# SEED TRANSFER ZONES FOR A NATIVE GRASS

# *Festuca roemeri*

## GENECOLOGICAL EVIDENCE

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## ABSTRACT

A common-garden study of *Festuca roemeri* (Pavlick) E. B. Alexeev (Poaceae) revealed substantial genetic variation within and among 47 populations from throughout its range in the Pacific Northwest, US, for growth, fitness, phenological, and morphological traits. Using climatic and physiographic variables, genetic patterns over the landscape were examined through principal component and regression analysis. Elevation and latitude of the seed source, and to a lesser extent temperature and precipitation, explained a significant proportion of the genetic variation, suggesting that observed variation was associated with adaptation to local environments. Most plants from the Willamette Valley exhibited poor growth and survival, perhaps due to inbreeding. *Festuca roemeri* variation clustered into seed transfer zones corresponding to Level III ecoregions, and one zone was further subdivided. High-elevation populations separated from lower-elevation populations but did not cluster into a single seed zone. Seed transfer zones reported here provide a guide for plant community restoration efforts using this species.

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**KEY WORDS**
common-garden study, habitat restoration, inbreeding depression, conservation genetics

**NOMENCLATURE**
Plants: Barkworth and others (2007)
Fungi: ITIS (2008)

*Festuca roemeri* (Pavlick) E. B. Alexeev (Poaceae) is a community dominant in grasslands and savannas west of the Cascade Range and Sierra Nevada from southern British Columbia through northern California at elevations of 30 to 1830 m (100 to 6000 ft). The species is valued for habitat restoration and erosion control projects, but its use has been limited by difficulties obtaining correctly identified seeds and by disagreements about genetically and ecologically appropriate seed transfer zones. Local seeds are preferred (McKay and others 2005), but how local those seeds should be may vary among species (Rogers and Montalvo 2004). The common-garden study reported here provides information to delimit *F. roemeri* seed transfer zones.

This grass was described relatively recently as *F. idahoensis* var. *roemeri* Pavlick (Pavlick 1983) and later treated as a species (Alexeev 1985). Populations from inland areas of the Klamath region differ from both northern and coastal populations in details of leaf anatomy and isozymes (Wilson 1999). The Klamath region populations have been recognized as a separate subtaxon, *F. roemeri* var. *klamathensis* B. L. Wilson (Wilson 2007).

The species now has 3 centers of distribution. *Festuca roemeri* var. *roemeri* grows in relatively extensive grasslands in the Puget Trough of western Washington. Most *F. roemeri* populations in the Willamette Valley and Douglas County are small and isolated (Wilson 1999). The *F. roemeri* var. *klamathensis* populations in northwest California and southwest Oregon vary in size but are often large and interconnected.

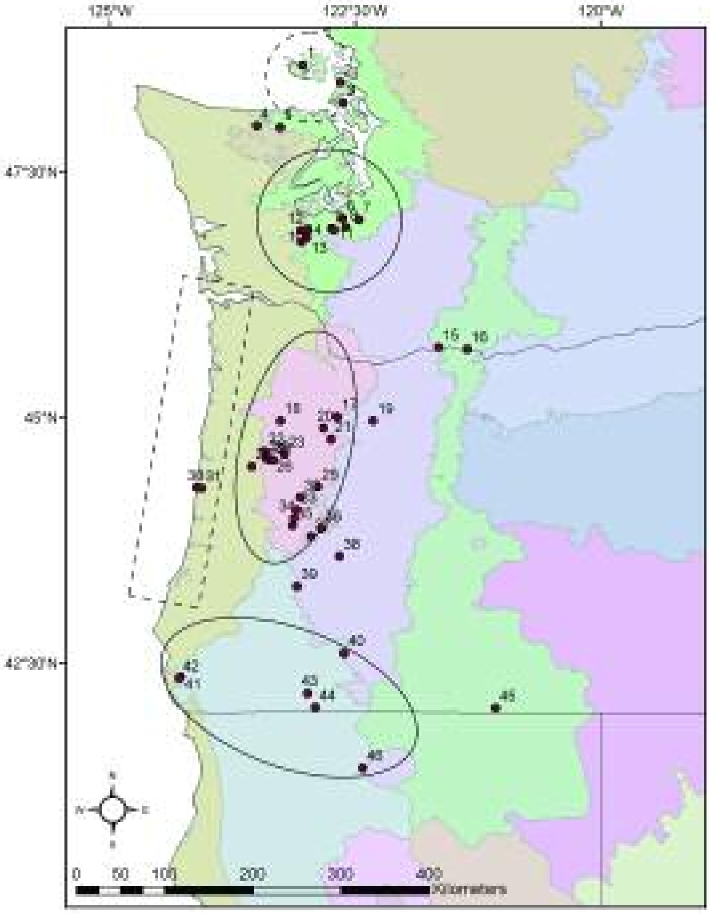

*Figure 1. Festuca roemeri* populations used in this study mapped on Level III ecoregions for Washington and Oregon, and proposed seed transfer zones. For the population numbers corresponding to the dots, see Table 1.

A practical problem limiting restoration use of *F. roemeri* is that it often grows in mixed populations with *F. rubra* L., and the 2 species are easily mistaken for each other (Wilson 1997; Dunwiddie and Delvin 2006). Ensuring that wild-collected seeds are actually *F. roemeri* requires careful examination of each individual plant from which seeds are collected, or growing the seeds in cultivation for at least one generation and removing contaminant plants. Therefore, the most practical way to obtain correctly identified seeds of *F. roemeri* is to buy them from cultivated sources that have been checked repeatedly for identification.

A more theoretical problem has been lack of consensus on appropriate seed transfer zones. This grass is variable in stature, foliage color, and morphological details. An earlier study of *F. roemeri* revealed that many leaf traits are plastic, varying in response to the environment, but that others are stable and presumably genetic (Wilson 1999). Therefore, much of the observed variation in *F. roemeri* could be purely phenotypic and of little value for delimiting seed transfer zones. The problem is particularly acute in the Willamette Valley, where the small remnant populations differ in color, height, and leaf width (B. Wilson, personal observation). It is unclear if the differences are genetic or phenotypic; if the differences are genetic, it is unclear whether they are adaptive or if they are merely non-adaptive results of inbreeding in small isolated populations.

This article reports on a study that compared *Festuca roemeri* populations in a common-garden setting, removing the effects of the environment and revealing patterns of genetic variation. The study concentrated on *F. roemeri* var. *roemeri* from the centers of distribution in the Puget Trough, Washington, and the Willamette Valley, Oregon. To provide context, populations of *F. roemeri* var. *klamathensis* were included, as well as *F. roemeri* var. *roemeri* populations from coastal, Columbia Gorge, and high-elevation sites (Figure 1). Results were used to delimit genetically appropriate seed transfer zones for *F. roemeri.*

## METHODS

### Common Garden

The common garden was established at Corvallis Plant Materials Center of the USDA Natural Resources Conservation Service. The site was in Benton County, Oregon, at 68 m (225 ft) elevation, on level, moderately well-drained Woodburn silt loam soil. The mean annual precipitation was 107 cm (42 in).

In 2001 and 2002, seeds were obtained from 55 wild populations. Each population was initially categorized into an ecological grouping based on known geographic and taxonomic factors (Table 1). Each individual plant had its own coin envelope in which seeds were stored, together with a few leaves from the parent plant. Thirty or more seeds were collected from at least 8 maternal plants in each population. Taxonomic identity of samples was checked by examining leaf cross-sections cut from the central third of a leaf from at least one-fifth of the samples, but additional specimens were examined when samples deviated from expected *F. roemeri* morphology (Wilson 1997, 1999).

On 16 and 17 December 2002, seeds from the 47 populations that were identified as *F. roemeri* and had mature seeds (Table 1) were planted in 164-ml tube containers, watered, and stratified by storing them at 3 °C (37 °F) in the dark. Eight half-sib families per population were used, except when only fewer than 8 were available. Eight or more tubes per family were planted with 3 seeds each. On 6 January 2003, the tubes were placed in the greenhouse in a randomized block design with each of the 3 greenhouse benches treated as a block to account for an environmental gradient in the greenhouse. The

TABLE 1

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Festuca roemeri *populations planted in the common garden. The number is the label for the corresponding dot on the map (Figure 1).*

| Site | No. | Ecological group | State | County | Elev (ft) | Latitude | Longitude | Analysis | Seed zone |
|---|---|---|---|---|---|---|---|---|---|
| Abiqua Road | 17 | Willamette Valley | OR | Marion | 450 | 45°00'76" | 122°67'52" | yes | Willamette Valley |
| Ace Williams Mt | 39 | roemeri south | OR | Douglas | 1900 | 43°28' | 123°08'98" | yes | |
| Bald Hill southeast | 26 | Willamette Valley | OR | Benton | 460 | 44°56'37" | 123°32'83" | yes | Willamette Valley |
| Bald Hill west | 25 | Willamette Valley | OR | Benton | 500 | 44°56'62" | 123°33'96" | yes | Willamette Valley |
| Baskett Butte | 18 | Willamette Valley | OR | Polk | 360 | 44°96'51" | 123°25'79" | yes | Willamette Valley |
| Beazel property | 22 | Willamette Valley | OR | Benton | 1000 | 44°65'56" | 123°41'89" | yes | Willamette Valley |
| Blue Mountain | 5 | High — NW WA | WA | Clallam | 5970 | 47°955 | 123°25'88" | yes | |
| Cape Perpetua | 30 | Coastal | OR | Lincoln | 700 | 44°28'68" | 124°10'72" | yes | Coastal |
| Cobleigh Road | 40 | roemeri south | OR | Jackson | 2270 | 42°59'91" | 122°60’88" | yes | |
| Cummins Creek Trail | 31 | Coastal | OR | Lincoln | 1000 | 44°27'98" | 124°06’22" | yes | Coastal |
| Drano Lake | 15 | Columbia Gorge | WA | Skamania | 240 | 45°71'37" | 121°64'63" | yes | |
| Duncan Spring | 45 | Klamath Co | OR | Klamath | 4775 | 42°04'11" | 121°06'72" | no | |
| Edgewood-Weed Road | 46 | Siskiyou | CA | Siskiyou | 3200 | 41°43'15" | 122°41'84" | yes | Siskiyou |
| Fairview Peak | 38 | High — WV | OR | Lane | 5800 | 43°58'53" | 122°65'35" | yes | |
| Fidalgo Island | 2 | Washington | WA | Skagit | 300 | 48°41'01" | 122°64'44" | yes | San Juan |
| Fire Knoll | 20 | Willamette Valley | OR | Marion | 700 | 44°89'25" | 122°81'53" | yes | Willamette Valley |
| Glacial Heritage | 14 | Washington | WA | Thurston | 100 | 46°78'64" | 123°04'31" | yes | Puget Trough |
| Horse Rock Ridge | 29 | Willamette Valley | OR | Linn | 3200 | 44°29'36" | 122°87'37" | yes | |
| Hulkill Hollow | 43 | Siskiyou | OR | Jackson | 2840 | 42°19'26" | 122°981 | yes | Siskiyou |
| Hurricane Ridge | 4 | High — NW WA | WA | Clallam | 5150 | 47°96'94" | 123°49'72" | yes | |
| Johnson Prairie | 9 | Washington | WA | Thurston | 400 | 46°92'31" | 122°73'46" | yes | Puget Trough |
| Kingston Prairie | 21 | Willamette Valley | OR | Linn | 780 | 44°78'03" | 122°74'17" | yes | Willamette Valley |
| Kloster Mountain | 36 | Willamette Valley | OR | Lane | 1875 | 43°867 | 122°84'28" | yes | Willamette Valley |
| Lodgepole Meadow | 42 | Siskiyou | OR | Curry | 2250 | 42°34'36" | 124°28'95" | yes | Siskiyou |

TABLE 1 (continued)

| Site | No. | Ecological group | State | County | Elev (ft) | Latitude | Longitude | Analysis | Seed zone |
|---|---|---|---|---|---|---|---|---|---|
| Mary's Peak | 28 | High — WV | OR | Benton | 4000 | 44°50'37" | 123°55'06" | yes | |
| Memaloose Park | 16 | Columbia Gorge | OR | Hood River | 400 | 45°69'38" | 121°35'18" | no | |
| Mima Mounds | 12 | Washington | WA | Thurston | 240 | 46°90'37" | 123°04'82" | yes | Puget Trough |
| Mortar Point 3 | 6 | Washington | WA | Thurston | 240 | 47°02'71" | 122°62'35" | yes | Puget Trough |
| Morton Farm | 23 | Willamette Valley | OR | Benton | 450 | 44°583 | 123°402 | yes | Willamette Valley |
| Mount Tamalpais | — | San Francisco | CA | Marin | 500 | 37°90389 | 122°43'03" | no | |
| Novick Property | 34 | Willamette Valley | OR | Lane | 975 | 43°97'47" | 123°11'69" | yes | Willamette Valley |
| Open Spaces Park | 24 | Willamette Valley | OR | Benton | 900 | 44°57'19" | 123°37'34" | yes | Willamette Valley |
| Pyramid Rock | 41 | Siskiyou | OR | Curry | 2400 | 42°35'79" | 124°27'02" | yes | Siskiyou |
| Rock Hill | 32 | Willamette Valley | OR | Lane | 800 | 44°18'43" | 123°05'31" | yes | Willamette Valley |
| Rocky Prairie | 10 | Washington | WA | Thurston | 200 | 46°91'58" | 122°97'83" | yes | Puget Trough |
| Row Point | 37 | Willamette Valley | OR | Lane | 840 | 43°78'82" | 122°94'37" | yes | Willamette Valley |
| San Bruno | — | San Francisco | CA | San Mateo | 1300 | 37°68'75" | 122°43'53" | no | |
| Scatter Creek | 13 | Washington | WA | Thurston | 190 | 46°83'23" | 122°98'63" | yes | Puget Trough |
| Silver Fork | 44 | Siskiyou | OR | Jackson | 6450 | 42°047 | 122°90'22" | yes | Siskiyou |
| Skinner Butte | 33 | Willamette Valley | OR | Lane | 400 | 44°05’81” | 123°09’45” | yes | Willamette Valley |
| Smith Prairie | 3 | Washington | WA | Island | 100 | 48°205 | 122°618 | yes | San Juan |
| Table Rock | 19 | Willamette Valley | OR | Clackamas | 4390 | 44°96'42" | 122°31'18" | yes | Willamette Valley |
| Thirteenth Div. Pr, | 7 | Washington | WA | Pierce | 400 | 47°01'43" | 122°45'84" | yes | Puget Trough |
| Upper Weir Prairie | 11 | Washington | WA | Thurston | 540 | 46°91'02" | 122°70'52" | yes | Puget Trough |
| Weiss Road | 35 | Willamette Valley | OR | Lane | 1100 | 43°89’88” | 123°13'29" | yes | Willamette Valley |
| Yellow Island | 1 | Washington | WA | San Juan | 40 | 48°58'61" | 123°03'06" | yes | San Juan |
| Yelm | 8 | Washington | WA | Thurston | 340 | 46°93'64" | 122°59'07" | yes | Puget Trough |

greenhouse was set at a 21 °C/18 °C (70 °F/64 °F) day/night temperature with a 14-h day length. Once emergence was complete, tubes were randomly thinned to one seedling each. After 12 wk in the greenhouse, plants were moved to a shade house for 5 wk to harden off before being planted in the field.

Plugs were planted in a fallow field during 8 May to 12 May 2003 using a completely randomized design with 8 plugs per family (that is, 8 replications of single plant plots) with a single row border. Slow-release fertilizer (Osmocote 14N:14$P_2O_5$:14$K_2O$; The Scotts Company, Marysville, Ohio) was placed into each hole prior to planting each *F. roemeri* plug, and weeds were controlled by hand tillage, herbicides, and bark mulch. The field was irrigated approximately once each month in 2003, but not in later years. To minimize infection by leaf and stem rusts (*Puccinia* spp. [Uredinales: Pucciniaceae]), chlorothalonil and propiconazole were applied 5 times in 2003 and 9 times in 2004. Each individual plant was top-dressed with slow-release fertilizer once each winter (2004 and 2005).

### Data Collection

Data were recorded for traits related to seedling growth, foliage and fertile culms, phenology, rust infection, and seed yield (Table 2). Emergence date was determined by examining every tube daily. Seedlings that emerged were marked with a small stick. Albinism was also recorded for cotyledons lacking pigment. Plant height was measured as the length of the third longest leaf, and culm height was the height of the tallest fertile shoot. Width was measured at ground level. Leaf and (or) stem rust infection, leaf color, leaf abundance, and culm abundance were estimated by scoring. Lemma measurements excluded the awn. Seedling height and width were measured in the greenhouse at 10 wk after planting. Morphological traits and rust infection were measured in the field between 28 July and 1 August 2003, in June 2004, and in June 2005 (Table 2). From March to November 2004, all plants were evaluated for their phenology (Table 2). Between March and August, the plot was monitored weekly, and biweekly thereafter. Seeds were harvested selectively on a plant-by-plant basis at the optimal time to maximize the amount of seeds collected from each plant, from 3 June until 20 July 2004. At the end of this period, seeds were bulked within families, dried, and weighed.

### Analysis

NCSS (Hintze 2001) was used to perform t-tests and Wilcox rank-sum tests. Analysis of variance (PROC GLM, SAS 1999) was conducted on all traits to identify statistical differ-

TABLE 2

*Summary of growth, morphology, yield, and rust infection traits recorded or calculated for Festuca roemeri in the common-garden study.*

| Category | Trait | Description | Date | Year | PCA? |
|---|---|---|---|---|---|
| Seedling | Emergence date | Julian date | January | 2003 | |
| Seedling | Germination rate | ratio of seeds emerged to planted | January | 2003 | |
| Seedling | Albinism | yes/no | January | 2003 | |
| Growth | Plant height (10 wk) | length of longest leaf (mm) | March | 2003 | |
| Growth | Crown width (10 wk) | width of crown (mm) | March | 2003 | |
| Growth | Plant height (29 wk) | length of longest leaf (mm) | July–August | 2003 | Y |
| Growth | Crown width (29 wk) | width of crown (mm) | July–August | 2003 | Y |
| Growth | Form 2003 | ratio of height to width (29 wk) | (calculated) | 2003 | Y |
| Foliage | Leaf color (29 wk) | score of 1 (blue) to 5 (green) | July–August | 2003 | Y |
| Rust | Rust infection (29 wk) | score of 1 (none) to 10 | July–August | 2003 | Y |
| Growth | Dormancy (29 wk) | yes/no | July–August | 2003 | |
| Survival | Survival 2003 | % (survivors / plants in family) | July–August | 2003 | |
| Growth | Culm height (74 wk) | height of tallest culm (cm) | June | 2004 | Y |
| Growth | Crown width (74 wk) | width of crown (cm) | June | 2004 | Y |
| Growth | Form 2004 | ratio of height to width (calculated) | | 2004 | |
| Growth | Leaf abundance (74 wk) | score of 1 to 10 | June | 2004 | Y |
| Phenology | Dormant or dead | score on each date | March–November | 2004 | |
| Phenology | Vegetative stage; no culms | score on each date | March–November | 2004 | |
| Phenology | Boot stage | score on each date | March–November | 2004 | |
| Phenology | Elongation (jointing) stage | score on each date | March–November | 2004 | |
| Phenology | Emergence of inflorescence | score on each date | March–November | 2004 | |
| Phenology | First anthesis | score on each date | March–November | 2004 | |
| Phenology | 50% anthesis | score on each date | March–November | 2004 | Y |
| Phenology | Seed milky stage | score on each date | March–November | 2004 | |
| Phenology | Seed soft dough stage | score on each date | March–November | 2004 | |
| Phenology | Seed maturity | score on each date | March–November | 2004 | Y |
| Phenology | March 3 phenology score | stage on this date | March | 2004 | Y |
| Growth | Culm abundance (74 wk) | score of 0 (none) to 10 | June | 2004 | |
| Growth | Leaf abundance (126 wk) | score of 1 to 10 | June | 2005 | Y |
| Growth | Culm abundance (126 wk) | score of 0 (none) to 10 | June | 2005 | |
| Rust | Rust abundance (126 wk) | score of 1 (none) to 10 | June | 2005 | |

| Category | Trait | Description | Date | Year | PCA? |
|---|---|---|---|---|---|
| Inflorescence | Lemma length | mm | N/A | 2004 | |
| Inflorescence | Awn length | mm | N/A | 2004 | |
| Inflorescence | Palea length | mm | N/A | 2004 | |
| Inflorescence | Lower glume length | mm | N/A | 2004 | |
| Inflorescence | Upper glume length | mm | N/A | 2004 | |
| Inflorescence | Seed Score | Lemma + palea + upper glume + lower glume lengths (calculated) | | 2004 | Y |
| Inflorescence | Distance to lowest branch of inflorescence | cm | N/A | 2004 | |
| Inflorescence | Inflorescence length | cm | N/A | 2004 | |
| Inflorescence | Spikelets per lowest node | number | N/A | 2004 | |
| Yield | Total seed yield per family | grams | June–July | 2004 | Y |
| Survival | Survival (2005) | % (survivors / plants in family) | July | 2005 | Y |
| Survival | Survival (2006) | % (survivors / plants in family) | May | 2006 | |

Y = used in principal components analysis.

ences among populations and families within populations. For the greenhouse traits, benches served as replications.

Principal component analysis (PROC PRINCOMP) was conducted using SAS (SAS 1999) on population means of 15 traits (Table 2) chosen to represent a range of morphological and phenological attributes (growth, form, phenology, leaf, and seed traits) and included variables with relatively strong population differences and some correlation to physiographic variables. The first 3 principal components (PCs) explained 68% of the variation in the 15 variables and were further examined for relationships to physiographic and climatic variables. Because the fourth PC explained only 8% of the variation, subsequent analyses examined only the first 3 PCs.

Environmental variables were determined for the population sources and used to examine the relationship of source environments to the PCs. Climatic data were extracted from the Parameter Elevation Regressions on Independent Slopes Model (PRISM) (PRISM Group 2007) statistical geographical model, which estimates monthly and annual average precipitation, minimum and maximum temperature, and the average dates of the last and the first frost for 4 x 4-km (2.5 x 2.5-mi) grid cells (Daly and others 1994). For each site, the following data were available: latitude, longitude, elevation, fall first frost day, spring last frost

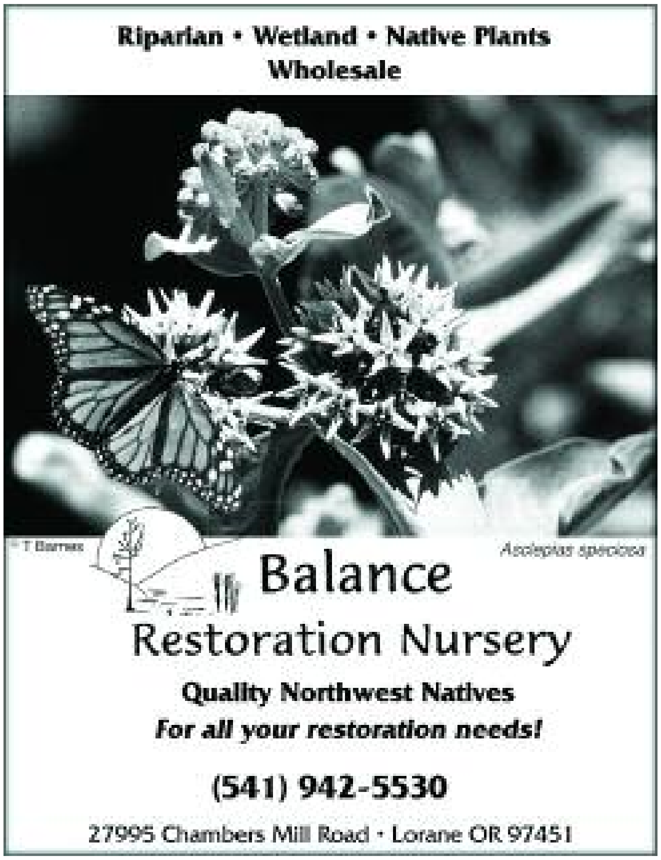


*Figure 2.* Variation in *Festuca roemeri* plants grown in the common garden.

Photo by Rob Fiegener

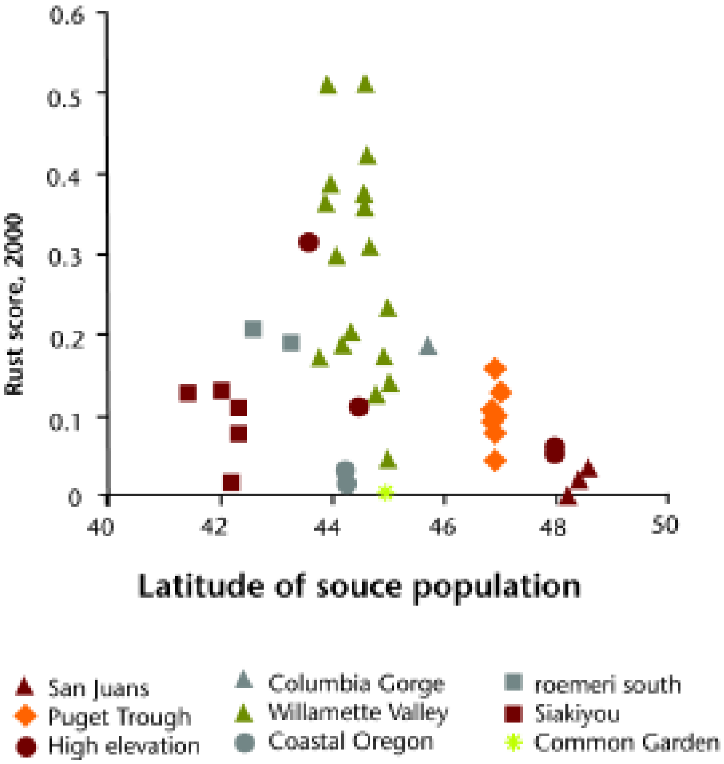


*Figure 3.* Rust incidence scores by latitude for *Festuca roemeri* in the common-garden study and the location of the common garden.

day, total annual frost free days, annual amount of precipitation, average annual temperature, average monthly precipitation, minimum, maximum, and average (mean of min and max) monthly temperatures. The following variables were also constructed: total annual precipitation, mean annual temperature, average temperature, and total precipitation for spring (March–May), summer (June–August), fall (September–November), and winter (December–February). Simple and multiple regression models were constructed by regressing the PC scores on geographic and climatic variables. Model-building was performed using the r-square selection method (PROC REG) that identifies the models with the largest regression coefficient of determination for the specific number of variables considered. The best 3- or 4-variable model is reported in each case, such that all variables were statistically significant ($P < 0.05$) and the number of independent variables did not exceed 10% of the total observations (hence 4 parameters).

## RESULTS

A few seedlings (0.6%) were emerging when the containers were moved to the greenhouse after stratification. Emergence peaked 4 d later, when 29% of seedlings emerged, and quickly tapered off although the last observed seedling emerged 22 d after containers were brought to the greenhouse.

Certain geographically peripheral populations included in the common garden were removed from analysis because they distorted analysis of more typical *F. roemeri* populations. The Duncan Spring and Memaloose Park plants were collected in transition zones between *F. roemeri* and *F. idahoensis*. Both had high mortality and very low seed yields in the common-garden study. The 2 populations collected in the San Francisco area, from Mount Tamalpais and San Bruno Mountain, were morphologically unusual and were located far south of the others.

Survival rates in the common garden were very high until early 2006. Over 99% of individuals survived the first summer. A year later, 95% had survived, and nearly 75% had survived to the end of summer 2005. Population survival rates through 2005 ranged from 29% (Hurricane Ridge, Olympic National Park) to 98% (Cummins Creek, coastal Oregon). During the winter of 2005–2006, the common-garden plot experienced severe flooding, the plants were inundated continuously for several weeks, and only 181 plants (6.5%) survived. Nine of the top 10 surviving populations were from the Oregon coast or western Washington.

Although the common-garden study was performed in the Willamette Valley, the Willamette Valley populations tended to perform more poorly for fitness-related traits than did Puget Trough and Oregon coast populations (Appendix 1). In 5 Willamette Valley populations, 4% or more of the seedlings were albino. The only Willamette Valley populations lacking albinos had seedling emergence rates of 11% or less. By contrast, the only Puget Trough population with more than 4% albinos was Yelm, an urban remnant population with less than 50 individuals. Of the 8 populations that did not survive the flooding in 2006, 7 were from the Willamette Valley.

Not all Willamette Valley populations performed poorly. Kloster Mountain, Horse Rock Ridge, and Rock Hill populations produced high seed yields and exhibited fairly high survival (82 to 85%) through 2005. These 3 were relatively large wild populations (500 to 3000 plants), while all other Willamette Valley populations had samples that were small (25 to 300 plants), except the Morton Farm population that grows on a patch of high-magnesium Dickson silt loam and has low yield both in the wild and in the common-garden study.

*Festuca roemeri* proved to be highly variable in leaf color, width, density, and length, as well as number and length of fertile culms (Figure 2). Some populations could be recognized easily. ANOVA showed that both the population and families within population effects were all statistically significant at $P < 0.05$ (data not shown).

Many of the traits showed some level of association (correlation) with the physiographic variables (Appendix 2). Growth-related traits such as plant height and width were correlated with latitude and elevation, particularly in 2003, the first year of growth (Appendix 2). Variation in seed quality in the first year might result from effects of seed source environment on seed quality, as well as on genetics. High-elevation and high-latitude plants were more compact, with shorter leaves and wider crowns. The high-elevation plants also had broader leaves, though that feature was not quantified. Plants from lower elevations and latitudes were variable but generally possessed longer leaves and narrower crowns. In 2004, plants from higher elevations had shorter culms than plants from low elevations. No significant correlations or trends were found for foliage in year 2004 or 2005; leaf abundance was correlated with latitude in 2004 (Appendix 2). Seed-source temperature appeared to be a stronger driver than precipitation for most traits, the exception being growth traits in 2003 and 2004.

Phenology in 2004 varied by ecological group. *Festuca roemeri* var. *klamathensis* from Hulkill Hollow, Jackson County, Oregon, flowered in March, and plants of other populations continued flowering into June. Average seed harvest date per population varied from 8 June to 1 July, and dates of seed ripening varied by ecological group. The Siskiyou populations

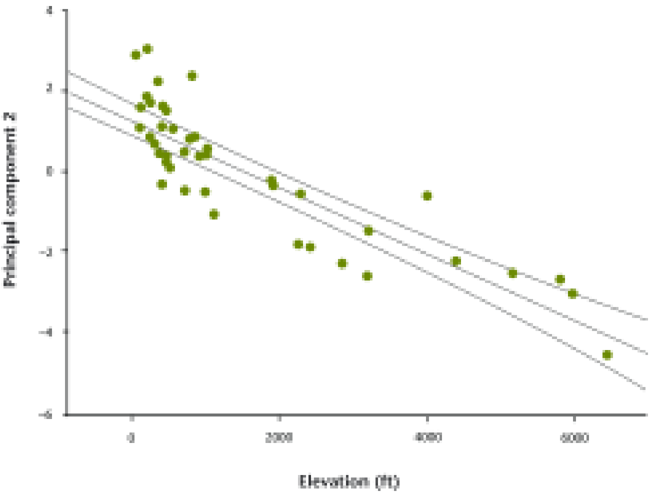


*Figure 4.* Regression of the second principal component and elevation of the source population for *Festuca roemeri*. $R^2 = 0.7522$. 95% confidence intervals shown.

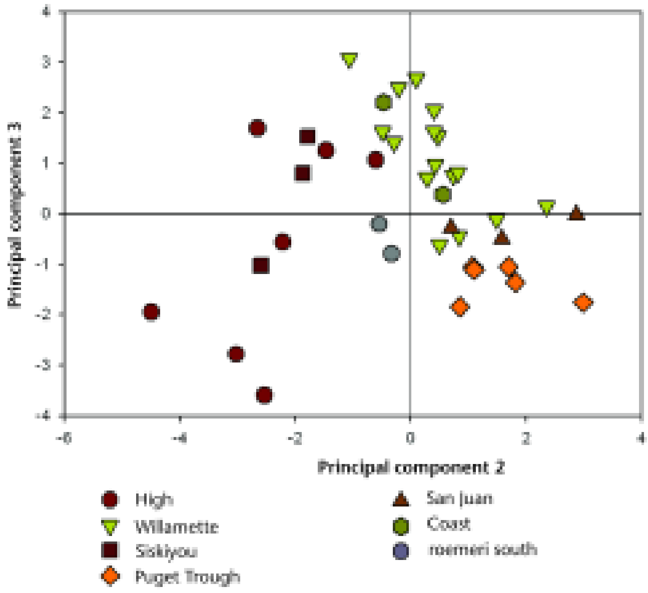


*Figure 5.* Plot of principal component 2 and 3 for *Festuca roemeri* in the common-garden study. The "Oregon" population cluster includes Willamette Valley and coastal populations. The "Washington" cluster includes both Puget Trough and San Juan populations.

were first, averaging 13 June, then, in order, high-elevation, Puget Trough, San Juan, Willamette Valley, Coastal, and Columbia Gorge populations with the average maturity date of 28 June. Anthesis and seed shatter were significantly correlated with latitude and elevation; linear regression of these traits shows that both occur earlier in higher-elevation populations, at a rate of 1.6 d per 305 m (1000 ft) elevation for anthesis and 0.9 days per 1000 ft for seed shatter. Nearly all populations included some plants that did not flower (7%), that set seeds and proceeded to go dormant (15%), and that set seeds but did not go dormant (78%). The Cummins Creek Trail population from coastal Oregon was unique in that every plant flowered and set seeds, but none became dormant.

Rust incidence rates in 2003 were correlated with latitude and highly associated with summer precipitation. Rates were low on southern and northern populations and highly variable on middle latitudes (Figure 3). Rust incidence was highest on populations from areas of lower summer precipitation and highest on populations collected in the Willamette Valley.

The first principal component (PC1) accounted for 32% of the total variation and was strongly correlated to many of the growth traits as well as to the emergence and phenology dates and survival (Appendix 2). Of the 3 PCs, PC1 was least associated with physiographic and environmental variables. PC2 accounted for 19% of the total variation and was strongly correlated to many of the growth traits and some of the seed traits (Appendix 2). It was strongly associated with elevation (r = –0.86; Figure 4) but also associated with latitude, temperature, and precipitation (Appendix 3). PC2 was strongly associated with the environment at the source location. PC3 accounted for 17% of the total variation and was strongly correlated to color and rust traits and most of the maturity date traits (Appendix 2). PC3 was associated with seed source latitude and temperature (Appendix 3).

PC2 and PC3 were strongly associated with the seed-source environments, indicating clinal variation patterns. In addition, the amount of the total variation of the original 15 traits explained by physiographic and climate variables was over 40% (sum of the r-squares × the percentage of variation explained in the PCs). This suggests that natural selection explains a substantial proportion of the genetic variation in this region (see Johnson and others 2004).

Before plots of PC2 versus PC3 were examined to look for clusters that might indicate populations with similarly adapted genotypes, principal components analysis was rerun without the 2003 height, width, and form measurements because early plant growth might have been affected by the environment in which the seeds were produced. Both analyses revealed an overall similarity among *F. roemeri* populations and both analyses revealed populations clustered in the same geography-based groups (Figure 5).

Because elevation was strongly associated with PC2 and latitude was associated with both PC1 and PC2, the populations were initially sorted into 4 broad categories: high-elevation (> 914 m [3000 ft]), Siskiyou (southern), Oregon (middle), and Washington (northern). The plot of PC2 versus PC3 (Figure 5) demonstrates that, in general, the Oregon and Washington sources have larger PC2 scores than the Siskiyou and high-elevation sources. The Oregon sources generally have higher PC3 scores than the Washington sources, but the Siskiyou and high-elevation sources are mixed together and cannot be separated on the basis of PC3 score. The PC1 score (not shown) does show some separation between the high elevation and Siskiyou sources. The Siskiyou sources also exhibited unique leaf morphology (Wilson 1999), so it seemed reasonable to keep the high-elevation and Siskiyou sources separate.

The 3 Washington sources that were intermixed with the Oregon sources (Figure 5) were all from small populations in the San Juan Islands. They were redefined as a “San Juan” zone. The remaining low-elevation Washington sources were all from relatively large populations in the Puget Trough or recently isolated remnants from them. This group was redefined as a “Puget Trough” zone.

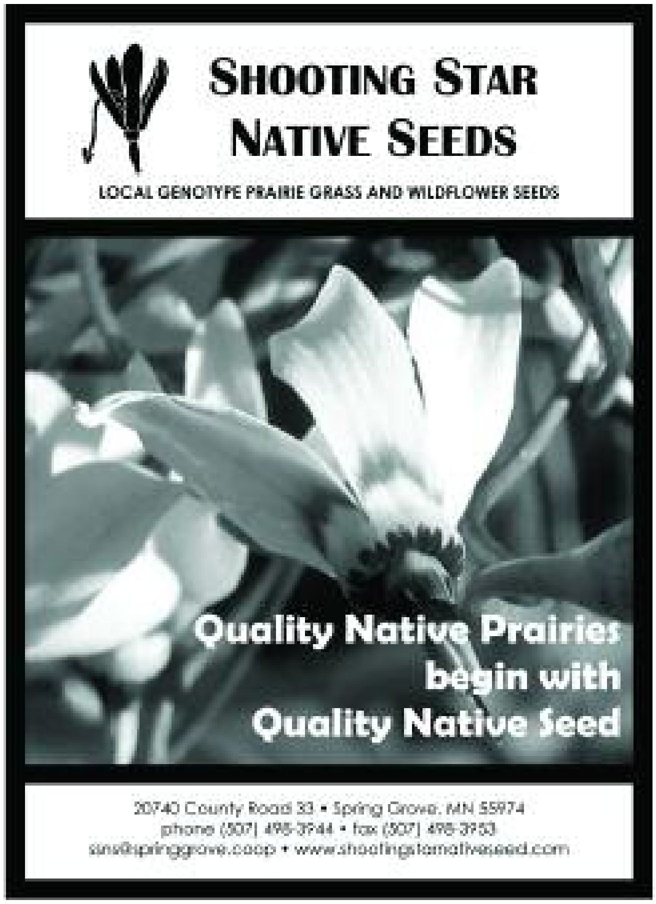
The 2 sources with the highest seed yields (Cummins Creek and Cape Perpetua) were the only sources sampled from the Oregon Coast (Figure 1). In principal components analysis they overlapped with the Willamette Valley populations (Figure 5). However, they were geographically separated from the Willamette Valley. They also had above-average survival in 2006 and above-average rust resistance (data not shown), which resulted in some of the largest PC1 scores. These 2 populations were taken out of the Oregon group and designated as “Coastal.” Nearly all the remaining low-elevation “Oregon” sources were from the Willamette Valley, so this group was designated as a “Willamette Valley” zone. There were no obvious geographic or ecological clusters within the Willamette Valley cluster (Figure 5).

## DISCUSSION

Common-garden studies provide a robust method for untangling genetic and environmental contributions to adaptive traits such as leaf morphology and growth rate. This study revealed substantial genetic variation in *F. roemeri* (Figure 2) but also revealed patterns that can be used to identify seed transfer zones.

The most important environmental variables for assigning *Festuca roemeri* populations to unique genetic groups (Figure 5) were elevation and latitude. High-elevation populations were too diverse to form a single cluster of similar populations, but they did differ from the low-elevation populations. The low-elevation (< 914 m [3000 ft]) populations clustered into 3 groups (Figure 5) that, when mapped on the landscape, corresponded with Level III ecoregions (Thorson and others 2003; Figure 1) to a large extent. A study of *F. roemeri* DNA also found that all populations were similar and samples from the same geographic area tended to cluster together (Jones and others 2008). In that study, the Siskiyou and Puget Sound clusters were recovered, but the variable Willamette Valley populations formed 2 clusters. A few populations, for example, the Ace Williams population isolated on a serpentine outcrop in northern Douglas County, Oregon, did not cluster closely with other sampled populations.

The populations of the Puget Lowlands Ecoregion in western Washington (Thorson and others 2003) were divided into 2 seed transfer zones because of small genetic differences between mainland and island populations (Figure 5; Jones and others 2008) and because of the geographic isolation between those groups (see Figure 1). The Puget Trough zone includes mainland populations, most of them very large with thou-



sands of individuals, on flat to gently rolling prairies. The San Juan zone includes smaller, more isolated populations on islands in Puget Sound.

Although the Willamette Valley populations were more genetically diverse than the Washington populations (Figure 5), they were treated as one seed transfer zone because no geographic or ecological patterns were detected within this cluster. Treating the Willamette Valley as a single seed zone is consistent with the conclusions of previous studies of *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae) below 914 m (1000 ft) elevation (St Clair and others 2005), *Alnus rubra* Bong. (Betulaceae) (R Johnson, personal observations), and the Poaceae grasses *Danthonia californica* Bol. (R Johnson, personal observations) and *Bromus carinatus* Hook. & Arn. (Doede, undated report).

In general, local populations are thought to be better adapted to local conditions than are plants from distant locations (Montalvo and Ellstrand 2001), but the Willamette Valley populations did not perform best in this Willamette Valley common garden. The 2 coastal populations performed much better, for example, having average seed yields 125% as great as the next highest-yielding population. The Willamette Valley plants performed poorly, tending to do worse than the populations from the Puget Trough (Appendix 1). This could be interpreted as evidence that Willamette Valley plants are not well adapted to the Willamette Valley, or at least to the common-garden site. The garden was located on the valley floor but most Willamette Valley populations were collected on slopes. A more careful look at the results, however, suggests an alternative hypothesis.

Three Willamette Valley populations yielded and survived well in the common-garden study on the valley floor, even though all 3 were collected on steep slopes. All 3 had relatively large populations, estimated at close to or more than 1000 plants each. All the other Willamette Valley populations sampled were small, with 25 to 300 plants, except for one from an unusual substrate (high-magnesium Dickson silt loam), which had low seed yield in the wild and in the common garden. We hypothesize that most Willamette Valley populations suffer from lowered fitness due to inbreeding in small populations. The hypothesis is consistent with unquantified observations in a cultivated field unrelated to this study, where wildland-collected seeds from several Willamette Valley populations crossed to produce F1 generation plants that were larger, more rust resistant, longer lived, and higher yielding than plants grown from wildland-collected seeds (K Kuykendall, B Wilson, personal observations). The hypothesis that the Willamette Valley populations are more inbred than most *F. roemeri* populations could be tested using molecular markers that identify heterozygotes and assess overall population diversity.

The history of *F. roemeri* populations in the Willamette Valley suggests that they would be susceptible to a buildup of harmful alleles. When the valley was covered by prairies (Habeck 1961), community dominant *F. roemeri* would have been abundant. It is probably an obligate outcrosser like its relatives in the *F. ovina* complex (Stace 2002). A large outcrossing population such as this would carry many potentially harmful alleles at low frequencies. As *F. roemeri* populations fragmented following Euro-American settlement, remaining populations became isolated, and harmful alleles may have increased in frequency. Small inbred populations can purge their harmful alleles, but apparently there has been inadequate time for that to occur in these populations.

We hypothesize that healthier Willamette Valley accessions of *F. roemeri* for habitat restoration and erosion control projects can be generated by crossing plants from the small, inbred, wild populations, thus producing a cultivated seed source that overcomes lowered fitness resulting from inbreeding in the small remnant wild populations.

The *F. roemeri* seed transfer zones delimited in this article provide a practical guide for restoration efforts using this species. Results of this study suggest that movement of propagules within each proposed seed transfer zone poses minimal risk of maladaptation. The seed zones can also be used for selecting seed sources to create ecologically appropriate *F. roemeri* populations for seed increase in cultivation. Such seed increases should lead to better supplies of correctly identified seeds for use in each zone. Creation of pre-varietal germplasm releases has been initiated at the USDA NRCS Corvallis Plant Materials Center (Darris and others 2008).

# APPENDIX 1

*Comparison of 17 Willamette Valley populations of* Festuca roemeri *with 9 Puget Trough and 2 Coastal populations for traits related to fitness, using the Wilcox Rank-Sum test for differences in medians.*

| | **Mean (SE), for WV** | **Mean (SE), for PT** | **Mean (SE) for Coastal** | **Wilcox test, Z** | **WV-PT probability** | **Wilcox test, Z** | **WV-Coastal probability** |
|---|---|---|---|---|---|---|---|
| Seedling emergence | 0.666 (0.050) | 0.788 (0.069) | 0.8656 (0.0625) | 1.4552 | 0.1456 | 1.5277 | 0.1266 |
| Albino seedlings | 0.026 (0.005) | 0.018 (0.007) | 0.0019 (0.0019) | –0.7013 | 0.4831 | –1.7973 | 0.0723 |
| Families with albino(s) | 0.334 (0.051) | 0.250 (0.070) | 0.0625 (0.0884) | 0.9012 | 0.3675 | –1.8142 | 0.0696 |
| Rust score 2003 | 0.283 (0.027) | 0.105 (0.038) | 0.0234 (0.0078) | –3.4506 | 0.0006* | –2.1919 | 0.0284 |
| Yield/plant 2004 | 11.193 (1.178) | 10.963 (1.620) | 34.4688 (0.2188) | 0.5390 | 0.5899 | 2.1919 | 0.0284 |
| Fertile tillers 2005 | 1.184 (0.146) | 1.931 (0.200) | 3.7310 (0.2645) | 2.5870 | 0.0097* | 2.1919 | 0.0284 |
| Survival 2004 | 0.856 (0.019) | 0.860 (0.026) | 0.9766 (0.0234) | –0.2436 | 0.8075 | 1.9949 | 0.0457 |
| Survival 2005 | 5.178 (0.243) | 6.700 (0.334) | 0.9766 (0.0078) | 3.2377 | 0.0012* | 2.1929 | 0.0283 |
| Survival 2006 | 0.021 (8.322) | 0.111 (0.011) | 0.2021 (0.0781) | 3.7583 | 0.0002* | 2.2530 | 0.0243 |
| Leaf length (seedling) | 17.944 (0.413) | 19.285 (0.568) | 20.8945 (0.8086) | 2.2637 | 0.0236* | 1.9262 | 0.0341 |
| Leaf length 2003 | 20.243 (0.398) | 21.138 (0.548) | 21.8056 (0.7147) | 2.3715 | 0.0177* | 1.7934 | 0.0729 |
| Leaf length 2004 | 87.156 (1.949) | 101.158 (2.678) | 98.4844 (11.2031) | 3.1260 | 0.0018* | 1.1292 | 0.2588 |

WV = Willamette Valley, PT = Puget Trough, * = significantly different from Willamette Valley.

## APPENDIX 2

*Correlations of traits with latitude, longitude, elevation, annual temperature, and annual precipitation of the seed source, and with the first 3 principal components.*

| **Trait** | **Latitude** | **Longitude** | **Elevation** | **Annual temperature** | **Annual precipitation** | **PC1** | **PC2** | **PC3** |
|---|---|---|---|---|---|---|---|---|
| Emergence date | –0.107 | –0.314 | –0.136 | 0.366 | 0.273 | **–0.396** | 0.022 | 0.200 |
| Leaf length (10 wk) | **0.429** | 0.313 | **–0.536** | 0.197 | –0.315 | **0.485** | **0.708** | 0.055 |
| Culm width (10 wk) | –0.243 | 0.220 | **0.448** | **–0.427** | –0.039 | –0.023 | **–0.384** | 0.018 |
| Culm height (2003) | **0.455** | 0.219 | **–0.572** | 0.228 | –0.324 | **0.461** | **0.768** | 0.130 |
| Crown width (2003) | **–0.406** | –0.031 | **0.668** | –0.362 | 0.417 | **0.444** | **–0.654** | 0.360 |
| Form (2003) | **0.559** | 0.254 | **–0.780** | 0.304 | **–0.540** | –0.059 | **0.931** | –0.201 |
| Rust (2003) | –0.344 | –0.037 | –0.114 | 0.283 | –0.083 | –0.308 | –0.066 | **0.643** |
| Color (2003) | –0.104 | –0.322 | –0.216 | 0.312 | –0.082 | –0.370 | –0.087 | **0.463** |
| Culm height (2004) | 0.323 | 0.332 | –0.506 | 0.241 | –0.276 | **0.549** | **0.611** | –0.054 |
| Crown width (2004) | –0.075 | –0.002 | 0.121 | 0.064 | 0.279 | **0.774** | –0.022 | **0.428** |
| Form (2004) | 0.186 | 0.226 | –0.326 | 0.042 | **–0.428** | –0.212 | 0.305 | **–0.504** |
| Anthesis date | –0.311 | –0.051 | **–0.507** | **0.666** | –0.106 | **–0.602** | 0.311 | **0.622** |
| Seed maturity date | –0.361 | –0.220 | –0.328 | **0.656** | 0.126 | **–0.421** | 0.129 | **0.740** |
| Mar 3 phenological score | –0.074 | 0.192 | 0.316 | **–0.401** | –0.167 | **0.657** | –0.263 | **–0.414** |
| Culm abundance (2004) | –0.242 | –0.206 | –0.006 | 0.188 | 0.071 | **0.756** | –0.099 | **0.407** |
| Foliage (2004) | 0.107 | –0.018 | –0.089 | 0.064 | 0.054 | **0.616** | 0.203 | 0.362 |
| Lemma | **–0.390** | –0.053 | 0.274 | –0.111 | 0.132 | 0.258 | **–0.443** | 0.102 |
| Awn | 0.311 | –0.063 | –0.044 | –0.227 | –0.024 | **0.434** | 0.039 | –0.111 |
| Palea | –0.485 | –0.092 | 0.247 | –0.019 | 0.119 | 0.224 | **–0.462** | 0.145 |
| Upper glume length | –0.203 | –0.187 | 0.058 | –0.009 | 0.085 | 0.125 | –0.199 | 0.098 |
| Lower glume length | –0.372 | –0.188 | 0.100 | 0.106 | 0.088 | 0.051 | –0.258 | 0.111 |
| Lowest infl. branch | 0.044 | 0.171 | –0.282 | 0.193 | –0.159 | 0.364 | 0.257 | –0.026 |
| Inflorescence | 0.270 | 0.244 | –0.294 | 0.061 | –0.151 | **0.568** | 0.370 | –0.134 |
| Seed score | **–0.401** | –0.135 | 0.191 | –0.011 | 0.116 | 0.182 | –0.378 | 0.124 |
| Survival (2005) | 0.247 | 0.017 | –0.022 | –0.062 | 0.071 | **0.768** | 0.157 | 0.024 |
| Foliage (2005) | 0.215 | –0.194 | **–0.393** | 0.294 | 0.004 | **0.484** | 0.423 | **0.434** |
| Culm abundance (2005) | –0.242 | –0.206 | –0.006 | 0.188 | 0.071 | **0.756** | –0.099 | **0.407** |
| Seed yield | –0.185 | –0.160 | 0.168 | –0.011 | 0.204 | **0.838** | –0.176 | 0.283 |
| Survival (2006) | **0.422** | –0.133 | –0.140 | –0.094 | 0.055 | **0.545** | 0.336 | –0.121 |

*Note:* Correlations >|0.30| are significant at $P = 0.05$; correlations >|0.39| are significant at $P = 0.01$ and are in bold.

APPENDIX 3

*Correlations of principal components (PCs) 1, 2, and 3 with physiographic and climatic variables.*

| | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| Latitude | 0.093 | **0.557** | **–0.411** |
| Longitude | 0.051 | 0.205 | –0.285 |
| Elevation | 0.134 | **–0.860** | –0.220 |
| Annual temperature | –0.174 | **0.456** | **0.564** |
| Annual precipitation | 0.084 | **–0.434** | 0.180 |
| Spring precipitation | 0.130 | **–0.473** | 0.203 |
| Summer precipitation | **0.406** | –0.186 | –0.095 |
| Fall precipitation | 0.079 | **–0.428** | 0.110 |
| Winter precipitation | 0.025 | **–0.420** | 0.226 |
| Spring temperature | –0.169 | **0.600** | **0.510** |
| Summer temperature | –0.382 | 0.310 | **0.405** |
| Fall temperature | –0.140 | 0.296 | **0.627** |
| Winter temperature | 0.012 | **0.464** | **0.563** |
| Frost free days | 0.033 | 0.308 | 0.189 |

*Note:* Correlations >|0.30| are significant at $P = 0.05$; correlations >|0.39| are significant at $P = 0.01$ and are in bold.

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