys vs. Western and High Cascades | 12.0 | — |
| Klamath region vs. Western Cascades vs. High Cascades (omit valleys) | 6.33 | >0.1 |
| Elevation; >1500 m vs. <1500 m | 53.03 | <0.001 |

[a]$\chi^2$ test invalid because at least one expected frequency was less than five.

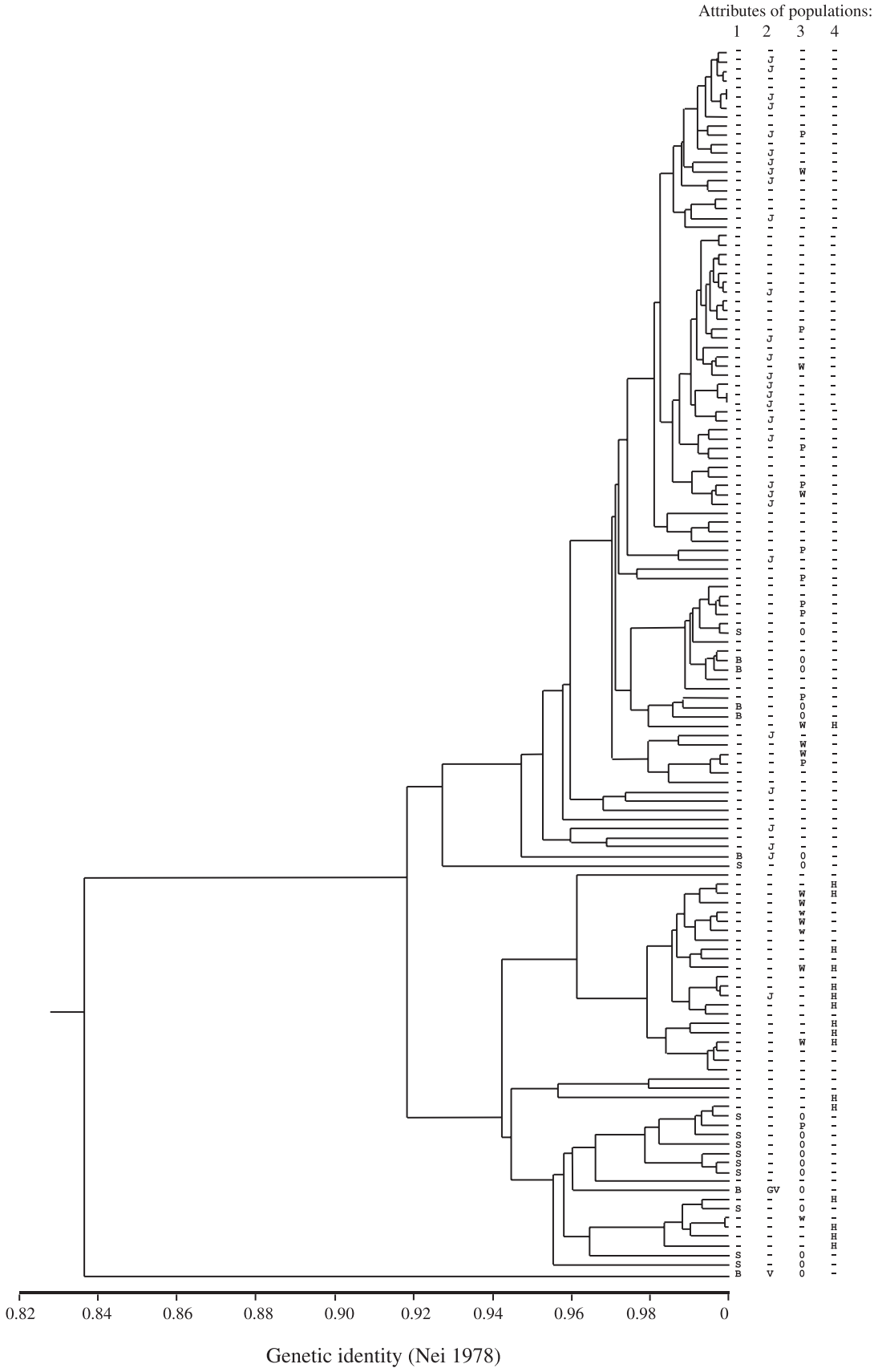
**Table 7.** Three ways to score band patterns of a set of homoeologous loci for a dimeric enzyme (such as TPI) in a known allotetraploid.

| Phenotype | Observed pattern | (1) Always score both homeologous loci | (2) Score both homeologous loci only if necessary | (3) Treat as diploid |
|---|---|---|---|---|
| Single band in every individual | All I | AA AA | AA | AA |
| Three bands in every individual | All I I I | AA BB | AA BB | AB |
| Most individuals with single band, some with three bands | Most I | Most AA AA | Most AA | Most AA |
| | Some I I I | Some AA AB[a] | Some AB | Some AB |
| | | Few AA BB[a] | | |

[a]AA AB genotype distinguished from AA BB genotypes by band intensity.

Omitting those loci too complex to interpret from a genetic analysis may produce a biased sample, because variable loci are more complex than invariant loci. A genetic analysis based on a sample biased toward invariant loci may be less useful than a phenotypic analysis, in some cases.

Once the decision to provide genetic analysis is made, further decisions are required about which loci to include, treatment of homeologous loci, and the distribution of alleles among homeologous loci. Studies of population genetics in polyploids usually employ one of three approaches to determining the number of loci scored for each enzyme (Table 7). (*i*) Assume that a tetraploid has two homoeologous loci for each enzyme and score both of them for every enzyme (Knapp and Rice 1996). This method maximizes the number of monomorphic loci and minimizes the number of alleles per locus. Although this is a logical inference from polyploidy, this approach is rarely used. (*ii*) Assume each enzyme is produced by one locus unless hypothesizing a second locus is necessary because of fixed heterozygosity or individuals with three or more alleles per apparent locus (Perez de la Vega 1994, Sanders and Hamrick 1980; Schierenbeck et al. 1995). This method may be chosen, because it does not require the inference of "invisible" loci (Nevo et al. 1982) and because it may be the most common way of scoring isozymes in polyploids. It is used "by default" when the chromosome number is unknown. It is the most parsimonious approach for diploidized ancient tetraploids that exhibit gene duplications (Inoue and Kawahara 1990). (*iii*) Treat the plants as if they were diploid and score each set of homeologous loci as one locus. This approach is commonly employed for autopolyploid taxa exhibiting multiple chromosome numbers and polysomic inheritance (Bayer 1989; Cai et al. 1990; Ehrendorfer et al. 1996; Hamrick and Allard 1972; McArthur et al. 1986). A variation on this approach is to score the plants as polyploids but report some gene diversity statistics per set of homeologous loci, rather than per locus (Garcia et al. 1989). However, treating autopolyploids as diploid becomes problematic if some individuals are heterozygous for three or four alleles at a set of homeologous loci. This approach may be chosen for taxa exhibiting fixed heterozygosity, because it allows comparisons between populations and taxa with



different chromosome numbers or in cases where fixed heterozygosity is due to agamospermy (Roy 1995). When plants exhibiting fixed heterozygosity are scored as if they were diploid, the number of polymorphic loci is artificially inflated, because the plants are all reported as heterozygous at one locus, when in fact they are all homozygous at two loci (in sexually reproducing plants). Authors who have done this with known polyploids have recognized this distortion and accepted it as necessary to allow statistical comparisons. The problem is greater when the distortion is unrecognized and a biological explanation other than polyploidy or gene duplication is sought for a statistical anomaly that is misunderstood as excess heterozygosity. Although most articles providing statistical analysis of isozyme genetic diversity in polyploids use one of these three approaches, other methods have been used. One approach has been to remove from the data entire sets of homeologous loci exhibiting fixed heterozygosity (Peterson et al. 1993). A particularly interesting approach with a nearly monomorphic allotetraploid was to partition the polyploid's genotype into two genomes and compare each genome independently with genetic diversity of the ancestral diploids (Sun 1996).

Following a previous study of *E. glaucus* isozymes (Knapp and Rice 1996), we chose to score each set of homeologous loci as two loci (Table 1). Three factors allowed consistent genetic interpretation of the band patterns observed in *E. glaucus*. First, *E. glaucus* is a tetraploid. Assigning variation to loci is easiest in low polyploids, both because band patterns are simpler than in higher polyploids and because variation in band intensity can be used to assess allele number. Dosage regulation of gene expression often acts jointly on all alleles of in a set of homeologous loci, and bands produced by a single allele may be too faint to be observed in a higher polyploid. For example, in a plant with genotype AA AA AB, the BB homodimers and perhaps even AB heterodimers may not show on a gel. In a tetraploid, bands produced by a heterozygote are usually visible or can be inferred from the observation of the heterodimer. Second, *E. glaucus* is self-fertilizing, and therefore, nearly all individuals are homozygous. Finally, *E. glaucus* is an allopolyploid. Therefore, inheritance is not polysomic, and the polyploid can be treated statistically as a diploid.

Although *E. glaucus* is an unusually easy polyploid for which to provide genetic interpretations of isozyme band patterns, assigning alleles to loci required some arbitrary decisions. First, we scored a single band as the product of two enzymes with identical mobility (genotype AA AA), produced at two homeologous loci. If one locus were silenced, the plant should theoretically receive the score AA 00 for this single band, but this possible error would not effect summary statistics. The more serious problem involves variable enzymes. Assigning alleles to loci is sometimes arbitrary. If most individuals are fixed heterozygotes with bands at positions A and B, while a few individuals have bands only at positions A and C, we can reasonably infer that the genotypes for the first are AA BB, and the genotypes for the second are AA CC. The problem is greater if most individuals have a single band at position A, a few have bands at positions A and D, and a few have bands at positions A and E. Are alleles producing bands D and E at the same locus or different loci? In outcrossing plants, progeny studies or band intensities in individuals with bands at all three positions might clarify the situation; however, in this self-pollinating species, nearly all individuals were homozygous, and the question could not be answered readily. Our allele assignments followed a previous study (Knapp and Rice 1996) when possible. When evidence was lacking, we arbitrarily assigned all variants to one of the two loci in a homeologous set. We did this because we assumed that one of a pair of homeologous loci must produce a functional allele, but the other need not, and therefore, mutations might be selected against in one locus but not in the other. Our inevitable errors would cause us to underestimate the percent polymorphic loci and would also affect expected heterozygosity. We felt the distortions in summary statistics caused by misassigning alleles to loci were acceptable, because the only alternative was to perform an entirely phenotypic analysis of these band patterns.

**Genetic variation**

Isozyme variation in *E. glaucus* combined some of the traits expected for both selfing and outcrossing plant taxa (Hamrick and Godt 1990). As expected from an outcrossing species, measures of genetic variability were high (Table 1). This great variability was similar to that reported previously for *E. glaucus* (Knapp and Rice 1996) and much higher than seen in most self-pollinating plants. It was in keeping with that reported for outcrossing species (Hamrick and Godt 1990). Grasses are more variable than average plants, but *E. glaucus* isozymes were more variable than the average grass, and its percent polymorphic loci and alleles per locus are even higher than the average for outcrossing grasses (Godt and Hamrick 1998). As expected of an outcrossing species, *E. glaucus* had a large number of multilocus genotypes per population and very few populations had unique alleles. In addition, natural interspecific hybrids of *E. glaucus* occur frequently in the wild (Stebbins et al. 1946).

In some ways, *E. glaucus* isozyme diversity was typical of selfing plants. Populations were highly differentiated genetically. In general, self-pollinating plants have 51% of their isozyme variation among populations; the figure is 41% for selfing grasses (Godt and Hamrick 1998). For *E. glaucus*, this figure was 42% (this study) or 54% in a study that covered a larger area but included fewer populations (Knapp and Rice 1996). As expected for a selfing plant, average variation observed within *E. glaucus* populations in this study was low. In fact, it was substantially lower than the average for previous studies of selfing grasses (Godt and Hamrick 1998). Most individuals in self-pollinating species tend to be homozygous; 98.5% of the *E. glaucus* individuals in this study were completely homozygous, as were 99.9% of the 766 individuals in a previous study (Knapp and Rice 1996).

The generalization that self-pollinating plants have low genetic variation at the species level is rarely true in common grasses sampled in their native ranges (Table 8). Even the apomictic *Pennisetum polystachion* (L.) Schult., and *Pennisetum subangustum* (Schumach.) Stapf & C. E. Hubb. were highly variable, with no significant differences among diploid, tetraploid, and hexaploid populations (Schmelzer and Renno 1997).

Why are the self-pollinating grasses in subfamily Pooidinae so much more variable at the species level than

**Table 8.** Isozyme diversity in self-pollinating grasses of subfamily Pooidinae studied in their native range.

| Species | Location | Chromosomes[a] | No. of populations | No. of individuals[b] | No. of enzymes[c] | Loci | *P* (%) | *A* | $A_p$ | $H_e$ | Homozygotes (%) | $F_{ST}$ | Loci counted[d] | Age[e] | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genetic interpretation provided** | | | | | | | | | | | | | | | |
| *Elymus canadensis* L. | Central N Am | 4*X*, allo | 63 | | 3 | 14 | 29 | 1.29 | | | | | 2 | P | Sanders et al. 1979 |
| *Elymus glaucus* | West coast N Am | 4*X*, allo | 20 | 766 | 8 | 20 | 80 | 3.3 | 3.9 | 0.194 | 99.9 | 0.549 | 1 | P | Knapp and Rice 1996 |
| *Elymus glaucus* | West coast N Am | 4*X*, allo | 133 | 3342 | 11 | 26 | 77 | 2.96 | 3.60 | 0.127 | 98.5 | 0.401 | 1 | P | This study |
| *Hordeum spontaneum* K. Koch | Israel | 2*X* | 28 | 1179 | 15 | 28 | 89 | 3.75 | | | | 0.97 | 0 | A | Nevo et al. 1979 |
| *Triticum dicoccoides* | Israel | 4*X*, allo | 12 | 457 | 18 | 50 | 68 | 2.8 | | | | | 2 | A | Nevo et al. 1982 |
| **Phenotypic interpretation provided**[f] | | | | | | | | | | | | | | | |
| *Avena barbata* Pott ex Link | SW Spain | 4*X*, allo | 42 | 4011 | 10 | 15 | 87 | 3.53 | 3.92 | | 99.8 | | 4 | A | Garcia et al. 1989 |
| *Avena barbata* Pott ex Link | Israel | 4*X*, allo | 35 | | 7 | 9 | 100 | | | | 99.95 | High | 6 | A | Kahler et al. 1980 |
| *Avena sativa* L. | Spain | 6*X*, allo | 267 | 3355 | 8 | 9 | 78 | 3.67 | 4.43 | | | | 5 | A | Pérez de la Vega et al. 1994 |
| *Pucinellia nuttaliana* (Schult.) Hitchc. | NW N Am. | 4*X*, 6*X*, 8*X* | 32 | 370 | 11 | 17 | 94 | 3.00 | | | | | 3 | | Davis and Manos 1991 |

**Note:** Statistics are reported at the species level (i.e., for the entire study). Homozygotes is the percentage of sampled individuals that were homozygous at all loci. *P*, percent polymorphic loci; *A*, mean number of alleles per locus; $A_p$, mean number of alleles per polymorphic locus; $H_e$, expected heterozygosity; $F_{ST}$, measure of differentiation among populations; values above 0.25 indicate very great differentiation (Hartl and Clark 1997).

[a]Chromosomes are given as diploid (2*X*), tetraploid (4*X*), etc. allo, allopolyploid.

[b]Number of individuals in the entire study.

[c]Number of enzyme systems.

[d]What loci were included: (0) taxon is diploid, all loci included once; (1) each area of activity on the gel was considered to involve two loci, even if only one band was observed; (2) an area of activity considered two loci if alternate alleles observed, but single bands were interpreted as a single locus; (3) polyploid, but treat each region of activity on the gel treated as one locus; (4) score as polyploid but report some statistics per pair of homoeologous loci; (5) genotypes inferred but genotypes that can't be distinguished are lumped; (6) statistical analysis of phenotype.

[e]A, annual; P, perennial.

[f]Variation in no. of loci, *P*, *A*, and $A_p$ scored as patterns per putative set of homeologous loci. The *Avena sativa* samples were accessions of Spanish land races.

the average self-pollinating plant? Both biology and sampling artifacts may be involved. First, many plants have circumvented barriers to self-compatibility by polyploidy. The average of reported diversity value is depressed by those recent polyploids that have little variation except that present in the one or few individuals which were their ancestors. Second, several of the grasses in Table 8 are members of polyploid complexes in which partially fertile hybrids form among different taxa and among plants with different chromosome numbers. Grasses such as these exchange genes fairly freely, even among ploidy levels, and tend to be variable (Keeler 1998). Third, isozyme studies cannot reveal the great genetic diversity of these selfing species unless they include many populations, because each individual population has low variation. Such extensive isozyme sampling is expensive and, therefore, rare. Fourth, the economically important plants studied in extensive, expensive detail include many economically important weeds. These plants are often self-fertilizing, but many of these plants have had their isozyme diversity reduced by population bottlenecks associated with long-distance dispersal (e.g., Garcia et al. 1989; Novak et al. 1991). Finally, grasses that are considered selfers may outcross to some extent (Kon and Blacklow 1990; Lönn 1993; Sanders and Hamrick 1980; Till-Bottraud et al. 1992), and even the apomictic *Pennisetum polystachion* (L.) Schult., and *P. subangustum* reproduce sexually to a limited extent (Schmelzer and Renno 1997).

**Isozymes, geography, and habitat**

Referring to morphological variation in *E. glaucus*, Snyder (1950, pp. 629–630) wrote, "Marked variability is found between the strains from a given locality as well as between strains from different localities. Similarities between types for one or more characters appear to occur at random …. not only is there an absence of a broad pattern of variation but it is also impossible to group the strains on the basis of broad similarities in floral and vegetative characters." Nearly the same comments could be made about *E. glaucus* isozyme variation (Knapp and Rice 1996; this study).

The geographic and habitat variables examined here had little value for predicting genetic distances among *E. glaucus* populations. On average, genetic distances between populations growing in close proximity (within 5 km) were smaller than those between geographically distant populations, but some pairs located within 5 km were genetically divergent. Distant populations might be similar or not. By and large, populations collected in wet or serpentine habitats are not genetically similar to other populations from the same type of habitat. No difference was detected between wetland and nearby upland populations; in fact, these pairs were significantly more similar to each other than the average paired population comparison.

Two main *E. glaucus* genetic groups were found (Fig. 2). Group 2 included nearly all the populations collected at high elevations (over 1500 m), and this difference between group 1 and group 2 was statistically significant. In addition, group 2 included the few coastal populations. Group 1 plants grew in low to moderate elevations, mostly in habitats classified by the collectors as mesic or dry.

Two lines of evidence suggest that the two genetic groups identified may have some biological importance beyond their differences in isozyme allele frequencies. First, most of the voucher specimens collected for Oregon populations can be classified into group 1 or group 2 by their gross morphology. This was not dependent on the elevation from which the voucher was collected. Morphological differences among populations of *E. glaucus* often persist in common garden studies (Snyder 1950; Adams et al. 1999). Second, the genetic groups are associated with elevation, and high-elevation plants have been shown to differ physiologically from low-elevation plants in central California (Snyder 1950). When planted in a common garden in Berkeley, low-elevation plants grew normally; however, the plants collected above 2100 m were small and had delayed flowering, and two of the five populations died.

**Seed transfer**

We concur with Knapp and Rice (1996) that management goals of preserving *E. glaucus* genetic diversity while planting well-adapted seed can best be met by using seeds from several sources in each habitat restoration project. We also agree that using seeds from local sources plays an important role in preserving the great genetic diversity present in *E. glaucus*. Unlike Knapp and Rice (1996), we consider *E. glaucus* seed transfer zones delimited by isozyme variation to be so small as to be impractical from a management perspective. *Elymus glaucus* populations are highly differentiated genetically, but that variation cannot be predicted from the geographic distance between populations, at any scale studied within the California Floristic Province. On average, populations within 5 km of each other were slightly more similar to each other than any two populations picked at random, but even nearby populations were sometimes very genetically different. Important genetic differentiation has been observed between populations growing within 200 m of each other (Knapp and Rice 1996).

The source for *E. glaucus* to be used in a habitat restoration project should be chosen not on the basis of isozyme frequencies or geographic proximity but on the practical basis of adaptation. Will the seeds grow where planted? If so (at least within the California Floristic Province), it might be used. Of course, seeds produced in a habitat similar to that of the restoration project are the most likely to grow. Adaptation could be tested in common gardens, but if the project schedule does not allow for that, seeds from many sources could be used. Seeds from some source populations might die, but others would grow.

The fact that *E. glaucus* is self-pollinating eliminates some genetic concerns often expressed about seed transfer, for two reasons. First, nonlocal seed will not break up coadapted gene complexes in the local plants, because most local plants will set seed by selfing, no matter what is planted near them. Second, the entire genome of a homozygous, self-pollinating plant functions somewhat like a gigantic linkage group. Beneficial alleles in different lineages may never come together to produce superior offspring. Therefore, the local plants may not be optimally adapted to the local environment (Rice and Mack 1991) and introducing nonlocal genes may not have a negative impact on adaptation.

**Taxonomic implications**

Isozyme allele frequencies in the one population of *E. glaucus* ssp. *virescens* included in this study was highly divergent from other populations sampled (Fig. 2). This supports the hypothesis that *E. glaucus* ssp. *virescens* is distinct enough to recognize taxonomically. Plant taxa often have geographically coherent ranges; if *E. glaucus* ssp. *virescens* is a taxon, why are plants identified as *E. glaucus* ssp. *virescens* in Colorado, as well as coastal California? One possible explanation involves genetic drift. Although the *E. glaucus* ssp. *virescens* sample was relatively large (45 plants), perhaps the population itself was small and isolated, and the divergent allele frequencies resulted from genetic drift. However, we favor an alternative explanation. Very short (or absent) lemma awns is used to diagnose specimens of *E. glaucus* ssp. *virescens*. However, the condition of having lemma awns short or absent is produced by a single dominant gene in *Elymus lanceolatus* Scribner & J.G. Smith (T. Jones, personal communication). We hypothesize that some coastal populations constitute a distinct taxon, *E. glaucus* ssp. *virescens*, that differs from *E. glaucus* ssp. *glaucus* in several traits including awn length. We hypothesize that inland reports of this taxon result from the rare occurrence of a gene suppressing long awn formation in *E. glaucus* ssp. *glaucus*. The inclusion of a single *E. glaucus* ssp. *virescens* population in this study is an inadequate test of these hypotheses.

*Elymus glaucus* ssp. *jepsonii* differs from the typical variety, because *E. glaucus* ssp. *jepsonii* has pubescent foliage (Barkworth 1993). Other characteristics, including lemma awn length, appear uncorrelated with leaf pubescence (Snyder 1950). Pubescent plants collected for this study did not share a distinctive environment. Pubescent plants do not cluster together on the basis of isozymes (Knapp and Rice 1996, Fig. 2). The range of *E. glaucus* ssp. *jepsonii* is entirely included within the range of *E. glaucus* ssp. *glaucus* (M. Barkworth, personal communication). Because glabrous and pubescent individuals of *E. glaucus* share the same range and habitat and are members of the same genetic group (Fig. 2), we see no compelling reason to treat them as belonging to two different taxa. We conclude that *Elymus glaucus* ssp. *jepsonii* is based on a single character. It is merely a pubescent morph of *E. glaucus* ssp. *glaucus*, not worthy of taxonomic recognition.

Although recognizing an intraspecific taxon in *E. glaucus* based on foliage pubescence is untenable, this study suggests that a taxonomic split within *E. glaucus* ssp. *glaucus* may be warranted based on other features. Superficial examination of the approximately 115 voucher specimens for the Oregon populations revealed that plants from genetic groups 1 and 2 generally differ in appearance. Plants of group 2 appeared to have wider leaves and more robust inflorescences with more divergent awns. These differences could result from differences in environment, but that seems unlikely because the populations in each genetic group were collected in diverse habitats. If the two genetic groups prove to be diagnosable on morphological grounds, giving them different names at some taxonomic recognition might be useful, because the two groups may be adapted to somewhat different habitats. This issue deserves further study, but a detailed morphological examination of these specimens goes beyond the scope of this project.

## Conclusions

Although *E. glaucus* is self-fertilizing and highly homozygous, it is genetically variable. Populations are genetically differentiated. Genetic similarity of *E. glaucus* populations cannot be predicted from the habitat or morphological traits examined in this study or from the geographic distance between populations. However, two genetic groups were found: one associated with high elevation, and the other found at low and moderate elevations. The hypothesis that the two genetic clusters identified in this study may form distinct taxa based on morphology should be tested further. The validity of recognizing pubescent plants as *E. glaucus* ssp. *jepsonii* is not supported by isozyme data. To protect *E. glaucus* genetic diversity and maximize the probability that seeds used in a habitat restoration project will grow, the seeds should be collected from several populations, including but perhaps not limited to populations in or near the restoration project.

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