

Effects of Competition and Life History Stage on the Expression of Local Adaptation in Two Native Bunchgrasses

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Abstract

Concerns about the use of genetically appropriate material in restoration often focus on questions of local adaptation. Many reciprocal transplant studies have demonstrated local adaptation in native plant species, but very few have examined how interspecific competition affects the expression of adaptive variation. Our study examined regional scales of adaptation between foothill and coastal populations of two California native bunchgrasses (*Elymus glaucus* and *Nassella pulchra*). By combining competitive manipulations with reciprocal transplants, we examined the importance of the vegetation at a site as a selective factor in the process of local adaptation. By monitoring survival and reproduction of reciprocally transplanted populations over the course of 3 years, we also studied the effect of life history stage on the expression of local adaptation. For most of the fitness components we measured, local adaptation was detected

and interspecific competition consistently amplified its expression. Expression of local adaptation was especially apparent in the more inbreeding species *E. glaucus* and suggests that with weaker gene flow, selection may be more effective in creating ecotypes within this species. Local adaptation was detected at all life history stages but was most strongly expressed in traits associated with adult reproduction and the viability of seeds produced by the transplants. Taken together, our results indicate that the importance of local adaptation will become more apparent in the later stages of a restoration project as the plants at a site begin to reproduce and as they experience greater interspecific competition from the maturing vegetation at the site.

Key words: breeding system, competition, *Elymus glaucus*, grassland restoration, local adaptation, *Nassella pulchra*.

Introduction

A major constraint to ecological restoration using native plants is the lack of information on distances that populations can be translocated and successfully grown (Knapp & Rice 1994; Montalvo et al. 1997; Hufford & Mazer 2003; Jones 2003; Gustafson et al. 2004; McKay et al. 2005). Reciprocal transplant and common garden studies have repeatedly shown that plant populations are adapted to local environmental conditions (Bradshaw 1984; Endler 1986; Linhart & Grant 1996; Fenster & Galloway 2000; Montalvo & Ellstrand 2000). Restoration guidelines therefore often specify planting locally adapted genotypes. However, the spatial scale over which plant populations are adapted is not well understood and most certainly varies with species, population, and the degree of environmental heterogeneity (Antonovics 1976; Endler 1986).

The importance of interspecific competition as a selective factor in forming locally adapted populations has

been recognized since the theory of natural selection was proposed (Darwin 1859). It is thus rather remarkable that there have been so few experimental studies on the expression of local adaptation under differing levels of interspecific competition; our review of the plant literature revealed only a very small number of relevant studies (Kindell et al. 1996; Knight & Miller 2004). To examine the relative importance of interspecific competition in the formation of ecotypes in native bunchgrasses, we conducted studies on local adaptation that combined reciprocal transplant approaches with experimental manipulation of the vegetation at our planting sites.

The likelihood of ecotype formation depends not only on the strength of the selective factors such as competition but also on rates of gene flow. The breeding systems of plant species used in restoration are diverse and thus affect rates of gene flow within and among populations. For example, because reduced gene flow is likely for species that are self-pollinating, inbreeding species are expected to exhibit more pronounced local adaptation than cross-pollinating species (Hufford & Mazer 2003; Rice & Emery 2003; McKay et al. 2005). To consider these possible effects of breeding system on formation of ecotypes, we compared two native bunchgrass species used widely for restoration in California: purple needlegrass

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(*Nassella pulchra*) and blue wild rye (*Elymus glaucus*). Molecular genetic data from prior isozyme studies on these grasses (Knapp & Rice 1996, 1998) suggest that *E. glaucus* exhibits higher levels of inbreeding than *N. pulchra*. We therefore predicted that expression of local adaptation should be stronger in this inbreeding bunchgrass compared to the more outcrossing species.

Methods

Study Sites and Population Sampling

Reciprocal transplant studies were conducted at the Bodega Bay Marine Reserve (hereafter, Bodega) at Bodega Bay, California (lat 38°18'N, long 123°03'W) and 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, California. Although average annual rainfall varies between Bodega and Sierra, differences between the locations in other climatic factors are even more pronounced (Table 1). The climate at Bodega consists of cool, foggy summers and moderate, wet winters, whereas the interior Sierra location is colder in the winter and much hotter in the summer. Coastal populations used for seed collections grew on coastal terraces and steep wind-swept hillsides dominated by typical coastal vegetation—annual and perennial grasses and shrubs that include major genera such as *Bromus*, *Danthonia*, *Festuca*, *Baccharis*, and *Lupinus*. Sampled Sierra foothill populations grew within a blue oak (*Quercus douglasii*) woodland community with variably spaced trees and a grassy annual understory typical of this area. We sampled seeds from plants of both species growing across a range of microenvironments within the general area at each site. At least 30 open-pollinated seeds of each species were collected in the spring of 1995 and 1996 from each of the 30 widely spaced plants (i.e., at least 10 m apart), resulting in 30 maternal families being sampled at each site. We also made a bulk collection of seeds of each population at each site, combining an

approximately equal number of seeds from many (>200) plants at a site.

Experimental Design

To evaluate the potential for local adaptation, we reciprocally transplanted populations of both species into common gardens at each location. To cover a range of potential habitats, three replicate common gardens were established at each location. The plantings at the University of California Bodega Marine Laboratory Natural Reserve were located less than 5 km away from where coastal seed was collected. Common gardens at the Sierra location varied in elevation from 250 to 500 m above sea level and were in the immediate vicinity of where the Sierra seeds were collected. All the common gardens were fenced to exclude deer and cattle.

Within each common garden, we used two planting protocols to examine competition and local adaptation at different life history stages. One planting examined adaptation from the seed stage to the 7-month-old seedling stage (hereafter, the seed-to-seedling experiment), and another planting examined adaptation of transplanted seedlings through the following three growing seasons (hereafter, the seedling-to-adult experiment). Seedling mortality in these perennial grasses can be extremely high (Dyer et al. 1996), so a large collection of bulked seeds from each population was used in the seed-to-seedling experiment.

Seed-to-Seedling Experiment. Seeds from the bulked collection for each population were planted at both locations on 31 October 1996 and 1 November 1996, after the first germinating rain of the season. To examine the effects of competition on the expression of local adaptation, a low-competition treatment was created by removing vegetation from half of the plots prior to planting. The remaining plots in each block were left undisturbed as a high-competition treatment. To ensure minimal competition in the low-competition treatment, germinating or resprouting

Table 1. Precipitation averages, temperature extremes, species composition, and availability of PAR at the coastal (Bodega) and foothills (Sierra) planting locations.

	Bodega Location	Sierra Location
Mean annual precipitation (cm) ($\bar{X} \pm \text{SD}$)	135.9 $\pm$ 43.1	97.6 $\pm$ 30.5
January minimum temperature (°C) ($\bar{X} \pm \text{SD}$)	7.3 $\pm$ 2.1	4.8 $\pm$ 2.0
July maximum temperature (°C) ($\bar{X} \pm \text{SD}$)	14.8 $\pm$ 0.5	32.8 $\pm$ 0.9
Species richness (mean number of species per 25-cm ² plot $\pm$ SD)	14.7 $\pm$ 7.8	9.7 $\pm$ 0.6
Percent perennial species ($\bar{X} \pm \text{SD}$)	26.7 $\pm$ 14.2	0.0 $\pm$ 0.0
Percent native species ($\bar{X} \pm \text{SD}$)	35.2 $\pm$ 16.3	10.0 $\pm$ 17.3
Biomass in g/m ² ($\bar{X} \pm \text{SD}$)	490 $\pm$ 193	226 $\pm$ 95
Percent native biomass ($\bar{X} \pm \text{SD}$)	8.1 $\pm$ 6.4	7.1 $\pm$ 12.4
PAR—percent of ambient ($\bar{X} \pm \text{SE}$) in high-competition plots	23.2 $\pm$ 5.7	39.7 $\pm$ 6.4
PAR—percent of ambient ($\bar{X} \pm \text{SE}$) in low-competition plots	94.0 $\pm$ 2.0	88.0 $\pm$ 2.9

Temperature and precipitation data are averaged across the 3 years of the study (1997–1999), whereas species composition and light data were taken during the year that the experiment was established (1997). Measurements of PAR were taken at ground level and above canopy (ambient) between 1100 and 1400 hours during May 1997 at eight locations within each plot.

competitors were periodically removed by hand over the course of the experiment. Thirty-six seeds (18 from each population) were planted in each treatment combination in each of the six blocks at each location. Two blocks were established at each of the three common garden sites at each location. Within an experimental plot, seeds were planted in a 6×6 grid, with a spacing of 5 cm between seeds. Seeds from each population were randomly allocated to grid points, and the location of each seed was marked with a different-color toothpick for each population.

We conducted seed germination tests of the populations in the laboratory using a random subsample of seeds from the bulked collections. One hundred seeds from each population were weighed, and equal numbers from each population were placed on four replicates of moistened germination towels. Towels were placed in a beaker covered with plastic wrap, and germination was monitored daily for 20 days.

Seedling-to-Adult Experiment. Seeds from each maternal family were separately planted into 15-cm-deep and 2.5-cm-wide plastic "conetainers" (Stuewe and Sons, Inc., Corvallis, OR, U.S.A.) and grown in a greenhouse for 3 months prior to transplanting into the field in mid-December 1996. Two weeks prior to transplanting seedlings into the field, low-competition treatments were created by spraying a randomly chosen half of each block with glyphosate to kill emerging annual weeds and other competitors. To minimize competition within the low-competition plots, competitors emerging after the herbicide application were periodically removed by hand during the remainder of the experiment. Populations of each bunchgrass species were planted separately in randomly assigned plots within each block. When planting, we attempted to disturb the soils (and vegetation in the competition treatment plots) as little as possible by forming planting holes with screwdrivers and a planting dibble with the same dimensions as the conetainers. A total of 12 randomly chosen seedlings from each open-pollinated maternal family were planted at each location, four seedlings per family per block with two seedlings planted into the high-competition treatment and two planted into the low-competition treatment. Families of both populations were randomly arranged within a plot, and seedlings were planted 30 cm apart to minimize intraspecific competitive effects.

Data Collection

Survival of seedlings was scored in June 1997, approximately 7 months after planting. To account for variation in seed viability among seed sources, percent survival for each seed source was calculated as the proportion of the 18 seeds planted per experimental unit divided by the germination rate for that seed source.

Survival of the seedling transplants was censused in late spring of 1997, 1998, and 1999. To estimate individual

reproductive output, the number of flowering stems (hereafter, referred to as culms) was recorded for each plant. This average number of culms was not per surviving plant but rather the number of culms for each seedling initially transplanted; plants that did not survive to reproduce were given a culm number of zero. One randomly chosen culm was subsampled from each plant in the low-competition treatments in spring 1997, and seed number per culm was counted to assess the relationship between culm number and seed number.

Germination rates were determined for seeds produced in 1997, 1998, and 1999. Germination rates were determined only for seeds produced from plots with low competition because seed production from high-competition plots was insufficient for germination trials. Seed germination rates were highly correlated with rates of seed fill in 1998 and 1999 for both *Nassella* ($r = 0.84$, $p < 0.001$) and *Elymus* ($r = 0.96$, $p < 0.001$), so there was little, if any, dormancy in filled seeds of either species. We used percent germination as an index of seed viability.

It is possible that differences in average seed size between populations in the initial field collections of seed in 1995 and 1996 could have been due to maternal environment effects. To check for this, we examined seed weight differences among populations from seeds produced in a common environment. As part of seed viability testing in 1999, seeds produced by experimental plants in the low-competition treatment were collected at both planting sites. Before testing for viability of these seeds, average seed weights were determined by dividing seed weight per culm by the number of seeds per culm.

Precipitation and air temperature data were collected from weather stations located near our study plots at each location and compiled monthly. We also measured the light environment in the plots with a light sensor bar (Decagon Devices, Inc., Pullman, WA, U.S.A.) containing a linear array of 80 sensors that detect photosynthetically active radiation (PAR). All light measurements were made at ground level at eight prespecified locations within each plot between 1100 and 1400 hours. Estimates of light availability within the canopy were calculated as the percent of ambient light (i.e., light levels measured just above the plant canopy).

Species composition and total aboveground biomass of vegetation within each plot at each location were measured in May 1997. Within the fenced exclosures, vegetation was clipped from within three 25-cm² quadrats randomly positioned along transects located just outside of the transplant area. Plant material was sorted by species and biomass determined after drying samples to a constant weight at 65°C.

Statistical Analyses

Proportion survival data for seedlings were arcsine square root transformed prior to all statistical analyses to satisfy distribution assumptions of parametric analyses.

A split-plot mixed-model analysis of variance (ANOVA, block nested within planting location) was used to calculate the significance of main and interaction effects. Block was considered a random factor, whereas planting location, population seed source, and competition treatment were analyzed as fixed factors.

Culm number data were log transformed prior to analyses to reduce the correlation between the mean and the variance. Because the proportion of plants of each maternal family surviving in each experimental unit could take on just three values (0, 0.5, and 1), we rank transformed these data prior to analysis. ANOVAs were first conducted on each variable in each year in order to confirm that data transformations reduced heterogeneity of variance. A mixed-model repeated-measures ANOVA, with block nested within planting location and family nested within population seed source, was then used to analyze plant survival and culm production responses across years. Blocks and families were considered to be random factors, whereas planting location, population seed source, and competition treatment were treated as fixed factors. Although family effects were estimated by this design, these results are not presented here but are discussed elsewhere (Knapp & Rice, in preparation). Germination rates of seed from culm subsamples were analyzed by a mixed-model repeated-measures ANOVA across years with block, population seed source, and planting location as treatment factors. Germination percentages were arcsine square root transformed before analysis to satisfy distribution assumptions of parametric analyses. All calculations were made using the SAS GLM procedure (SAS Institute, Inc., Cary, NC, U.S.A.).

Cumulative Fitness Differentials

To better gauge the fitness consequences of local adaptation in both species, we calculated a cumulative fitness index that combined components of fitness measured at different life history stages. We first multiplied culm production for each year by seedling survival rates from the seed-to-seedling study. Because culm production estimates included zero culm production values from plants that did not survive to reproduction, these estimates incorporated mortality occurring during the seedling-to-adult stage. In order to estimate seed output, average seed production per culm was calculated from the culm subsamples harvested in 1997. Finally, to calculate the number of viable seeds produced during the course of the study, estimates of seed production per culm were multiplied by seed viability estimates obtained for each year. The resulting seed output estimates were then summed across years. We did not analyze this cumulative fitness index statistically because of the multiplicative nature of its derivation. Obviously, this index cannot fully represent total fitness in a long-lived herbaceous perennial, but we felt it was valuable to calculate because it represents a more integrative "seed-to-seed" measure of fitness differentials.

Results

Vegetation Species Composition and Light Availability

The vegetation at the coastal and foothill planting locations was different in both species composition and productivity (Table 1). Species diversity and productivity were higher at the Bodega location with a higher percentage of native species at this coastal site. An especially striking difference between the two locations was the lack of perennial species at the Sierra location, whereas over a quarter of the species at the Bodega site were perennials. As might be expected, vegetation removal treatments strongly altered the light environment in the plots and significantly increased the availability of PAR to transplanted seedlings within the low-competition treatments (Table 1).

Seedling Survival and Initial Seed Size

Seedling survival was significantly dependent on the interactive effects of population source, planting location, and level of competition (Table 2). This interaction did not differ significantly between species (i.e., there was not a significant four-way interaction). Averaged across species, seedling survival was higher for the local population most consistently when seedlings were exposed to stronger competition within undisturbed vegetation (Fig. 1). With competition, both the Bodega and the Sierra populations exhibited greater survival in their "homesite." In contrast, without competition, the seedling survival for the Bodega population in its homesite was actually less than that of the Sierra population ($p < 0.05$).

Because the effects of initial seed size and thus maternal influences are most likely at early life stages (e.g., seedling emergence and survival), we compared seed size differences among field-collected populations in both species. For both species, size (mean $\pm$ SD) of field-collected seeds from the Sierra location was significantly larger ($p < 0.05$; *Nassella* 7.1 ± 0.9 mg, *Elymus* 4.6 ± 0.8 mg) than from the Bodega location (*Nassella* 3.5 ± 0.5 mg, *Elymus* 3.8 ± 0.8 mg).

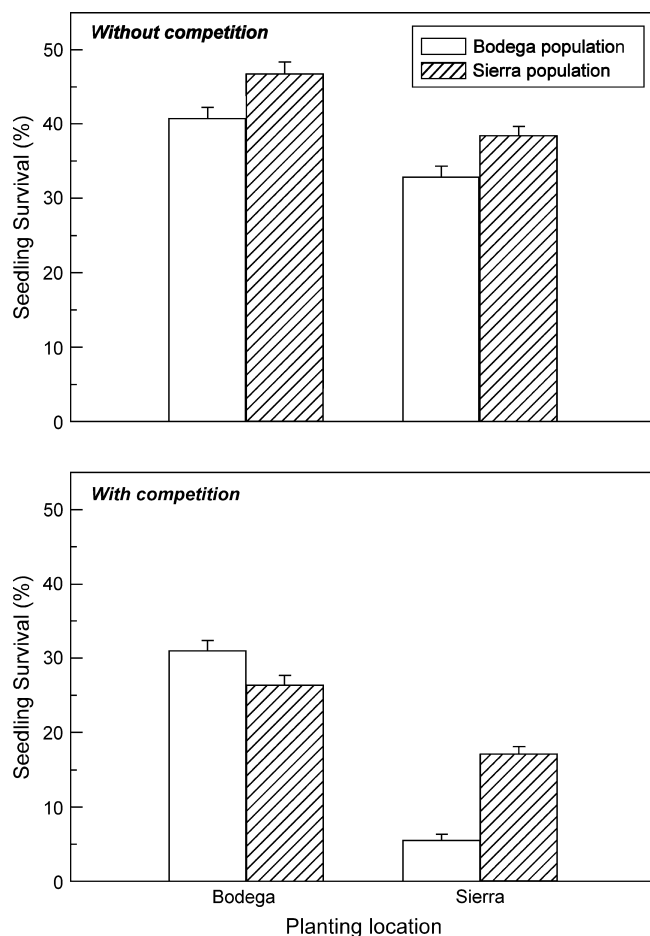
Adult Survival

Patterns of survival over time were affected by the interaction of seed source, planting location, and competitive environment, and these interactions differed between species. As a result, separate repeated-measures ANOVAs on survival over the 3 years of the experiment were conducted for each species (Table 3).

For *Nassella*, survival at both sites was much lower in populations experiencing high levels of competition in undisturbed vegetation (Fig. 2A & 2B). However, the effect of competition on the expression of local adaptation varied with planting location. At Bodega (Fig. 2A), survival under low competition did not differ significantly between local and nonlocal populations. Under high competition, however, survival at Bodega was much greater for local populations by the end of the experiment. At Sierra (Fig. 2B),

Table 2. ANOVA of percent seedling survival (arcsine square root transformed) for Bodega and Sierra populations of *Elymus glaucus* and *Nassella pulchra* planted reciprocally at both locations with and without vegetation removal (i.e., competition treatment).

Source	df	Sum of Squares	Mean Square	F Value	P Value
Block	10	3.349	0.335	19.51	<0.0001
Species (Sp)	1	0.979	0.979	57.07	<0.0001
Location (Loc)	1	0.693	0.693	40.37	<0.0001
Competition (Comp)	1	1.615	1.615	94.09	<0.0001
Population (Pop)	1	0.113	0.113	6.61	0.0139
Sp × Loc	1	0.015	0.015	0.89	0.3508
Sp × Comp	1	0.011	0.011	0.67	0.4184
Loc × Comp	1	0.179	0.179	10.43	0.0025
Comp × Pop	1	0.003	0.003	0.22	0.6396
Sp × Pop	1	0.003	0.003	0.21	0.6484
Loc × Pop	1	0.122	0.122	7.12	0.0110
Sp × Loc × Comp	1	0.032	0.032	1.92	0.1737
Sp × Loc × Pop	1	0.013	0.013	0.80	0.3754
Loc × Comp × Pop	1	0.105	0.105	6.15	0.0175
Sp × Comp × Pop	1	0.008	0.008	0.48	0.4922
Sp × Loc × Comp × Pop	1	0.022	0.022	1.27	0.2670
Error	40	0.686	0.017		

**Figure 1.** Significant interactive effects of population seed source, planting location, and competition treatment on seedling survival (averaged across data for both bunchgrass species). Error bars represent standard errors.

there was no difference between local and nonlocal populations in survival in either competition treatment.

Effects of competition on the expression of local adaptation in *Elymus* also varied between planting sites (Fig. 2C & 2D). At Bodega, there was no indication that competition significantly influenced the expression of local adaptation. Whether the population was local or nonlocal appeared to be more important than competition in affecting survival (Fig. 2C). This lower survival in the nonlocal population at Bodega may reflect the effect of disease because over the course of the experiment at the Bodega site, the rate of rust infection appeared to be more frequent in the nonlocal population (E. Knapp, U.S. Forest Service, personal observations, 1998). In addition, other common garden studies of these populations indicate that the Sierra population is more susceptible to rust infection than the Bodega population (Rice & Knapp, in preparation). In contrast, survival of *Elymus* at Sierra appeared to be more strongly influenced by competition (Fig. 2D). Additionally, survival by the end of the experiment under the high-competition treatment was much greater for the local population. Survival did not differ significantly between local and nonlocal populations in the low-competition treatment.

Reproductive Output

Similar to survival data, reproductive output (culm production) of both species over the 3 years of the study was affected significantly by the interaction of year, seed source, planting location, and competitive environment (Table 3). By the end of the experiment, the local *Nassella* population at Bodega was producing more culms per plant than the nonlocal population (Fig. 3A). A relative reproductive advantage for the local population was more pronounced in plants exposed to high competition. At the Sierra site,

Table 3. Summary of repeated-measures ANOVAs for (A) *Nassella pulchra* and (B) *Elymus glaucus* over 3 years showing *F* test values for all variables in the model.

	<i>df</i>	<i>Culms 1997</i>	<i>Culms 1998</i>	<i>Culms 1999</i>	<i>×Time</i>	<i>Survival 1997</i>	<i>Survival 1998</i>	<i>Survival 1999</i>	<i>×Time</i>
(A) <i>Nassella pulchra</i>									
Time					2248.83***				670.64***
Block	4	2.08	1.10	1.05	41.59***	0.09	0.67	0.69	26.18***
Location (Loc)	1	0.23	2.50	3.82	139.39***	3.51	1.04	1.08	25.06***
Competition (Comp)	1	67.41**	238.24***	24.20**	592.27***	0.00	10.72*	10.81*	349.87***
Loc × Comp	1	0.00	8.84*	0.05	132.49***	1.45	0.38	0.02	3.79*
Population (Pop)	1	0.00	33.34**	31.05***	38.24***	4.50*	14.05***	12.09***	9.17**
Comp × Pop	1	6.25*	0.37	1.05	1.23	1.59	16.70***	6.71*	6.22**
Comp × Block	4	51.90***	28.58***	92.51***	31.28***	1.09	23.34***	57.27***	32.57***
Loc × Pop	1	5.97*	11.95***	0.67	3.66*	0.39	19.21***	11.71**	8.62***
Loc × Pop × Comp	1	1.92	1.95	13.49***	15.33***	0.00	25.58***	12.76***	9.22***
Family	58	2.20**	2.39**	2.25*	1.31*	0.78	1.14	1.23	1.19
Loc × Family	58	0.97	0.93	1.01	0.96	1.01	1.44	1.00	0.91
Comp × Family	58	2.17**	0.94	0.98	1.06	1.00	1.41	1.18	0.94
Loc × Comp × Family	58	0.74	1.25	0.91	1.14	1.00	0.58	0.89	0.73
Error	472								
(B) <i>Elymus glaucus</i>									
Time					816.2***				904.24***
Block	4	1.58	4.31	3.76	11.12***	1.80	0.73	0.68	7.12***
Loc	1	1.50	2.24	0.17	72.14***	0.37	57.25**	1.97	124.37***
Comp	1	174.84***	269.55***	47.59***	115.65***	1.87	47.92**	16.30*	169.68***
Loc × Comp	1	0.15	3.42	2.90	30.77***	0.29	24.62**	8.72*	98.59***
Pop	1	5.77*	142.83***	131.15***	106.68***	0.75	0.83	92.02***	68.52***
Comp × Pop	1	29.06***	106.60***	174.36***	50.53***	0.00	1.62	14.97***	7.37**
Comp × Block	4	24.21***	8.16*	11.34***	19.44***	8.77***	7.48***	15.52***	13.78***
Loc × Pop	1	87.96***	247.06***	221.22***	49.95***	4.77*	36.63***	143.64***	88.82***
Loc × Pop × Comp	1	2.37	0.39	8.23**	4.79***	0.54	15.56***	2.19	7.48**
Family	58	3.21*	3.53	2.22	1.14	0.87	2.01	1.75	1.03
Loc × Family	58	0.71	0.66	0.71	0.81	1.02	0.73	0.76	0.92
Comp × Family	58	0.82	0.78	0.79	0.97	0.81	0.83	0.66	0.86
Loc × Comp × Family	58	1.06	0.36	1.04	0.99	1.22	0.96	1.17	0.95
Error	472								

Significance of the interactions with the Time effect was determined with the Wilks' lambda statistic. The symbols *, **, and *** denote effects significant at 0.05, 0.01, and 0.001 *p* values, respectively.

reproductive output overall was reduced strongly by increased competition, and under competition, there was a significant reproductive advantage for the nonlocal seed source in the final year of the study (Fig. 3B).

In *Elymus* populations at Bodega, by the end of the study, the reproductive output from the nonlocal population was much lower than that of the local population regardless of competitive environment (Fig. 3C). This lower reproductive output from the nonlocal Sierra source may have resulted from the higher rates of rust infection noted above for this population at Bodega. At Sierra, differences in reproduction between local and nonlocal *Elymus* populations were most apparent under high competition (Fig. 3D). Under high competition, reproductive output was greater in the local population, whereas the nonlocal population under low competition produced slightly more culms per plant than the local population.

Variation in the Size and Viability of Seed

For both species, we compared population variation in seed size within the initial field collections to common

garden collections taken from the experimental plots (Table 4). Results from the analyses support the conclusion that population differences in seed size in the initial collection (i.e., Sierra seeds were larger) are not caused by maternal environmental effects. For both species, a highly significant ($p < 0.001$) main effect of population source coupled with nonsignificant interaction terms indicates that larger seed size in Sierra populations was robust across different growth environments. Although overall seed size was greater in the common gardens (perhaps due to the reduced competition or reduced pollen limitation in *Nassella*), the relative difference between populations was consistent in both the field and the garden collections. In *Nassella*, the seed size difference between Sierra and Bodega sources was 51% in the initial collection and 37% in the common garden. In *Elymus*, the population seed size differences from the two collections were smaller with Sierra seeds 13% larger in the field collections and 16% larger in the common garden plants.

In both species, there were strong interactive effects of seed source and planting location on seed viability during

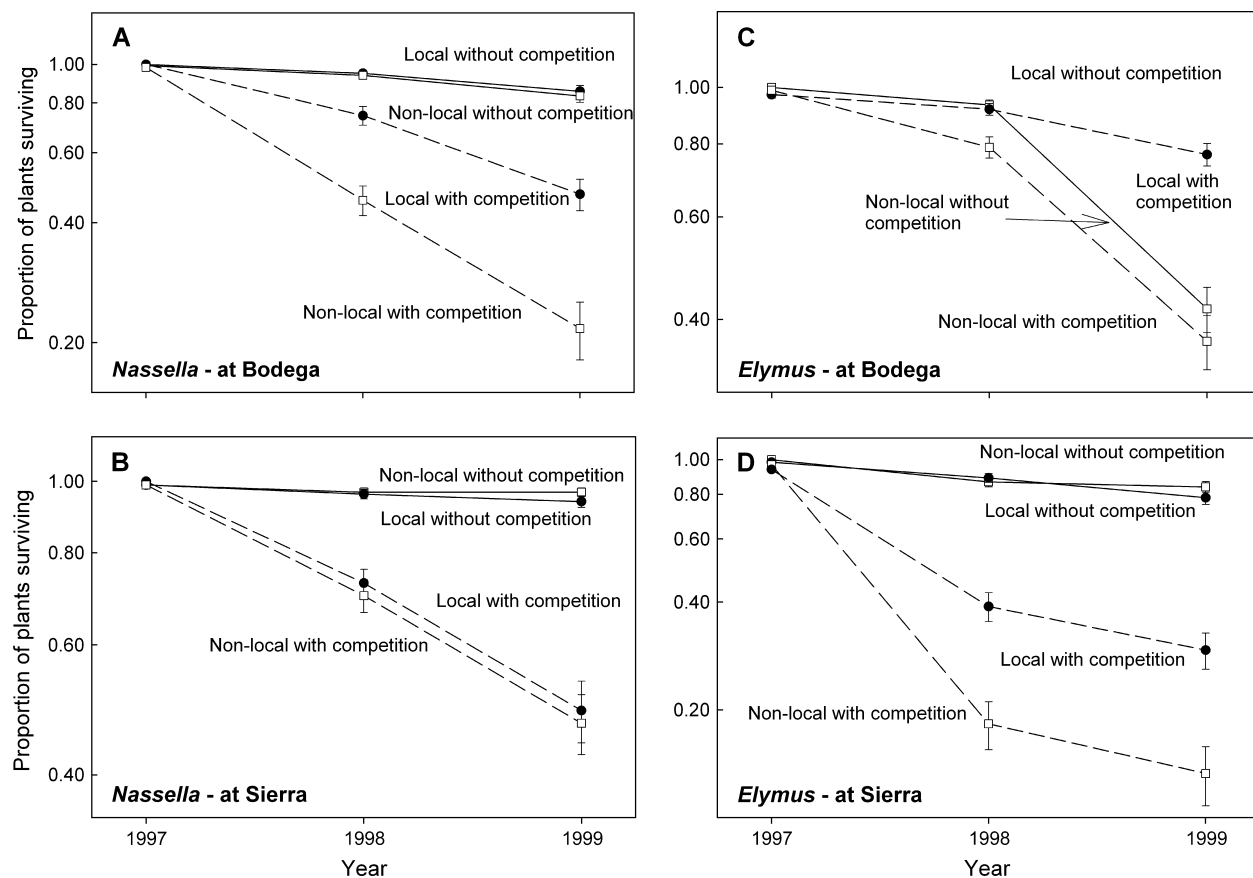


Figure 2. Significant interactive effects of population seed source and competition treatment on *Nassella* adult survival at the (A) Bodega and (B) Sierra planting locations and *Elymus* adult survival at the (C) Bodega and (D) Sierra planting locations across the 3 years of the study. Filled circles represent the local seed source, whereas open squares represent the nonlocal source. Solid lines indicate the low-competition treatment, whereas dashed lines represent the high-competition treatment. Error bars represent $\pm$ one standard error.

most years of the study (Table 5). As indicated by the highly significant ($p < 0.001$) seed source by planting location interaction (Table 5), seed viability in *Elymus* was always much higher for seeds produced by the local population (Fig. 4A). The lack of a three-way interaction of year, seed source, and planting location (Table 5) suggests that this expression of local adaptation was consistent across all years of the study. This local population advantage in seed viability was especially pronounced in years with reduced overall seed viability (1997 and 1999). In 1998, overall seed viability was higher, especially at the Sierra location, possibly due to above-average rainfall.

For *Nassella*, the highly significant interaction of seed source and planting location on seed viability ($p < 0.001$; Table 5) indicated higher viability for the local population at the Bodega location but not for the local population at the Sierra location (Fig. 4B). In fact, viability of seeds produced by the Bodega population at Sierra was the same or higher than that of the local Sierra population. A lack of a year, seed source, and planting location interaction indicates that this location difference in the expression of local adaptation was consistent across all years of the study (Table 5). In general, seed viability in *Nassella* was not as

variable among populations and locations although there was a general increase in viability at the Sierra location during 1998 that may reflect effects of higher rainfall for that year.

Cumulative Fitness Differentials

In *Elymus*, the cumulative fitness index indicated that the expression of local adaptation was dependent on both planting location and competitive environment (Fig. 5A & 5B). At Bodega, expression of local adaptation was more consistent in that the local population performed better than the nonlocal population under both low and high competition (Fig. 5A & 5B). At Sierra, under low competition (Fig. 5A), there were no significant differences between local and nonlocal sources in either adult survival or reproduction. As a result, local adaptation was only expressed in the high-competition treatment (Fig. 5B).

Expression of local adaptation in *Nassella* was less consistent across both sites and competition treatments (Fig. 5C & 5D). The local population at Bodega had higher relative fitness than the nonlocal population under both competition treatments. This difference was somewhat more pronounced under high competition (Fig. 5D). In contrast,

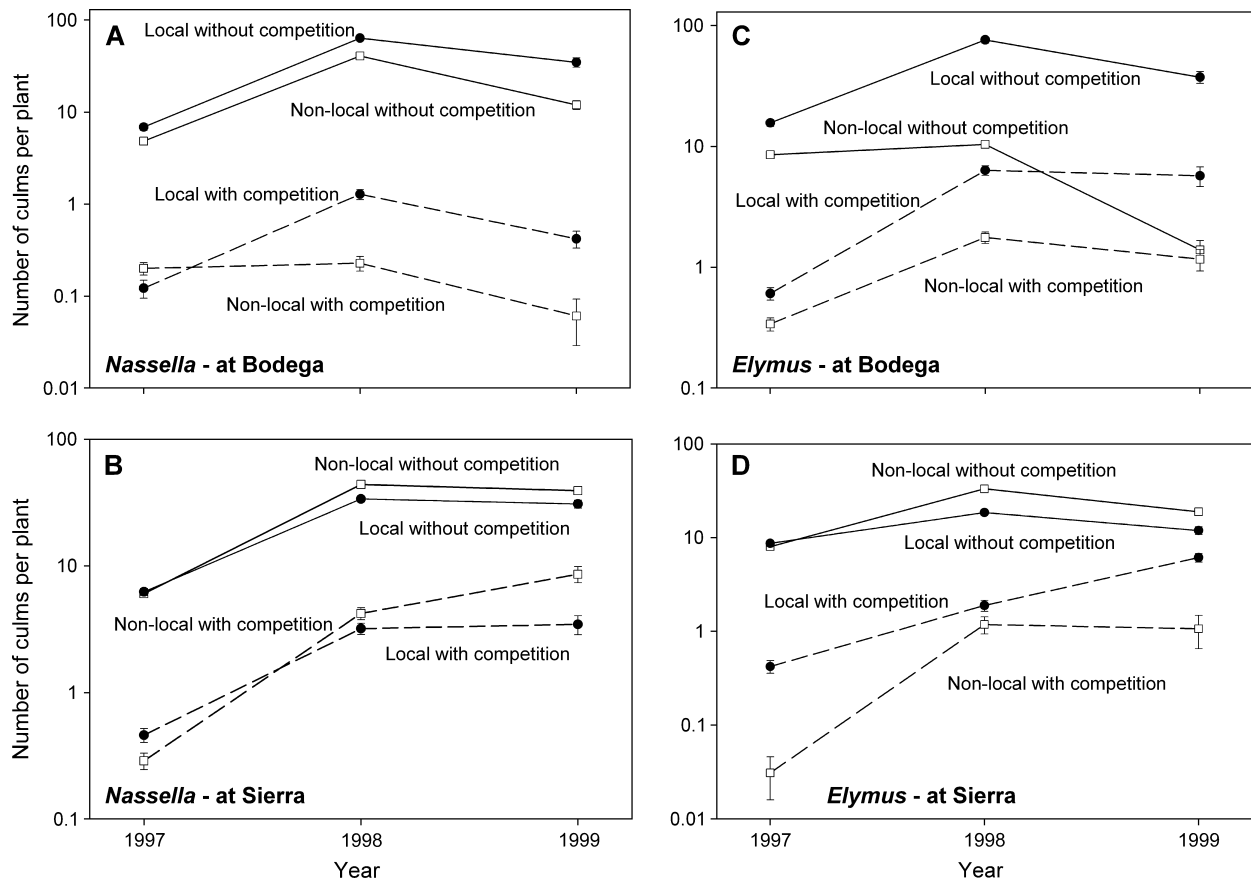


Figure 3. Significant interactive effects of population seed source and competition treatment on *Nassella* reproduction (culms per plant on log scale) at the (A) Bodega and (B) Sierra planting locations and *Elymus* reproduction (culms per plant on log scale) at the (C) Bodega and (D) Sierra planting locations across the 3 years of the study. Filled circles represent the local seed source, whereas open squares represent the nonlocal source. Solid lines indicate the low-competition treatment, whereas dashed lines represent the high-competition treatment. Error bars represent $\pm$ one standard error.

although seedling survival was higher for the local population at Sierra, the nonlocal Bodega population exhibited higher adult survival, reproduction, and seed viability at the Sierra site regardless of competition treatment. As a result, at Sierra, the nonlocal Bodega population had slightly higher relative fitness overall than the local population under both low and high competition (Fig. 5C & 5D).

Discussion

Competition and the Expression of Local Adaptation

In our study, increased interspecific competition consistently and significantly amplified the expression of local adaptation at multiple life history stages for both species. These results suggest that interactions with other plants at a site are important factors in the process of local adaptation. Most of the potential competitors at either site were non-native, so it is likely that any adaptation to competition represents a generalized selective response to diffuse competition (Dyer & Rice 1997) rather than an evolution-

ary response to competition from a particular suite of species. Although the use of reciprocal transplants to examine local adaptation has a long history (Clausen & Hiesey 1958, others), competition has rarely been experimentally manipulated within a reciprocal transplant design. In one of the very few studies available, Kindell et al. (1996) tested for local adaptation in seedling survival and biomass in the perennial grass *Aristida stricta* with vegetation removal as an experimental treatment. Although they found evidence for local adaptation in both seedling survival and biomass, removal of vegetation had no significant effect on its expression. The authors found local adaptation to be stronger for older aged seedlings, so it is possible that effects of competition on the expression of local adaptation might have surfaced if the study had been conducted for a longer period of time. In contrast, a study by Knight and Miller (2004) on within-population local adaptation in a clonal dune plant (*Hydrocotyle bonariensis*—Apiaceae) found results very similar to ours. Local adaptation over small spatial scales was amplified when vegetation remained intact, suggesting that

Table 4. Effects of population seed source and growth conditions on the individual weight of seeds from initial field collections and on the individual weight of seeds produced by (A) *Nassella* and (B) *Elymus* maternal plants grown in common gardens.(A) *Nassella* Seed Size ANOVA and Least Square (LS) Means

Source	df	Mean Square	F Ratio	Prob > F
Population seed source	1	1.7597	262.02	<0.0001
Growth conditions (field vs. garden)	1	0.5408	80.52	<0.0001
Growth conditions $\times$ population seed source	1	0.0049	0.73	0.3948
Error	71	0.0067		

Growth Conditions and Population Seed Source Individual Seed Weight (mg) ($LS\bar{X} \pm SE$)

Field-produced Bodega seed	3.49 $\pm$ 0.29
Field-produced Sierra seed	7.12 $\pm$ 0.21
Garden-produced Bodega seed	5.58 $\pm$ 0.17
Garden-produced Sierra seed	8.85 $\pm$ 0.15

(B) *Elymus* Seed Size ANOVA and LS Means

Source	df	Mean Square	F Ratio	Prob > F
Population seed source	1	0.0866	25.47	<0.0001
Growth conditions (field vs. garden)	1	0.0547	16.09	<0.0001
Growth conditions $\times$ population seed source	1	0.0027	0.79	0.3687
Error	91	0.0034		

Growth Conditions and Population Seed Source Individual Seed Weight (mg) ($LS\bar{X} \pm SE$)

Field-produced Bodega seed	3.44 $\pm$ 0.10
Field-produced Sierra seed	3.95 $\pm$ 0.10
Garden-produced Bodega seed	3.82 $\pm$ 0.13
Garden-produced Sierra seed	4.56 $\pm$ 0.14

Table 5. Repeated-measures analyses of population source and planting location effects on seed viability in (A) *Elymus glaucus* and (B) *Nassella pulchra* across the 3 years of the study (1997–1999).

Source	Numerator df	Denominator df	F Value	Prob > F
(A) <i>Elymus glaucus</i>				
Location (Loc)	1	4	41.0718	0.0030
Block	4	4	1.8263	0.2870
Population (Pop)	1	4	0.0006	0.9814
Pop $\times$ Loc	1	4	619.9445	<0.001
Year	2	3	68.1423	0.0032
Year $\times$ Loc	2	3	13.5421	0.0315
Year $\times$ Pop	2	3	5.9865	0.0897
Year $\times$ Loc $\times$ Pop	2	3	1.1007	0.4380
(B) <i>Nassella pulchra</i>				
Loc	1	4	111.0902	0.0005
Block	4	4	18.7658	0.0074
Pop	1	4	275.2095	<0.0001
Pop $\times$ Loc	1	4	22.6420	0.0089
Year	2	3	32.8214	0.0091
Year $\times$ Loc	2	3	59.4494	0.0039
Year $\times$ Pop	2	3	0.4492	0.6751
Year $\times$ Loc $\times$ Pop	2	3	3.8745	0.1474

Because of limited seed production in plants growing under competition, seed viability was tested for plants growing without competition only.

interspecific competition contributed to the evolution of local adaptation over very small scales. Obviously, it is difficult to make any generalizations from such a small number of available studies, but we suspect that further work combining competitive manipulations with reciprocal

transplants is likely to support our contention that interspecific interactions are a largely unmeasured and unappreciated selective force in the process of local adaptation. The practical implications of this conclusion are fairly clear. It is well known among restoration scientists

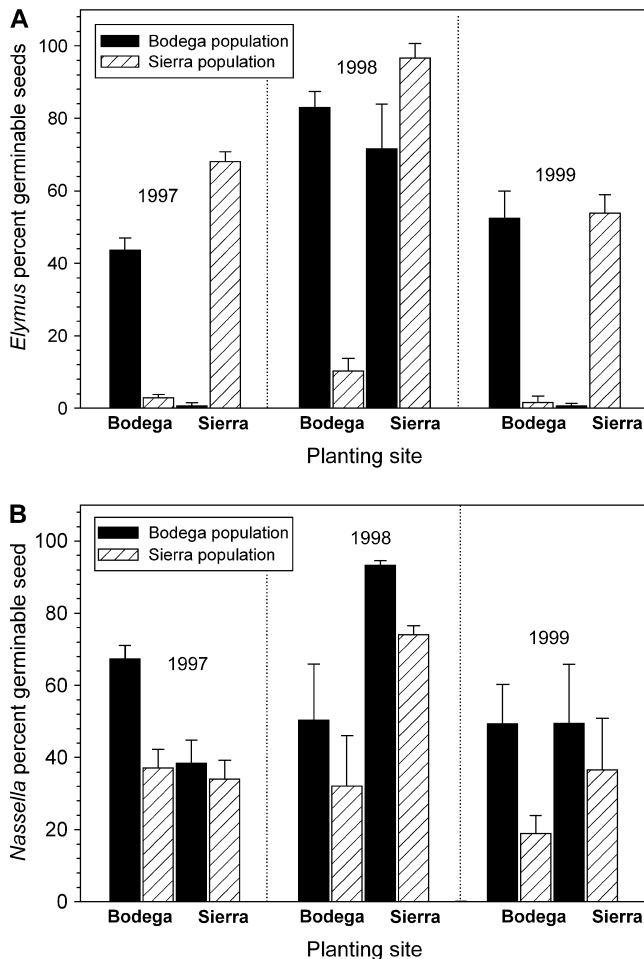


Figure 4. Significant interactive effects of population seed source and planting location on the germinability (i.e., viability) of (A) *Elymus* and (B) *Nassella* seeds produced at the Bodega and Sierra planting locations across the 3 years of the study. Seeds were collected from the low-competition treatment only. Error bars represent $\pm$ one standard error.

and practitioners that competition from the background vegetation at a site can strongly influence the success of a restoration project. Our research results demonstrate that competition at a restoration site and the origins of plant material introduced at that site may be interlinked by local adaptive processes. The ultimate success of the restoration may depend critically on recognition of this evolutionary history and the importance of interspecific competition as a selective agent.

Local Adaptation and Breeding Systems

For both bunchgrass species, the resident populations usually performed better than the nonresident populations when grown at their homesites. A significant "homesite advantage" in both survival and reproduction was particularly pronounced for both species at the Bodega location. However, the intensity and consistency of the expression of this homesite advantage were greater in *Elymus* especially when one compares estimates of cumulative fitness

between the species. This difference between species may, in part, result from differences in breeding system. Analysis of molecular markers and field measurements of hybridization indicate that *Elymus* is highly selfing (Knapp & Rice 1996; Wilson et al. 2001), where *Nassella* exhibits higher rates of outcrossing (E. Knapp, unpublished data; Knapp & Rice 1998). Theoretical and empirical studies have demonstrated that, under similar selection pressures, inbreeding populations are more likely to diverge genetically (Slatkin 1985; Hufford & Mazer 2003; Rice & Emery 2003; McKay et al. 2005) because effects of local selection are less likely to be countered by gene flow.

Seed Size Variation and Potential Maternal Environmental Effects

Because of the potential importance of seed size in determining initial seedling establishment, maternal environmental effects can often be important nongenetic contributors to population differences in seedling survival (Roach & Wulff 1987). However, it is very unlikely that larger seed size in Sierra populations was due to purely maternal environmental effects. Our results indicate that differences in seed size among populations observed for the field-collected seed persisted in seed grown in common gardens. It is possible that larger seed size could explain the greater seedling survival observed for the Sierra population under reduced competition at both sites. However, these initial differences in seed size did not result in a similar advantage for Sierra seedlings under competition, even though one might expect that larger size would be even more advantageous in this environment (Black 1958; Stanton 1984). Although overall seedling survival in both species was reduced in the interspecific competition treatment, the expression of local adaptation in seedling survival was significantly greater under these conditions. Studies on patterns of seedling survival have typically focused on concepts such as "safe sites" that emphasize environmental determinants of successful establishment rather than genetic differences (Harper 1977 and references therein). Given the potential vulnerability of newly germinated seedlings to microsite variation, it is surprising that environmental variation did not completely override genetic differences between populations. These results further emphasize the importance of considering genetic as well as ecological processes in the initiation and establishment of restoration projects.

Expression of Local Adaptation at Different Life History Stages

Local adaptation may occur at any time during plant development, and strength may vary greatly among different life history stages. Moreover, the expression of local adaptation in adult survival and reproduction may require many years to become manifest. Although a homesite advantage was often found for both adult plant survival and reproduction

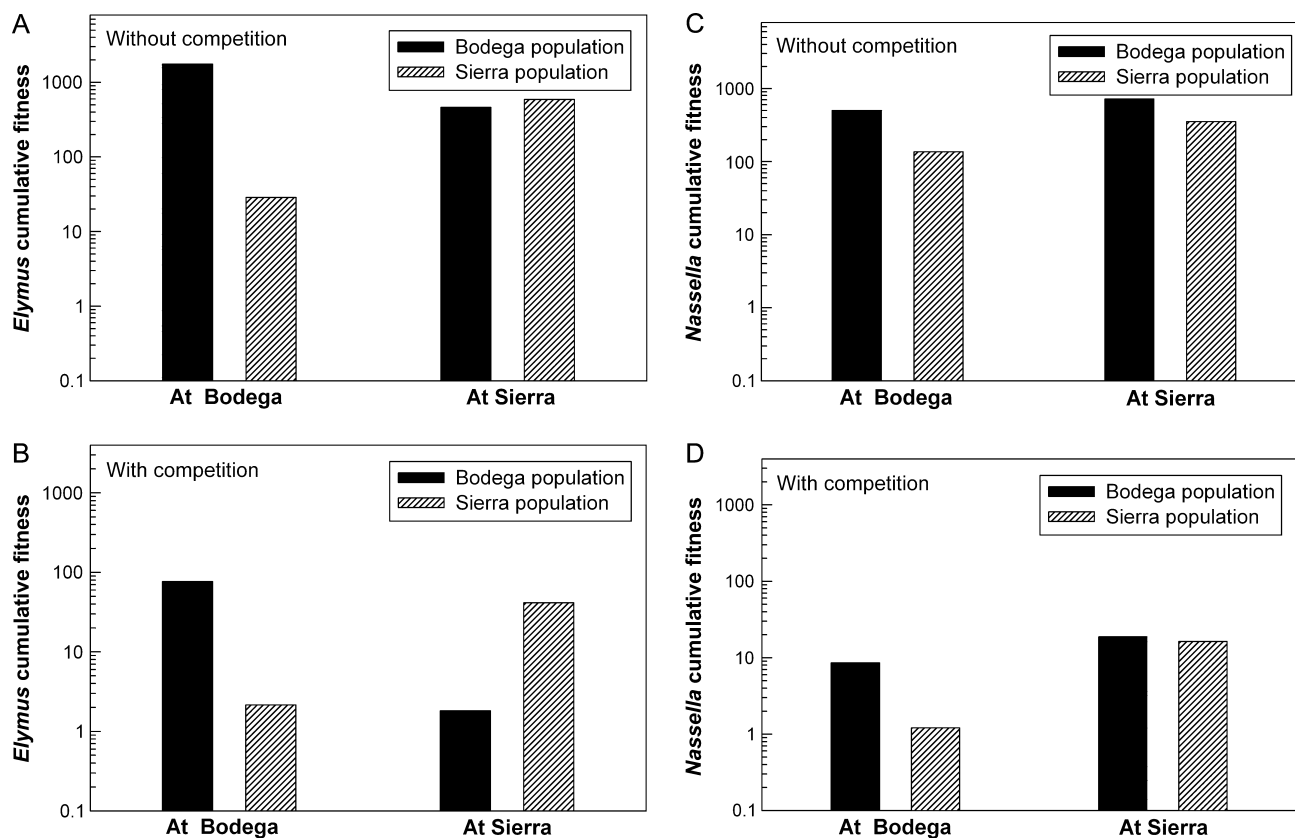


Figure 5. Significant interactive effects of population seed source and planting location on *Elymus* cumulative fitness (note log scale) under low competition (A) and high competition (B) and *Nassella* cumulative fitness (note log scale) under low competition (C) and high competition (D). See text for derivation of cumulative fitness index.

in our study, local adaptation was expressed most strongly in traits associated with reproduction. This was especially apparent in the results from the seed viability analysis, particularly in *Elymus*, where germination rates were much higher for seeds produced from plants grown in their home environment. This result is notable not just for the intensity of expression of local adaptation but also because this trait is rarely reported in studies of local adaptation. Seed quality is obviously important for the development of a sustainable population, and if it is overlooked, it may jeopardize the chances for a successful restoration. For example, the fact that some Sierra plants often grew very large at Bodega might lead to the conclusion that the planting was a success. However, low viability in the seed produced by these nonlocal plants would severely limit recruitment in the next generation.

Conclusions

Our results indicate that the importance of local adaptation in ecological restoration is most likely to become obvious in the later stages of a restoration project. The consistently more pronounced expression of local adaptation under conditions of higher competition would suggest

that as the vegetation at a restoration site reestablishes, the advantage of local genotypes is likely to increase over time. Similarly, as the restored vegetation becomes established and reproductively mature, selection intensity will increase for local adaptation in traits associated with reproduction such as seed number and viability.

Implications for Practice

- Two native grass species used widely in restoration in California exhibited marked regional adaptation between coastal and foothill populations.
- The expression of local adaptation was stronger under more competitive conditions.
- Although local adaptation was expressed at all stages in the life cycles of both species, it was strongest in traits associated with reproduction and seed viability.
- Local adaptation was more pronounced in the grass species with lower rates of outcrossing.

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