

RESEARCH ARTICLE

Effects of Competition and Temporal Variation on the Evolutionary Potential of Two Native Bunchgrass Species

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Abstract

The capacity of restored plant populations to adapt to new environmental challenges depends on within-population genetic variation. We examined how much genetic and environmentally based variation for fitness-associated traits exists within populations of two native grasses commonly used for restoration in California. We were also interested in understanding how phenotypic expression of genetic variation for these traits varies with growth environment. Thirty maternal families of *Elymus glaucus* (Blue wild rye) and *Nassella pulchra* (Purple needlegrass) were sampled from both coastal and interior populations and reciprocally transplanted into three replicated common gardens with and without interspecific competition at each site. Reproductive output of families differed both among years and with competition treatments. Phenotypic expression of genetic variation in culm

production differed among populations and was very low when families were grown with interspecific competition. Without interspecific competition, the degree of genetic determination peaked in year two in both species (8.4 and 15.1% in *E. glaucus* and *N. pulchra*, respectively). Significant genetic differences in reproduction and phenotypic plasticity were found among *N. pulchra* subpopulations sampled less than 3 km apart, further highlighting the importance of thoroughly sampling available genetic variation in populations used for restoration. The variable and generally low expression of genetic variation indicates that rates of adaptation in restored populations of these native grasses may vary temporally and may be especially slow within competitive environments.

Key words: *Elymus glaucus*, genetic variation, *Nassella pulchra*, phenotypic plasticity, selection response.

Introduction

Much has been written about choosing and assembling populations used for restoration (Fenster & Dudash 1994; Knapp & Rice 1994; Linhart 1995; Knapp & Dyer 1997; Lesica & Allendorf 1999; Hufford & Mazer 2003; McKay et al. 2005). Restoration success may depend on planting local genotypes because reciprocal transplant studies have shown that plant populations are frequently adapted to local environmental conditions (Antonovics & Primack 1982; Bradshaw 1984; Van Tienderen & Van der Toorn 1991; Linhart & Grant 1996; Montalvo & Ellstrand 2000; Joshi et al. 2001). Sampling enough individuals so that the population contains sufficient genetic variation may also be critical to restoration success (Bradshaw 1984; Huenneke 1991; Rice & Emery 2003). High levels

of genetic variation potentially enable populations to exhibit greater “ecological amplitude” (Bradshaw 1984) meaning that they may be successful over a wider range of environments.

In choosing or constructing a population for restoration, a balance is needed between adaptation to present environments and preservation of genetic variation for response to future conditions. Unfortunately, locally adapted genotypes are not always readily available in quantities required for restoration projects. Growing locally adapted plant material is a challenge for seed producers because the market is often limited. With low volume, cost of local seed is also typically higher. Regional population mixtures have been suggested as one means of enabling a broader planting of available restoration material, especially in situations where the selection pressures in the environment being restored are uncertain (Fenster & Dudash 1994; Knapp & Dyer 1997; van Andel 1998; Lesica & Allendorf 1999; Rice & Emery 2003). It is assumed that, over time, selection will “fine-tune” the population to be adapted to local conditions (Burton & Burton 2002; Rice & Emery 2003). The extent to which translocated populations evolve at novel sites and the length of time required for this to occur depends on the strength of selection and the presence of genetic variation for traits associated with fitness. If selection is

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weak, poorly adapted genotypes may be removed very slowly from the population. In turn, the presence of maladapted genotypes can negatively impact the restored population's fitness and thus increase the genetic load in the restoration planting.

The amount of genetic variation within natural populations can be estimated by planting progeny of randomly sampled individuals from a population (e.g., families) in a common garden. The observable phenotypic variation in traits of interest among families is a measure of genetic variation. The amount of among-family variation relative to the amount of within-family variation allows predictions to be made about the ease with which the population can change in response to selection. Although not as widely recognized as it should be, the expression of genetic variation can depend critically on the growth environment (Mazer & Schick 1991b; Conner et al. 2003; Etterson 2004). Because of this, common gardens are ideally replicated across relevant environments.

In this study, we used techniques associated with the field of evolutionary ecology to estimate the amount of genetic variation for a component of reproductive fitness (culm production) in two native perennial grass species (*Elymus glaucus* [Blue wild rye] and *Nassella pulchra* [Purple needlegrass]) planted in two locations and under different levels of competition. A primary goal was to make predictions about the ability of translocated populations to evolve in novel environments. Both of these grass species are used extensively for restoration purposes in California and both exhibit local adaptation (Rice & Knapp 2008). *E. glaucus* is self pollinating (Snyder 1950; Knapp & Rice 1996; Wilson et al. 2001), whereas evidence from molecular markers (Knapp & Rice 1998; Larson et al. 2001) and hybridization studies (Love 1954) indicate greater rates of outcrossing in *N. pulchra*.

Methods

Study Sites and Population Sampling

Seeds of *Elymus glaucus* and *Nassella pulchra* were collected at two locations in northern California located approximately 190 km apart. The coastal location at the Bodega Bay Marine Reserve (hereafter, Bodega), Bodega Bay, CA (lat 38°18'N, long 123°03'W) has a climate of cool, foggy summers, and wet winters. Vegetation consisted of a mixture of introduced and native annual and perennial grasses and forbs and native shrubs (Rice & Knapp 2008). The interior site was located at the University of California Sierra Field Research and Extension Center (hereafter, Sierra) (lat 39°24'N, long 121°41'W) in the Sierra Nevada foothills near Browns Valley, CA. This site is colder during the winter and much hotter during the summer than Bodega. Vegetation at Sierra is dominated by introduced annual grasses and some native grasses within a matrix of widely spaced Blue oak (*Quercus douglasii*) trees. Elevation at seed collection locations ranged from 30 to 175 m at Bodega and 250–500 m at Sierra; see Rice and Knapp (2008) for a more complete description of the study sites. A minimum of 30 open-pollinated seeds from each of 30 plants (i.e., maternal families) per species were collected

at each location. *E. glaucus* seeds were collected along a single long transect at both locations (at least 15 m between plants), while *N. pulchra* seeds were obtained from separate subpopulations within 3 km of each other at each location, due to population discontinuities across the landscape. We sampled three *N. pulchra* subpopulations at Sierra and two subpopulations at Bodega.

Experimental Design

Seeds from each family were initially planted into 15-cm-deep and 2.5-cm-wide plastic "conetainers" (Stuewe & Sons, Inc., Corvallis, OR, U.S.A.). After 3 months in the greenhouse, seedlings were transplanted into three replicated common gardens (i.e., blocks) at both the Bodega and Sierra sites. The Bodega plantings were located in an area with similar vegetation and climate to the collection site at an elevation of 20 m, less than 5 km from where seed was collected. Plantings at the Sierra location were adjacent to the seed collection sites, and covered a range of environments, varying in elevation from 250 to 500 m. All common gardens were enclosed in a fence to exclude deer and cattle. Half of the seedlings were planted into a low competition treatment, created with a single application of glyphosate 2 weeks prior to planting, and the other half were planted into plots where interspecific competitors were left in place (high competition treatment). Newly germinating plants emerging from the seed bank in the low competition treatment were periodically removed by hand for the remainder of the experiment. A total of 12 randomly chosen seedlings from each open-pollinated maternal family were planted at each site. Within each of the three blocks at a site, two seedlings from each family were planted into the high competition treatment and two seedlings were planted into the low competition treatment. Families of both populations were randomly arranged within each species-block-treatment combination and plants were planted 30 cm apart to minimize intraspecific competition.

Data Analyses

Reproductive output was estimated in each environment and across competition treatments by counting the number of flowering culms produced in the late spring of 1997–1999. Culm number was averaged over the total number of plants planted and is therefore a composite fitness measure that includes both fecundity and survival. Within each treatment by year combination, distributions of family means approximated normality. A split-split plot mixed model ANOVA (block nested within location and family nested within population) with repeated measures (SAS PROC GLM) was employed to calculate the significance of family nested within population, as well as main and interaction effects on culm production. Blocks and families were considered to be random, whereas location, population, and competition were treated as fixed factors. Analyses of variance were conducted for both untransformed and log transformed data. While transformation lessened the correlation between mean and variance, it also obscured some of the

interactions of scale (Falconer 1981, pages 262–269) that were a focus of our investigation. The significance of family variation and interactions of family by environment were determined using *F*-tests. Consistency in the rank order of family culm production across locations and competition treatments was additionally tested with Spearman’s rank correlation.

To estimate the proportion of genetically and environmentally based variation, variance components were computed (SAS PROC VARCOMP). Phenotypic variance was the sum of all variance components—among-family, within-family, and all among-family by environment interactions. Variance components can sometimes be negative and this is usually attributed to sampling error. To avoid bias, negative variance components were included in calculations of the phenotypic variance. The among-family variance component is an estimate of genetic variation, while the sum of the variance components for all factors in the model provides an estimate of phenotypic variation. An estimate of the degree of genetic determination (proportion of the total variation that is genetically based) is obtained by dividing the among-family variance by the total phenotypic variance. Differences in the degree of genetic determination across environments and years are due to differences in the amount of among-family variation expressed, and/or the total amount of phenotypic variation (i.e., the numerator and denominator in the degree of genetic determination ratio, respectively).

The amount of variation expressed by families as a result of growing in different locations and under different competitive environments was further explored by calculating the absolute value of the residuals of family means for culm number. The residual is the family mean subtracted from the population mean within each location × competition combination. While similar in concept to the among-family variance components, the residuals can be readily analyzed statistically with

Levene’s test (Levene 1960). The absolute values of the residuals for family means were analyzed using a similar statistical design as for culm number. Families and all interactions with families drop out of the model when the residual is considered the dependent variable, thus simplifying the analysis.

Differences in culm production among subpopulations of *N. pulchra* were tested using a one-way ANOVA on family means of plants grown in their home environment. Significance of differences among all pairs of subpopulations was evaluated with Tukey’s test.

Results

Within-population Variation in *Elymus glaucus*

Within populations, the time × family interaction was highly significant ($p = 0.003$), indicating that culm production of families differed across years (Table 1). In 1998, the competition × family interaction was also significant ($p = 0.022$), and the time × competition × family interaction was nearly significant as well ($p = 0.068$). Log transforming the dependent variable eliminated the magnitude of these interactions with the competition treatment, suggesting a scaling effect. Overall culm production was much greater for families grown under low interspecific competition and variation in culm production among families was also much greater under low competition (Fig. 1a).

Because the competition × family interaction was significant in 1998 and the competition × family × time interaction was nearly significant, variance components were calculated by splitting the data set in accordance with the three-way interaction. The degree of genetic determination was much lower in populations under high competition in 1998 and 1999 (Fig. 2). By 1999, degree of genetic determination in populations under high competition was reduced to zero. Under low competition,

Table 1. Analysis of variance of culm number over a 3-year period in *Elymus glaucus* and *Nassella pulchra*.

Source	df	Culms 1997	Culms 1998	Culms 1999	× Time
		<i>F</i> (level of significance)			
<i>Elymus glaucus</i>					
Family (Popn)	58	1.30 (0.251)	1.25 (0.238)	1.81 (0.152)	1.43 (0.003)
Loc × Fam (Popn)	58	0.93 (0.601)	1.08 (0.390)	0.82 (0.771)	0.80 (0.618)
Comp × Fam (Popn)	58	1.18 (0.264)	1.71 (0.022)	0.82 (0.796)	1.22 (0.068)
Loc × Comp × Fam (Popn)	58	0.95 (0.594)	0.75 (0.915)	1.14 (0.229)	1.03 (0.412)
Error	472				
<i>Nassella pulchra</i>					
Family (Popn)	58	1.21 (0.245)	1.19 (0.281)	1.31 (0.170)	1.95 (0.001)
Loc × Fam (Popn)	58	1.05 (0.423)	0.94 (0.586)	1.00 (0.503)	0.96 (0.614)
Comp × Fam (Popn)	58	3.64 (0.001)	2.16 (0.002)	2.78 (0.001)	1.76 (0.001)
Loc × Comp × Fam (Popn)	58	0.62 (0.987)	1.21 (0.145)	0.47 (0.999)	0.96 (0.590)
Error	472				

F-ratios, with significance in parentheses, are calculated according to a mixed model and a repeated measures split–split plot design. Only the family and family interactions (tested with the block × family(popn) interaction, 216 *df*) are shown here; the full model, with population, location, and competition main and interaction effects were published by Rice and Knapp (2008). Significance of interactions with time was tested with the Wilks–Lambda statistic. Factors significant ($p < 0.05$) when the same model was used with log transformed culm number data are shown in bold.

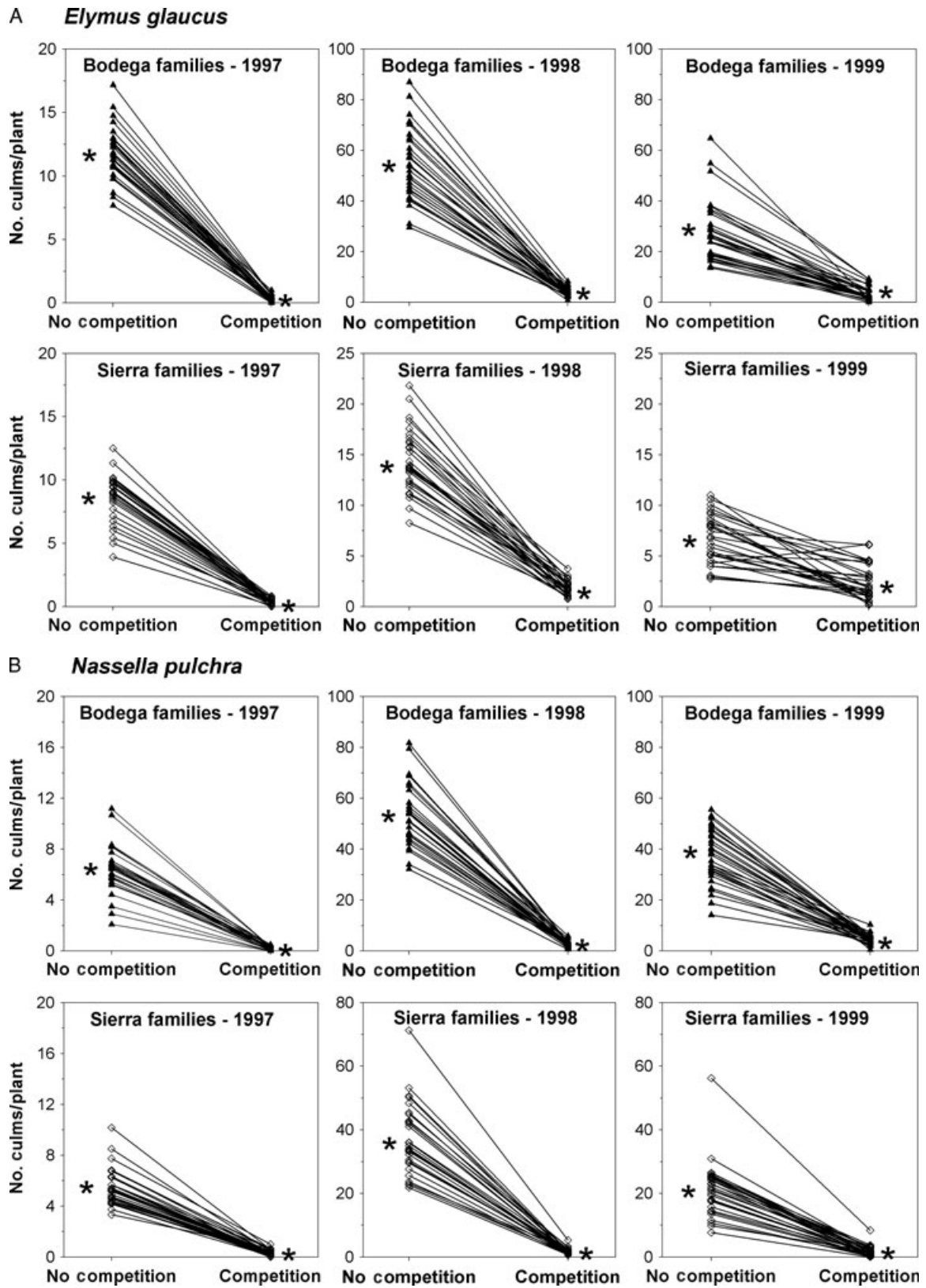


Figure 1. Culm production norms of reaction for (a) *Elymus glaucus* and (b) *Nassella pulchra*, showing 30 Bodega families (top three panels) and 30 Sierra families (bottom three panels), when grown with high- and low-interspecific competition over a 3-year period. Potential amount of genetic variation expressed in each environment is indicated by the range of family mean values. Asterisk (*) indicates average across families in each environment.

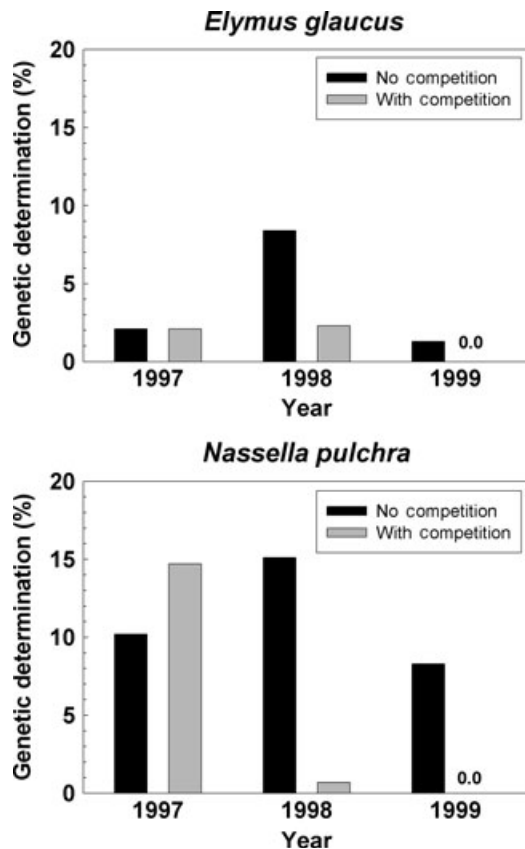


Figure 2. Degree of genetic determination for culm number in (A) *Elymus glaucus* and (B) *Nassella pulchra*, when planted with high and low interspecific competition over a 3-year period.

the degree of genetic determination was highest in 1998 and lowest in 1999.

Levene's test of the absolute value of the residuals indicated that the population \times location \times competition \times time interaction was significant ($p < 0.05$). At the Bodega site, the local Bodega population expressed more variation among families (as shown by the higher residuals) than the Sierra population under both low and high competition treatments (Fig. 3). This difference between populations in the amount of variation expressed was not statistically detectable at the Sierra site. The amount of variation among families was generally much higher under low competition than under high competition, regardless of population and location. While the magnitude of among-family variation under low competition conditions peaked in the second year of the experiment, the magnitude of among-family variation under high competition continued to rise. Plants grown with competitors grew much more slowly and many only began to produce a substantial number of culms near the end of the experiment.

Within-population Variation in *Nassella pulchra*

In the analyses of variance of culm number, both the time \times family and the time \times family \times competition interactions were highly significant (Table 1). All families produced

far more culms under low competition than under high competition. Relative culm production among families differed under the two competition treatments and across the 3 years of the study. Norm of reaction diagrams showed that several of the families producing the most culms under low competition performed poorly under high competition (Fig. 1b), indicating fitness tradeoffs between the environments may exist. Log transforming culm number did not eliminate the family by competition interaction in all years (Table 1), suggesting that crossing interactions exist in addition to the expected scaling interactions.

Variance components were calculated by splitting the data set in accordance with the competition \times family \times time interaction, the highest order interaction that was significant in the analysis of variance. In all but 1997, the degree of genetic determination was much greater under low competition than under high competition (Fig. 2). The degree of genetic determination also decreased from 1997 to 1999. Random mortality of plants over the course of the experiment may have been one cause of the changes in degree of genetic determination. Averaged across families, 10% of the plants in the low competition treatment and 58% of the plants in the high competition treatment died by the 1999 census.

The location \times competition \times time interaction was significant ($p < 0.05$) when analyzed with Levene's test. In contrast to the results for *E. glaucus*, there was not a significant population source effect. The amount of variation among families was much greater under low competition than under high competition at both Bodega and Sierra (Fig. 4). The suppression of variation among families resulting from high competition was especially pronounced at the Bodega site.

Subpopulation Variation in *N. pulchra*

Variation in culm production was also evident at the subpopulation level in *N. pulchra*, with significant differences among the three Sierra subpopulations. Plants from families of the Roadside subpopulation produced significantly fewer culms (average of 10.5) than the Forbes Hill A and Forbes Hill B subpopulations (average of 20.4 and 19.4, respectively) when planted at Sierra ($F = 8.71$, $p = 0.001$; Fig. 5a). The trend of the Sierra Roadside subpopulation producing fewer culms was evident when planted at Bodega as well (Fig. 5a), but the difference was not statistically significant ($F = 1.87$, $p = 0.172$). The Bodega *N. pulchra* families also originated from two separate subpopulations within 3 km of each other; the Community Center subpopulation grew closer to the ocean, at a lower elevation, and on flatter ground than the Bay Hill Road subpopulation. However, mean culm production of the two subpopulations did not differ (Fig. 5b).

Potential differences in genetic variation for phenotypic plasticity were found among subpopulations at each site. This is demonstrated by differences in the strength of the correlation of culm production for individual families within subpopulations across locations (Fig. 5). For the Sierra subpopulations, the Spearman's rank correlation in family culm number in the Roadside subpopulation across locations was significant

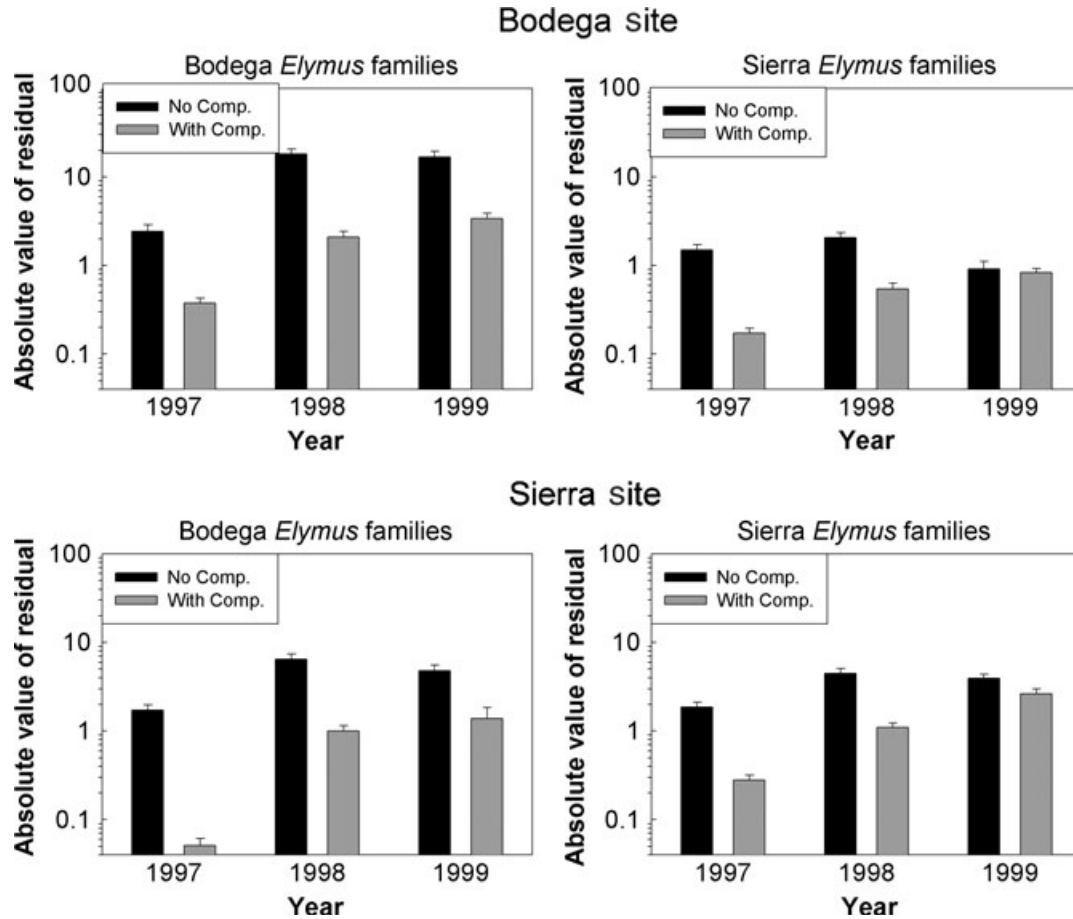


Figure 3. Absolute value of residuals for culm number among Bodega and Sierra *Elymus glaucus* families planted at both locations, under high and low interspecific competition, over a 3-year period. The residuals are an index of the relative amount of genetic variation expressed in the different environments and years. Error bars represent one SE.

($r = 0.703$, $p = 0.035$); rank correlations were not significant for the Forbes Hill A subpopulation ($r = -0.173$, $p = 0.610$) or Forbes Hill B subpopulation ($r = 0.309$, $p = 0.380$). For the Bodega subpopulations, family culm number in the Community Center subpopulation was not significantly correlated across locations ($r = 0.290$, $p = 0.314$) while family culm number in the Bay Hill Road subpopulation was correlated across locations ($r = 0.759$, $p < 0.001$). These estimates of genetic correlations across environments are approximate due to unknown maternal effects and possible downward bias caused by any inflation of family variances due to measurement error and environmental variance (Lynch & Walsh 1998).

Discussion

Genetic Variation and Degree of Genetic Determination

Genetic variation is necessary for natural or restored populations to evolve in new environments, and the rapidity with which evolution can occur is indicated by the degree of genetic determination for a trait. Genetic variation for culm number, measured by the amount of among-family variation, was found

in both species. We should note that using among-family variation as an estimate of genetic variation may overestimate the amount of true genetic variation if maternal environmental effects are important. However, maternal environmental effects typically become reduced over time and are usually not expressed in reproductive output (Roach & Wulff 1987). Further, results from Rice and Knapp (2008) on effects of seed size on phenotypic expression in these species suggest that the importance of maternal effects relative to among-family genetic effects was likely to be quite small. However, because potential effects of maternal environment cannot be completely ruled out, we consider our measures of genetic variation in *Elymus glaucus* to be maximal estimates. In *Nassella pulchra*, uncertainties about the mating system make the relationship among individuals unknown, while polyploidy (Stebbins & Love 1941) further complicates interpretation. As with *E. glaucus*, any effect of maternal environment would tend to cause genetic variation to be overestimated, while outcrossing would tend to reduce estimates of genetic variation. For this reason, estimates of genetic determination for both species should be considered approximate and preliminary. However, even with these uncertainties, within-species comparisons of differences

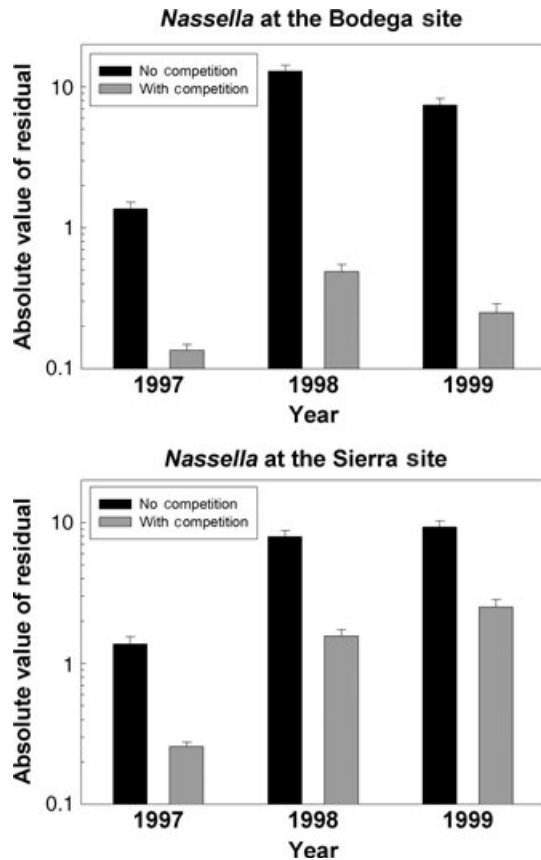


Figure 4. Absolute value of residuals for culm number among *Nassella pulchra* families planted at the Bodega and Sierra sites, under high and low interspecific competition, over a 3-year period. The residuals are an index of the relative amount of genetic variation expressed in the different treatments and years. Error bars represent one SE.

in expression of genetic variation across years and under varying levels of interspecific competition remain valid.

The amount of genetic variation within populations can vary for several reasons. Some populations may simply contain less genetic variation than others, as a function of their evolutionary history. For example, population bottlenecks resulting from founder events can substantially reduce the amount of genetic variation within a population (Lynch & Walsh 1998; Knapp & Connors 1999; Frankham et al. 2002). In addition to demographic processes, other ecological factors can play a role. Populations growing across spatially and/or temporally heterogeneous environments experience variable selection regimes that can lead to the build-up and maintenance of higher levels of genetic variation over time (Via & Lande 1987; Mazer & Schick 1991a; Prati & Schmid 2000). Phenotypic expression of existing genetic variation also can vary with environmental conditions. In this experiment, more genetic variation was expressed when populations were grown under low levels of interspecific competition. Previous studies have demonstrated similar environment-dependent expression of genetic variation at different planting densities where

intraspecific competition was manipulated (Shaw 1986; Mazer & Schick 1991b; Donahue et al. 2000).

Several factors may account for the strong among family by competition interaction we documented with *N. pulchra*, and to a lesser extent with *E. glaucus*, in this study. This interaction may be, in part, a matter of scale. Mean culm production was much greater without than with competition, and the variance often increases as the population mean increases (Falconer 1981). If the relative performance of families remains the same across environments (i.e., no crossing interactions), response to selection is expected to be faster in the environment with greater mean culm production and greater variance among families.

We found that the degree of genetic determination was quite low in both *E. glaucus* and *N. pulchra*, especially with interspecific competition, suggesting that natural selection acting on the phenotype in environments typical of where native grasses are planted will not readily lead to genetic change. The actual change resulting from such selection is likely less than the degree of genetic determination, given that our estimates of genetic variance consist of both additive and dominance components (in addition to any maternal effects), and the dominance component is generally much less readily transmitted to the progeny (Falconer 1981). By the third year of the study, none of the phenotypically expressed variation that remained in either species was genetically based, and response to selection under these conditions would not be expected to occur at all.

Why was the degree of genetic determination so low? One reason is that the California grassland is an extremely patchy matrix of environments that may reduce the efficacy of natural selection. Even when interspecific competition is removed, micro-scale soil moisture differences and mortality factors, such as gopher herbivory, result in a heterogeneous selective environment. The difference between plants that produce many culms and plants that do not may be more a result of these chance environmental factors than genetic superiority. Background environmental variability is even more pronounced with interspecific competition. The interspecific competitors vary spatially in composition and density, presenting very different above ground and below ground environments. A poorly adapted genotype that becomes established by chance in a patch with low levels of competition can produce much more seed and have a greater probability of transferring genes to the next generation than a "favorable" genotype that happens to land in an area with high competition. This capacity for phenotypically plastic fitness responses to favorable microsites has long been argued to be an important factor reducing the effectiveness of selection (Sultan 1987; Miner et al. 2005; Ghalambor et al. 2007). In our study, the degree of genetic determination diminished over time because of increasing mortality (mostly caused by gophers), especially under competitive conditions. Mortality seemed to be more a function of proximity to existing gopher activity rather than genotype.

In the absence of active management to eliminate interspecific competition, the environment for many restoration plantings is likely to be strongly influenced by weedy species. With culm production often an order of magnitude greater for

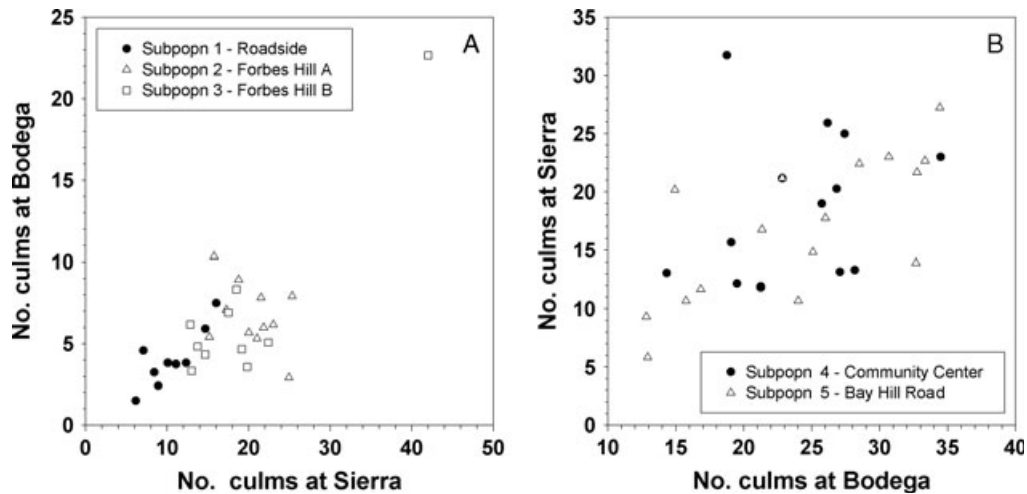


Figure 5. Scatterplots showing culm number of *Nassella pulchra* families from the different subpopulations sampled at the Sierra (A) and Bodega (B) sites, when planted in the subpopulations' home (x-axis) and away environment (y-axis).

Table 2. Spearman's rank correlation coefficients for mean number of culms per family across locations (home vs. away) and treatments (with high vs. low competition) in both *Elymus glaucus* and *Nassella pulchra*.

	Bodega families			Sierra families		
	1997	1998	1999	1997	1998	1999
<i>Elymus glaucus</i>						
Home versus away	0.081	0.675***	0.318	0.359	0.052	0.073
High versus low competition	0.429*	0.518**	0.346	0.415*	0.193	-0.014
<i>Nassella pulchra</i>						
Home versus away	0.485***	0.381*	0.618***	0.504**	0.636***	0.530**
High versus low competition	0.418*	0.286	0.209	0.391*	0.430*	0.579***

*, **, *** = significant at the 0.05, 0.01, and 0.001 levels, respectively.

plants experiencing low competition, a considerable portion of the seed for future generations is likely to come from plants in patches or gaps with reduced competition. If the number of plants growing in these more favorable environments is limited, little opportunity for selection may exist. Another factor that can constrain evolution in such patchy environments is the presence of crossing interactions, whereby genotypes perform differently with and without competition. Several of the families that produced the most seed without competition produced among the fewest seed, relative to the other families, with competition.

The long life and generation time of these native grasses may also substantially slow the adaptive “fine-tuning” of populations planted in new environments. It is thought that individual *N. pulchra* plants can live for 100 years or more (Hamilton et al. 2002). Established stands of these native grasses can resist invasion by exotic grasses (Lulow 2006) and it seems likely that adult stands would similarly suppress the recruitment of native seedlings. Although the generation times of these species have not been estimated, slow seedling recruitment should lengthen the generation time and reduce

the influx of new genetic variation available within the local seed pool.

Differences in Culm Production Among *N. pulchra* Subpopulations

Variability among subpopulations suggests possible population sub-structuring that could have an impact on restoration success. Culm production of plants from the Sierra Roadside subpopulation was significantly lower than culm production of plants from the other two subpopulations collected 3 km (or less) away. This was unexpected given how robust the plants of the Roadside subpopulation appeared at the time of seed collection. Plants from the Roadside subpopulation were growing from cracks on an abandoned road bed, while plants from the other two Sierra subpopulations were growing on more typical grassland substrates. This suggests that the phenotypic robustness of progeny from the Roadside subpopulation did not have a genetic basis and may have been due to reduced competition.

Our data indicate that differences in genetic variation for plasticity may exist; plasticity has been shown to vary within

populations and is a trait itself capable of evolution (Schlichting 1986; Scheiner 1993; Via et al. 1995; Ghalambor 2007). The degree to which values of traits or characters (i.e., character states) are independent across environments suggests the potential for genetic variation in plasticity and can be quantified by assessing the genetic correlation of character states across environments (Via & Lande 1985). Families from the Roadside subpopulation at Sierra showed a significant correlation in culm production across environments, whereas such correlations were not significant for families from the other two Sierra subpopulations. Some families from these latter two subpopulations were very plastic, producing many culms in the home environment and few culms in the away environment; other families exhibited little plasticity and produced similar numbers of culms in both environments. Differences in genetic variation in plasticity, as suggested by differences in the correlation of family performance across locations, were also found between the two Bodega *N. pulchra* subpopulations. Cross-environment genetic correlations differing significantly from zero indicate that character states are not independent and that genetic constraints may reduce the capacity for independent adjustment of mean phenotypes within each environment. This, in turn, would limit the evolution of adaptive plasticity across environments. Theory and experimental work suggests that temporally variable environments may select for increased phenotypic plasticity (Sultan 1987). If future selective regimes are characterized by more temporal variation in environmental conditions, the capacity of restored populations to adapt may depend on the existence of genetic variation in plasticity within populations.

Restoring Evolutionary Potential

Despite evidence for local adaptation in these two species of native grasses (Erickson et al. 2004; Rice & Knapp 2008), non-local populations are often planted because of the limited number of native seed sources generally available. Mixing populations originally collected from different environments is one means of potentially broadening the suitable planting area. Whether translocating individual populations into new environments or planting population mixtures, long-term evolutionary success depends on natural selection being able to adaptively mold the restored population—favoring genotypes suited to the new environment and removing poorly adapted ones. While some populations may respond relatively rapidly to selection (Reznick & Ghalambor 2001; Rice & Emery 2003), results from this study indicate that adaptation in these two native grasses may, in fact, occur quite slowly. Extreme patchiness of the selective environment, crossing interactions, and low rates of seedling recruitment in many native perennial grass species all reduce the capacity of a population to respond to natural selection. Poorly adapted genotypes may therefore persist for a long time, continuing to contribute to the seed pool and reducing the overall population fitness. In sites heavily dominated by exotic species, the persistence of native grass populations is already precarious and even a small reduction in population fitness could mean the difference between the

success and failure of a restoration planting. Our results highlight the importance of planting populations that are at least somewhat adapted to the local site conditions. While population mixtures are still a viable strategy, it is important to consider the breadth of such mixtures. Mixtures that are too broad with respect to genetic sources may impair the long-term persistence of a population if large numbers of seed are poorly adapted to the local site. Recognizing that natural selection may be a slow process, all components should ideally be relatively well adapted at planting. Natural selection need then only “fine-tune” the population to optimize performance in the new environment.

Managing the evolutionary potential of restored plant populations has taken on new urgency given the changing selection pressures associated with global change (Rice & Emery 2003). Climate change certainly represents an important evolutionary challenge for the restoration of plant communities. However, we suspect that novel competitive pressures associated with the global problem of invasive species may be of more immediate concern for the restoration of California grasslands. Aggressive non-native annual vegetation has caused the dominant resource limitation in California grasslands to switch from soil moisture to light, and the season of greatest resource limitation to switch from summer to winter and spring—a major shift in selection pressures (Dyer & Rice 1999). Given the long life span of many perennial bunchgrasses, poor population regeneration, and a potentially slow response to selection, it is possible that genotypes best adapted to deal with this new selective environment have yet to emerge. Results from this study suggest that such genotypes may exist because we found substantial differences among families in culm production under high levels of interspecific competition. Although not statistically significant, one *N. pulchra* family consistently produced over twice the number of culms of any other family.

Uncertainties about future selective environments highlight the importance of genetic variation in populations used for restoration. Genetic variation is necessary for populations to evolve to new environments, whether seeds are moved from one location to another, or when changes in climate or competitive environment occur in situ. Although the proper genetic sampling of populations is necessary to maintain the evolutionary potential of restored populations, it does not guarantee that this genetic variation will be expressed. When considering the evolutionary aspects of restoring native plant populations, it is important to remember that interactions between genotypes and environmental conditions can strongly influence both the expression of genetic variation and the capacity of a restored population to respond to new selective challenges.

Implications for Practice

- Results show that much of the phenotypic variation in these native grass populations is due to environmental variation and not genes. Factors such as small-scale differences in competition, random gopher mortality,

and yearly climatic variation, all can cause natural selection to operate very inefficiently. As a result, poorly adapted (non-local) populations or population mixtures may be slow to adapt to new environments, especially when grown with competition from other species. This highlights the need to select restoration source populations from areas with similar soils and climate, and not depend on natural selection to rapidly eliminate poorly adapted material.

- Significant genetic differences in culm production were found among subpopulations sampled a short distance apart. Thus, it may be beneficial to collect seed from as many subpopulations within the restoration target area as possible, to improve the probability of restoration success in changing and challenging environments.
- Populations may differ in their capacity to respond to variable environments; such plasticity may be especially important when seed sources are planted in different locations or with global climate change at the same location. Populations growing in temporally variable environments are possibly more likely to contain genes for greater plasticity, and collecting seed from such populations may enhance restoration success.

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