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Ecotypic Variation in *Elymus elymoides* subsp. *brevifolius* in the Northern Intermountain West

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Abstract

Bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) is an important native bunchgrass for rangeland restoration in western North America. This species is taxonomically complex and has diverged into as many as four subspecies, including subsp. *brevifolius*, for which four geographically distinct races have been described (A, B, C, and D). Of these four races, only C occurs in the northern Intermountain West. Our objectives were to describe phenotypic and genetic variation within C and to ascertain its taxonomic status. We evaluated 32 populations of C collected across the northern Intermountain West for a battery of biomass, phenological, and functional traits in common-garden settings in the field and greenhouse. Genetic variation was assessed with the use of amplified fragment length polymorphism (AFLP) markers, and correlations were calculated among phenotypic, genetic, environmental, and geographic distance matrices with the use of Mantel tests. Values for these four distance measures were positively correlated, suggesting that environmental heterogeneity and isolation by distance are shaping ecotypic divergence driven by natural selection. We describe three phenotypic zones for C that correspond to previously established ecoregion boundaries. Because genetic data group C apart from subsp. *brevifolius* races A, B, and D, which originate in the Rocky Mountains and western Great Plains, the so-called race C merits description as a new subspecies apart from subsp. *brevifolius*.

Resumen

El pasto nativo amacollado (*Elymus elymoides* [Raf.] Swezey) es importante para la restauración de los pastizales de Norte América. Esta especie es taxonómicamente compleja y se ha separado al menos en cuatro subespecies incluyendo la especie *brevifolius* de la cual se han descrito cuatro razas geográficamente distintas (A, B, C, y D). De estas cuatro razas solo la C se encuentra en la parte norte de las Intermontañas del Oeste. Nuestro objetivo fue describir la variación fenotípica y genotípica dentro de C y determinar su estatus taxonómico. Evaluamos 32 poblaciones de C recolectadas a lo largo de la parte norte de las Intermontañas del Oeste de una pila de biomasa fenológica y rasgos funcionales establecidos en jardines comunes en el campo y el invernadero. La variación genética se evaluó usando marcadores AFLP y las correlaciones fueron calculadas entre matrices de distancia fenológica, genética, medioambiental y geográfica, usando la prueba de Mantel. Los valores de esas cuatro distancias medidas estuvieron correlacionados positivamente, sugiriendo que la heterogeneidad e asilamiento por la distancia están moldeando la diferencia ecotípica conducida por la selección natural. Describimos tres zonas fenotípicas para C que corresponden al establecimiento de las fronteras de la ecoregión. Debido a que el grupo genético C esta aparte de la especie *brevifolius* las razas A, B, y D que se originaron en las Montañas Rocosas y la parte oeste de las Grandes Planicies, la llamada raza C tiene el merito descriptivo como una nueva subespecie independiente de la especie *brevifolius*.

Key Words: AFLP, common garden, Great Basin, ecotype, squirreltail

INTRODUCTION

Restoration practices are valuable for mitigating the effects of habitat loss (McKay et al. 2005), and in recent decades there has been an increased availability of native grass seed for the western United States that makes it possible to seed extensive areas (Knapp and Rice 1998; Dunne and Dunne 2002, 2003). The widespread use of such material potentially introduces nonlocal genetic material into local populations, which may be problematic if the seed is maladapted to local conditions and/or if novel plant materials negatively impact a remnant population through gene flow (McKay et al. 2005). Site maladaptation is especially a concern for populations of autogamous species because they are particularly prone to genetic and ecological differentiation (Allard 1988; Hamrick et al. 1991; Allard 1999). On the other hand, negative impacts from gene flow are primarily problematic for allogamous species and can lead to outbreeding depression in progeny between local and nonlocal individuals (Waser and Price 1994; Keller et al. 2000; Hufford and Mazer 2003).

The squirreltail bunchgrasses, bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) and big squirreltail (*E. multisetus* [J.G. Sm.] Burtt Davy), formerly constituted the genus *Sitanion*

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Raf. (Cronquist et al. 1977; Barkworth 1997). Members of the squirreltail complex may be found from the Pacific Coast to long 90°W, and from British Columbia to Mexico (Wilson 1963). Spanning a wide variety of landscapes and ecoregions, this extensive distribution sets the stage for an autogamous group, like the squirreltails (Jensen et al. 1990), to diverge both genetically and ecologically. Autogamous species typically possess greater among-population and less within-population genetic variation than allogamous species (Karron 1991). For example, phenological variation among squirreltail populations varies considerably depending on geographic origin (Clary 1975), which suggests the importance of ecotypic variation in this group.

Bottlebrush squirreltail, by far the more widely distributed of the two squirreltail species, is divided into four subspecies, namely, subsp. *elymoides*, *californicus*, *hordeoides*, and *brevifolius* (Wilson 1963). Subsp. *brevifolius* is further divided into at least four races, A–D, which have been described based on neutral genetic markers and morphological and phenological variation (Jones et al. 2003; Larson et al. 2003). The Larson et al. (2003) AFLP analysis showed that *E. elymoides* subsp. *brevifolius* is paraphyletic, with race C of this subspecies being phylogenetically distinct from races A, B, and D. Race C, found in the northern Intermountain West, is geographically disjunct from races A and B, which originate in the Rocky Mountains, and race D, which originates in the western Great Plains (Wilson 1963; Larson et al. 2003).

Bottlebrush squirreltail is important for restoration of degraded sagebrush-steppe communities of the Great Basin desert (Jones 1998). Our objectives were 1) to discern relationships among traits by multivariate factor analysis of C populations distributed across the northern Intermountain Region and to correlate these trait-based factors with physiographic and environmental variables describing the population sites; 2) to delineate geographically based groups of populations based on analysis of C phenotypic data; 3) to characterize genetic variation within and among C populations; 4) to assess the importance of natural selection in shaping ecotypic variation by examining correlations among genotypic, phenotypic, environmental, and geographic distances among populations; and 5) to clarify the relationship between C and other bottlebrush squirreltail taxa.

METHODS

Phenotypic Data

Thirty-two putative race C populations were collected from 1995 to 2004 in California, Idaho, Nevada, and Oregon (Table 1). Sites were sampled by harvesting from at least 50 randomly selected plants (no closer than 5 m apart) along two or more transects that traversed the length of the sampled site, and seed was bulked to represent the plant population. Populations were evaluated in an unirrigated field trial at the Utah State University Millville research site (lat 41°39′23.8″N, long 111°48′51.9″W) south of Millville, Utah (Table 2). We transplanted greenhouse-grown seedlings on 13 May 2005 to 14-plant plots (7 by 2 plants on 0.4-m centers) in a randomized complete block design with three replications. A relatively small number of plants (42) were evaluated per population, as previous work had shown that bottlebrush squirreltail is highly autogamous (Jensen et al. 1990; Larson et al. 2003). For four populations, additional samples were taken from minority individuals that were visually distinctive in the field trial for awn color, leaf glaucousness, or phenological development. For each of these four populations, we referred to the minority individuals as a biotype relative to the population majority. No phenotypic data were collected on the four biotypes.

In 2006 and 2007, heading date was measured as the number of days after April 30 that at least two-thirds of the plants in a plot had at least two spikes emerging from the boot. The same protocol was used for two other phenological characters, anthesis date and awn-divergence date. Leaf-area index (LAI) was estimated in July 2006 and June 2007 by dividing a single above-canopy reading for each plot by the mean of three under-canopy readings with an AccuPAR LP-80 ceptometer (Decagon, Pullman, WA). Canopy height was measured on 22 June 2006 and 18 June 2007 midway between members of five to seven adjacent pairs of plants within a plot. To determine 2006 grain yield, grain was collected from each plot every few days as it ripened, bulked over the season, weighed, and divided by the number of surviving plants per plot. Subsamples of grain were cleaned, and a 200-grain sample was weighed to calculate individual grain mass. Neither grain yield nor grain mass was measured in 2007. Biomass for each plot was clipped at a 5-cm height on 24 July 2006, shortly after harvest, and also on 2 July 2007. Harvested biomass was dried in a forced-air oven at 40°C and weighed.

Additional data were collected in a greenhouse evaluation on the campus of Utah State University, Logan, UT (Table 3). We planted 1.9-L cups on 2 November 2006, each with five squirreltail seedlings of a single population, and thinned to three seedlings on 7 December 2006. Cups (experimental units) were arranged in a randomized complete block design with six replications. Thus, each population was represented in the greenhouse by 18 individuals. Soil, one part screened Ricks gravelly loam (coarse–loamy over sandy or sand–skeletal, mixed, superactive mesic Calcic Haploxerolls) to three parts Kidman fine sandy loam (coarse–loamy mixed mesic Calcic Haploxerolls), was steam sterilized before planting.

After 60 d we counted tillers and leaves, measured plant height, and determined above- and below-ground biomass. Leaves and roots at harvest were scanned on an Epson Expression 10000 XL scanner (Epson America, Inc., Long Beach, CA), and leaf area and leaf and root lengths were measured with the use of WinRHIZO software (Regent Instruments Inc., Saint-Foy, Québec, Canada). From these data, we calculated specific leaf area (SLA, leaf area/leaf dry-mass), specific root length (SRL, root length/root dry mass), leaf number per tiller (leaf number/tiller number), and mass per leaf (leaf dry mass/leaf number).

Thirteen of the 14 field-measured traits, all except 2006 grain mass, displayed significant differences among populations. Because some of these traits were biologically similar, and hence were highly correlated, we used a data-reduction technique to generate four new composite variables from 12 of the 13 traits (all except 2006 grain yield) to represent phenology, 2006 biomass, 2007 biomass, and canopy height. These 12 variables were standardized and subsequently

Table 1. Location, elevation, maximum and minimum temperature, average annual precipitation, and three factor scores for 32 C populations.

Population number	Location	Elevation (m)	Latitude (°N)	Longitude (°W)	T_{max} (°C)	T_{min} (°C)	Average annual precipitation (mm)	Factor 1	Factor 2	Factor 3
Acc:1123	Prairie City/Dixie Pass, Oregon	1 194	44.49	118.66	14	0	497	0.629	0.904	0.723
Acc:1135	Wildhorse Reservoir, Nevada	1 895	41.65	115.78	13	−4	362	−0.679	−0.479	0.486
Acc:1137	Carroll Summit, Lander County, Nevada	2163	39.25	117.77	15	0	328	−2.897	1.603	−0.447
T-1202	Hwys 75 and 20, Blaine County, Idaho	1 512	43.30	114.29	14	−1	327	−1.097	−0.305	0.012
T-1203	E Fairfield, Idaho	1 533	43.34	114.69	13	−3	350	−1.380	−1.297	−0.257
T-1204	E Hill City, Idaho	1 551	43.32	115.01	13	−2	355	−0.219	1.122	−0.823
T-1205	W Hill City, Idaho	1 665	43.32	115.26	13	−1	573	−0.367	−0.801	−0.694
T-1206	E Dixie, Elmore County, Idaho	1 523	43.32	115.34	13	−1	567	0.257	−1.039	−1.193
T-1306	E Dixie, Elmore County, Idaho	1 523	43.32	115.34	13	−1	567	−0.162	−0.839	−1.503
T-1345	E Baker, Oregon	1 121	44.73	117.80	14	0	341	1.359	−1.242	1.046
T-1346	Pleasant Valley, Oregon	1 166	44.67	117.62	14	1	361	1.280	−1.898	1.372
T-1358	Nevada County, California	1 827	39.38	120.07	15	−3	631	−1.195	−0.279	−0.168
T-1375	Tahoe City, California	1 944	39.20	120.10	13	−1	821	−0.734	−0.442	0.120
T-1452	Clover Creek Valley, Oregon	1 031	45.08	117.95	14	0	417	0.389	−1.399	1.838
T-1472	E Antelope, Oregon	1 120	44.90	120.60	13	2	360	0.702	0.106	0.558
T-1477	Shaniko, Oregon	1 019	45.00	120.76	15	1	353	0.905	0.202	−1.359
T-1478	S Redmond, Oregon	949	44.21	121.22	16	0	242	0.630	0.572	1.053
T-1483	W Dairy, Oregon	1 259	42.20	121.60	15	0	357	0.972	0.624	0.146
T-1485	NE Dairy, Oregon	1 449	42.32	121.40	15	0	449	−0.503	1.314	0.347
T-1489	Bly, Oregon	1 326	42.40	121.04	16	−1	371	0.283	0.568	0.673
T-1502	N Lakeview, Oregon	1 473	42.23	120.37	14	0	406	1.133	0.527	−1.106
T-1504	Plush cutoff, Oregon	1 723	42.29	120.07	14	0	435	−1.357	−0.008	1.314
T-1529	S Adin, California	1 525	41.01	120.83	15	0	447	−0.100	−0.110	−0.274
T-1586	SE Owyhee, Nevada	1 663	41.92	116.06	13	−1	440	0.493	−0.432	−0.919
T-1591	N Jordan Valley, Oregon	1 401	43.14	117.04	15	0	403	0.034	−1.134	0.179
T-1595	Jordan Valley, Oregon	1 339	42.98	117.06	16	0	356	0.190	−1.216	−2.105
T-1600	E Burns, Oregon	1 325	43.65	118.62	14	−1	315	−1.501	−0.557	1.350
T-1614	Fox, Oregon	1 468	44.67	119.14	13	0	449	−0.235	0.725	−1.106
T-1618	Ukiah, Oregon	1 008	45.13	118.96	15	−1	444	1.398	0.865	−0.782
GV	Grandview, Oregon	794	44.53	121.25	16	1	274	0.646	0.923	−0.668
SHPL	Shaniko Plateau, Oregon	1 019	45.00	120.76	15	1	353	1.289	2.024	0.950
CRNG	Crooked River National Grassland, Oregon	794	44.53	121.25	16	1	274	−0.161	1.399	1.238

consolidated with the use of PROC PRINCOMP (SAS Institute 1999). We used principal-component analysis (PCA) instead of factor analysis for this initial step because the primary objective was data reduction (Hair et al. 2006).

Second, common-factor analysis was conducted with the use of SAS PROC FACTOR on the four composite variables and the remaining original field variable (2006 grain yield), as well as eight greenhouse-measured variables: tiller number, plant height, leaf area, SLA, root length, SRL, leaf number per tiller, and mass per leaf. For this second step, we used common-factor analysis, as opposed to PCA, because the primary objective was not data reduction, but instead description of innate relationships among variables (Hair et al. 2006).

Next, we extracted three factors and then applied a SAS VARIMAX rotation. The purpose of such an orthogonal rotation is to generate high factor loadings for a few variables and low loadings for the majority of variables in order to derive a simpler and more interpretable result. Then, with the use of the same 13 variables as the factor analysis (four composite field variables, 2006 grain yield, and eight greenhouse variables), we created a phenotypic Euclidean-distance matrix with the use of SAS PROC DISTANCE. With this matrix we conducted a cluster analysis with the use of the average linkage method (UPGMA) in SAS PROC CLUSTER and then generated a phenotypic-distance dendrogram with the use of PROC TREE.

Genotypic Data

An AFLP analysis was conducted on the 36 populations (including the four biotypes) from the field study. We sampled DNA of the four biotypes separately from the majority

Table 2. F-test results and associated statistics for 14 field-measured putative adaptive traits measured in 2006 and 2007 on 32 C populations.

	Numerator, denominator df	F	Mean (SE)	Range
2006 field traits				
Heading date (d after 30 April)	31, 59	34.67[1]	29.7 (0.7)	20.7–36.0
Anthesis date (d after 30 April)	31, 59	30.82[1]	37.3 (0.8)	30.0–43.3
Awn-divergence date (d after 30 April)	31, 60	34.82[1]	54.0 (0.7)	46.0–59.0
Biomass (g plant^{-1})	31, 62	5.55[1]	24.0 (2.0)	8.9–31.7
Canopy height (cm plant^{-1})	31, 61	6.10[1]	57.6 (1.8)	48.5–66.1
Leaf-area index	31, 62	2.66[2]	0.50 (0.07)	0.28–0.69
Grain yield (g plant^{-1})	31, 62	2.52[2]	10.7 (1.7)	3.0–16.6
Grain mass (mg)	31, 58	1.51[3]	2.64 (0.27)	1.94–3.28
2007 field traits				
Heading date (d after 30 April)	31, 58	34.72[1]	29.2 (0.8)	21.3–36.0
Anthesis date (d after 30 April)	31, 61	10.88[1]	36.5 (1.3)	30.0–45.3
Awn-divergence date (d after 30 April)	31, 62	10.77[1]	56.3 (0.9)	52.0–62.0
Biomass (g plant^{-1})	31, 62	5.95[1]	40.2 (4.8)	5.8–67.6
Canopy height (cm)	31, 62	11.1[1]	59.1 (1.4)	40.5–66.8
Leaf area index	31, 62	2.59[2]	0.75 (0.11)	0.24–1.16

[1]Significant at $P < 0.0001$.
[2]Significant at $P < 0.01$.
[3]Not significant ($P \geq 0.05$).

individuals. We identified the four minority biotypes with their respective population identifier preceded by BT. For example, BTT1600 is the minority biotype found in the T-1600 population. DNA was also sampled from Pueblo, Wapiti, and Tusas germplasms (*E. elymoides* subsp. *brevifolius* race A; Tilley et al. 2006); Toe Jam Creek germplasm (Jones et al. 2004b) and T-1735 (origin: Gooding County, Idaho; *E. elymoides* subsp. *californicus*); Fish Creek (Jones et al. 2004a) and Rattlesnake (Jones 2010) germplasms (*E. elymoides* subsp. *elymoides*); and Sand Hollow germplasm (*E. multisetus*; Jones et al. 1998).

Plant tissue for DNA extraction of up to eight plants for each of the 36 populations was collected from the field plot after fall green-up in 2006. After exclusion of outliers, i.e., those individuals that grouped distantly from the population majority based on the neighbor-joining tree described below, 12 populations were represented by eight plants, 12 by seven plants, 8 by six plants, 2 by five plants, and 2 by four plants.

Table 3. F-test results and associated statistics for 21 greenhouse-measured putative adaptive traits measured on 32 C populations.

Greenhouse traits	Numerator, denominator df	F	Mean (SE)	Range
Cumulative leaf length (m) after 14 d	31, 155	6.41[1]	0.125 (0.006)	0.095–0.148
Cumulative leaf length (m) after 35 d	31, 155	5.77[1]	0.845 (0.052)	0.627–1.149
Cumulative leaf length (m) after 60 d	31, 155	2.58[1]	2.09 (0.12)	1.74–2.38
Tiller number after 35 d	31, 153	2.06[2]	2.97 (0.29)	2.33–4.22
Tiller number after 60 d	31, 155	2.12[2]	5.47 (0.41)	4.44–6.61
Leaf number (plant^{-1})	31, 155	2.73[1]	18.9 (1.2)	16.0–21.5
Plant height (m plant^{-1})	31, 155	8.01[1]	0.234 (0.010)	0.180–0.283
Leaf area (mm^2 plant^{-1})	31, 154	1.95[2]	1 928 (137)	1 635–2 299
Dry shoot mass (g plant^{-1})	31, 153	2.94[1]	0.178 (0.012)	0.138–0.206
Dry leaf mass (g plant^{-1})	31, 149	3.04[1]	0.122 (0.007)	0.093–0.140
Specific leaf area (m^2 kg^{-1})	31, 154	1.69[3]	15.8 (1.2)	13.5–17.5
Leaf area ratio (m^2 kg^{-1})	31, 151	1.60[3]	11.0 (0.8)	9.3–12.0
Root length (m plant^{-1})	31, 153	3.99[1]	6.53 (0.46)	4.72–8.30
Dry root mass (mg plant^{-1})	31, 155	4.10[1]	65.8 (9.1)	33.3–95.6
Specific root length (m g^{-1})	31, 155	3.41[1]	107.6 (10.2)	83.0–154.2
Leaf number (tiller^{-1})	31, 149	1.57[3]	3.46 (0.18)	3.00–3.86
Mass per tiller (mg)	31, 153	2.63[1]	33.7 (3.2)	24.2–44.5
Mass per leaf (mg)	31, 152	4.82[1]	6.61 (0.41)	4.96–8.59
Root-to-shoot ratio	31, 152	3.11[1]	0.362 (0.009)	0.227–0.434

[1]Significant at $P < 0.0001$.
[2]Significant at $P < 0.01$.
[3]Significant at $P < 0.05$.

Tissue of the remaining eight populations was obtained in the greenhouse. Population T-1204 was removed from the AFLP data set because several of the individual plants included in the sampling procedure were *E. multisetus* contaminants. This led to an inflated estimate of genetic variation within this group and therefore an inability to place it in the dendrogram accurately.

DNA of individual plants was extracted with the use of the DNeasy 96-well extraction kit (QIAGEN, Valencia, CA). Quantity and quality of DNA were assessed by spectrophotometry and agarose gel electrophoresis. The AFLP technique was performed as described by Vos et al. (1995), except that *Eco*RI selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on the 5′ nucleotide. Also, the preamplification primer combination (i.e., E.AC/M.CT) included two selective nucleotides in addition to the universal *Eco*RI and *Mse*I adapter sequences. The selective primers used were E.AAG/M.CAG, E.AAT/M.CTC, E.ACG/M.CAC, E.AGG/M.CAG, and E.AGT/M.CAA. Amplicons were separated on a capillary ABI 3730 instrument with the GS-500 LIZ size standard and Genescan software (Applied Biosystems, Foster City, CA). Selective amplification reactions were replicated for each preamplified DNA sample. The 6-FAM–labeled amplification products were size-fractionated by the Utah State University Center for Integrated Biosystems with the use of an ABI 3100 instrument with 50-cm capillaries, POP-6 polymer, Genescan 400HD [ROX (rhodamine X)] internal size standards, and Genescan software (PE Applied Biosystems). The Genescan sample files were visually analyzed for the presence or absence of DNA fragments between 50 and 400 base pairs with the use of Genographer version 1.5 (Benham et al. 1999). Only bands with at least 100 relative fluorescent units were included in the analysis.

Neighbor-joining genetic distance analysis (Saitou and Nei 1987) and analysis of molecular variance (AMOVA; Excoffier et al. 1992) were used to analyze and test AFLP variation within and among groups. Genetic distances between individual plants were determined by pairwise comparison of the absolute number of differences between AFLP profiles of individual plants (Euclidean distances). The average proportion of shared fragments was computed as $2N_m/(N_x + N_y)$, where N_m was the number of pairs of bands matching between individuals, and N_x and N_y were the total number of bands amplified from the two individuals (Dice 1945; Nei and Li 1979; Lynch 1990). Neither Euclidean distances nor the average proportion of shared fragments count shared null alleles.

Population subdivision was tested with the use of AMOVA. Raw binary data were converted to Euclidean distance, with the resulting distance matrix constituting the input file for AMOVA with the use of Arlequin 2.0 software (Genetics and Biometry Laboratory, Department of Anthropology and Ecology, University of Geneva, Geneva, Switzerland). With this matrix, we created a genetic-distance dendrogram by the neighbor-joining method with midpoint rooting in PAUP* version 4.0 (Sinauer Associates, Inc., Sunderland, MA). We developed a graphic display of the neighbor-joining tree with the use of TREEVIEW (Page 1996) and reported bootstrap values ≥ 50.

Correlations between genetic, phenoptypic, environmental, and geographic distances (see below) were evaluated by the Mantel (1967) test statistic (Z), with the use of the MxComp procedure of NTSYS-pc (Rohlf 1998). Significance tests for these correlations were determined by comparing observed values to values obtained by 1 000 random permutations (Smouse et al. 1986). Therefore, the upper-tail probability (p) that 1 000 random Mantel test statistic (Z) values are (by chance) less than observed values of Z equals 0.002 or greater.

Environmental, Physiographic, and Geographic Data

Latitude and longitude coordinates (NAD 83) of collection sites were collected using a Sony Pyxis IPS-360 global positioning system (Sony Corporation, Minato, Tokyo, Japan). Coordinates were uploaded to derive elevation and 30-yr average (1971–2000) annual minimum temperature (T_{min}), annual maximum temperature (T_{max}), and annual precipitation (AAP; Table 1), as well as to identify Omernik Level III ecoregions (Western Ecology Division, United States Environmental Protection Agency 2007), with the use of the Data Extraction Tool (Forage and Range Research Laboratory and Utah State University RS/GIS Lab 2008).

Correlation coefficients among physiographic variables (elevation, latitude, and longitude), environmental variables (T_{min}, T_{max}, and AAP), and the three previously described orthogonal multivariate factors were calculated from the phenotypic data set. Linear regression described the relationship between the populations' phenotypes, as reflected by their factor scores, and the physiographic and environmental characteristics of the populations' collection sites. A Euclidean-distance matrix was constructed from the environmental data with the use of PROC DISTANCE, which was then used to conduct a UPGMA cluster analysis (PROC CLUSTER). Then, an environmental-distance dendrogram was created with the use of PROC TREE. Geographic distance (straight-line distance) between populations was calculated following the protocol of Larson et al. (2004). Finally, to quantify the relationship between the populations' environments, phenotypes, and genotypes, we calculated correlation coefficients between environmental, phenotypic, genotypic, and geographic distance matrices. This was accomplished with Mantel Asymptotic Approximation tests, with the use of PC-ORD version 5 (MjM Software Design, Gleneden Beach, OR).

RESULTS

Site Characteristics

We found several significant correlations among the collection-site physiographic and environmental variables (Table 4). Elevation was negatively correlated with latitude, longitude, T_{max}, and T_{min}, and positively correlated with AAP. Average annual precipitation was negatively correlated with T_{max}, and T_{max} was positively correlated with T_{min}. To summarize, sites of northerly populations were at lower elevations that tend to receive less precipitation and experience higher maximum temperatures than sites of southerly populations. Sites of westerly populations were also at lower elevations that experience higher maximum and minimum temperatures than those of easterly populations. Higher-elevation populations

Table 4. Pearson's correlation coefficients (r) among three physiographic variables (elevation, latitude, longitude), three environmental variables (average maximum temperature [T_{max}], average minimum temperature [T_{min}], and average annual precipitation [AAP]), and three factor scores.

	Elevation	Latitude	Longitude	T_{max}	T_{min}	AAP
Latitude	−0.836[1]					
Longitude	−0.410[2]	0.003[3]				
T_{max}	−0.469[4]	0.044[3]	0.611[4]			
T_{min}	−0.597[4]	0.459[4]	0.540[4]	0.374[2]		
AAP	0.518[4]	−0.473[4]	−0.118[3]	−0.447[2]	−0.304	
Factor 1	−0.708[1]	0.612[4]	0.281[3]	0.161[3]	0.450[2]	−0.144[3]
Factor 2	−0.155[3]	−0.011[3]	0.541[2]	0.390[2]	0.261[3]	−0.239[3]
Factor 3	−0.267[3]	0.177[3]	0.300	0.128[3]	0.167[3]	−0.242[3]

[1]Significant at $P < 0.0001$.
[2]Significant at $P < 0.05$.
[3]Not significant at $P < 0.10$.
[4]Significant at $P < 0.01$.

were generally located further south and east and exposed to colder and wetter climatic conditions.

Relationships Among Plant Traits and Their Relationships to Site Characteristics

All six phenological measurements (heading dates, anthesis dates, and awn-divergence dates in 2006 and 2007) were positively correlated ($r = 0.752$–944, $P < 0.0001$). Consequently, they were reduced into a single composite variable representing phenology. The composite variable was the first principal component of the six original phenological variables, which described 89.6% of their variation. Biomass and LAI from 2006 were positively correlated ($r = 0.795$, $P < 0.0001$), and these were also combined to represent 2006 biomass-related measurements, with the first principal component describing 82.2% of that variation. Biomass and LAI from 2007 were positively correlated as well ($r = 0.879$, $P < 0.0001$) and were likewise combined into a new composite variable representing 70.9% of that variation. Also, canopy heights from 2006 and 2007 were positively correlated ($r = 0.727$, $P < 0.0001$), and these were combined into a single composite variable representing 55.9% of that variation. Grain yield, measured only in 2006, was uncorrelated ($P > 0.05$) with 11 of the 12 other variables, with the sole exception being 2006 biomass ($r = 0.361$, $P < 0.05$). Thus, 2006 grain yield was not combined with other variables but was instead considered separately.

After common-factor analysis was conducted on the four new composite variables and nine directly measured standardized variables (2006 grain yield and eight greenhouse variables), we extracted three factors. Together, these factors explained 65.0% of the variation for these 13 variables (Table 5). Factor 1, which explains 37.7% of the variation among all variables, has highly positive factor loadings for phenology and 2006 and 2007 biomass and negative loadings for leaf area and root length (Table 5). Factor 1 was positively correlated with latitude and T_{min}, negatively correlated with elevation, and uncorrelated with longitude, T_{max}, and AAP (Table 4).

In order to correct for the confounding effects of latitude and longitude on elevation, we regressed the residuals of Factor 1

Table 5. Factor loadings for three orthogonal factors derived from four composite (C) and nine directly (D) measured variables in the field (F) and greenhouse (GH).

Variable	Factor 1	Factor 2	Factor 3
Phenology (C, F)	**0.815**[1]	0.244	0.004
2006 biomass (C, F)	**0.733**	−0.392	0.253
2007 biomass (C, F)	**0.712**	−0.112	0.275
Canopy height (C, F)	−0.360	0.370	**−0.590**
2006 grain yield (D, F)	−0.008	**−0.597**	0.085
Leaf area (D, GH)	**−0.747**	0.228	0.119
Root length (D, GH)	**−0.535**	−0.136	−0.415
60-d tiller number (D, GH)	0.011	**−0.774**	0.283
Plant height (D, GH)	−0.388	**0.641**	−0.419
Leaf number per tiller (D, GH)	−0.048	**0.750**	0.248
Specific leaf area LA (D, GH)	−0.235	0.189	**0.883**
Specific root length (D, GH)	0.362	−0.305	**0.563**
Mass per tiller (D, GH)	−0.403	0.382	**−0.725**
% variance explained	37.7	14.5	12.8

[1]Factor loadings greater than 0.5 or less than −0.5 are bolded.

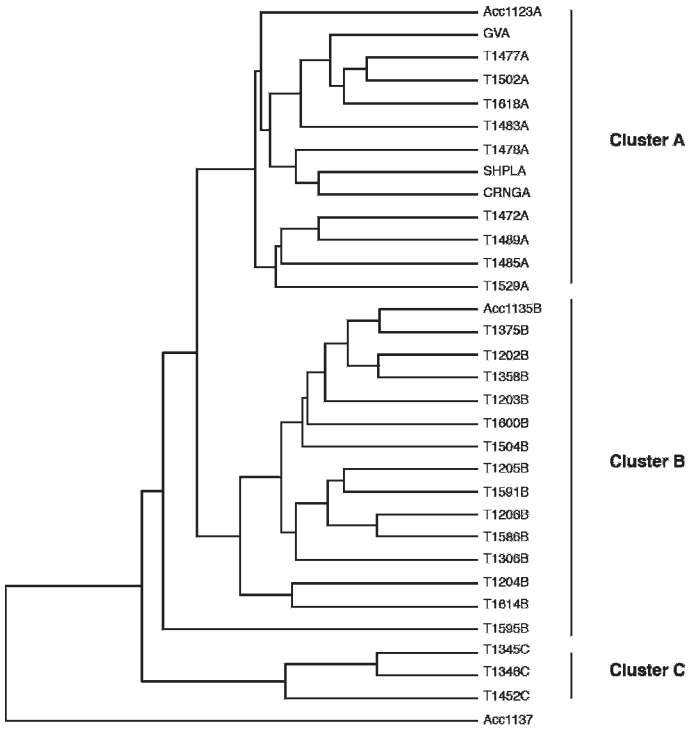

Figure 1. Phenotypic-distance dendrogram of 32 C populations showing three phenotypic clusters (A, B, C).

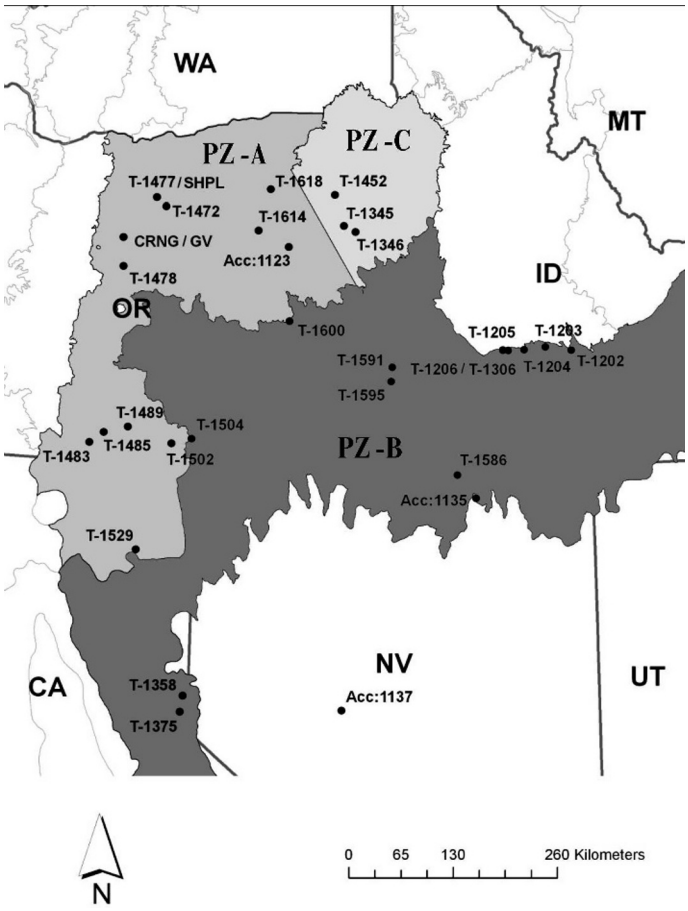

Figure 2. Map of 32 C population locations across three phenotypic zones (PZ-A, PZ-B, and PZ-C).

after accounting for latitude and longitude on the residuals of elevation after accounting for latitude and longitude. The new model still showed a negative relationship between Factor 1 score and elevation, but accounted for only 9% of the variation in Factor 1 ($P < 0.10$). There was no significant ($P > 0.10$) effect of latitude on Factor 1 after correcting for elevation and longitude.

Factor 2, which explains 14.5% of the variation among all of the variables, had positive factor loadings for greenhouse plant height and leaf number per tiller and negative loadings for grain yield and greenhouse tiller number (Table 5). Positive correlations (Table 4) were found between Factor 2 and both T_{max} and longitude. Factor 2 was uncorrelated with T_{min}, elevation, latitude, and AAP. Longitude remained positively correlated with Factor 2 after accounting for elevation and latitude ($r = 0.500$, $P < 0.01$).

Factor 3, which explains about 12.8% of the variation among all of the variables, had positive factor loadings for SRL and SLA and negative loadings for canopy height and mass per tiller (Table 5). However, Factor 3 was not correlated with any physiographic or environmental variables (Table 4).

Populations with high Factor 1 scores (late maturity, high biomass, low greenhouse leaf area, and root length) tended to originate at lower elevations and higher latitudes and experience higher T_{min} (Table 4). Populations with low Factor 2 scores (high grain yield, high greenhouse tiller number, short greenhouse plant height, and few greenhouse leaves per tiller) tended to originate at westerly longitudes and experience lower T_{max}. Populations with high Factor 3 scores tended to exhibit short field canopy height, high SLA, high SRL, and low greenhouse tiller mass. Average annual precipitation was not correlated with any of the three factors.

Geographically Based Groups of Populations

The results of the phenotypic-distance dendrogram, constructed from the 13 original field-measured variables and 8 original greenhouse-measured variables, indicated that all populations except Acc:1137 grouped into one of three clusters, designated A, B, and C (Fig. 1). When each pairwise combination of factors was plotted, populations separated into three clusters without overlap (data not shown). Factor 1 separated populations with high biomass, late maturity, and low surface area (clusters A and C) from populations with low biomass, early maturity, and high surface area (cluster B). Factor 2 separated populations with few, tall greenhouse tillers and low grain yield (cluster A) from populations with many, short greenhouse tillers and high grain yield (clusters B and C). These clusters correspond very closely with geographic origin (Fig. 2), specifically Level III ecoregions (Western Ecology Division, United States Environmental Protection Agency 2007).

Genetic Characterization

A total of 982 fragment-size categories were detected across 296 plants, or approximately 196 per primer pair. Of these, 61 were monomorphic and 31 were present in only one plant, leaving 890 that were informative. Individual samples were not resampled in different runs, but plants within an accession generally grouped together.

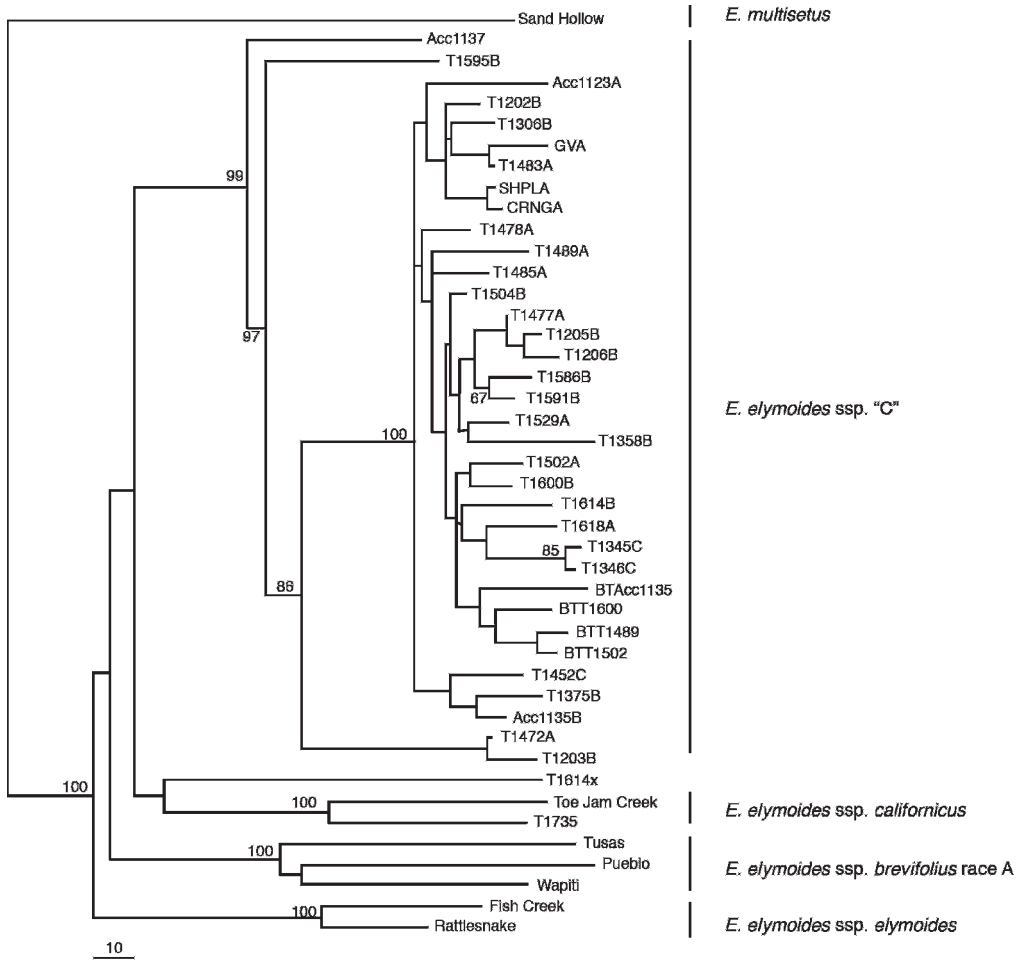

Figure 3. Genetic-distance dendrogram with bootstrap values ≥ 50. Each population identifier is followed by a letter designating its cluster in the phenotypic-distance dendrogram (see Fig. 1). Four minority biotypes are designated by the prefix BT.

Twenty-seven of the putative race C populations, accompanied by the four biotypes, clustered into a single well-defined major clade supported by a bootstrap value of 100 (Fig. 3). None of the minority biotypes grouped with their sympatric majority companions, but all grouped within this major clade. Four populations, T-1472, T-1203, Acc:1137, T-1595, which were not in geographic proximity to one another (Table 1), grouped apart from the major clade (Fig. 3). These four populations are not as genetically homogeneous as those in the major clade, and their taxonomic position is unclear. They appear to be more closely related to C than the other taxa examined here, including race A, yet they may be distinct from C. One of the 27 populations, T-1614, was genetically mixed, with four plants falling within the major clade and four others, designated T-1614x, grouping apart from the major clade.

Table 6. Mantel asymptotic approximation correlation coefficients among four distance measures.

	Phenotypic distance	Genetic distance	Environmental distance
Genetic distance	0.900[1]		
Environmental distance	0.723[1]	0.778[1]	
Geographic distance	0.801[1]	0.776[1]	0.803[1]

[1]Significant at $P < 0.0001$.

Overall, the AMOVA results indicated that 49.7% (33 df) of the genetic variation was partitioned among the C populations with 50.3% (202 df) remaining within populations. The AMOVA indicated that 3.2% ($P < 0.05$) of that 49.7% among-population variation was accounted for by the three clusters (2 df) identified by the phenotypic data, leaving 46.5% among populations within clusters.

Correlations Among Four Distance Measurements

Based on the Mantel tests, we report positive correlations for all pairwise combinations of the four distance matrices (phenotypic, genetic, environmental, geographic), ranging from $r = 0.723$–0.900 ($P < 0.0001$; Table 6). This means that more distantly related populations were more phenotypically different, and that these distances were positively associated with distances in physical (geographic) and ecological distance.

Genetic Distances Between C and Other Taxa

The genetic distance between C and subsp. *brevifolius* race A (78.7) was found to be comparable to the genetic distance between C and subsp. *californicus* (78.4) and that between C and subsp. *elymoides* (97.5; Fig. 3). Furthermore, the genetic distance between race C and subsp. *brevifolius* race A (78.7) was of the same order of magnitude of that between subsp.

californicus and subsp. *brevifolius* race A (99.9), subsp. *brevifolius* race A and subsp. *elymoides* (105.9), and subsp. *californicus* and subsp. *elymoides* (118.7).

DISCUSSION

Because our squirreltail populations closely correspond to their respective environments, it is desirable for land managers to match C plant material to target site, at least when the target site has not been severely disturbed (Jones and Monaco 2010; Johnson et al. 2009). However, because we conducted phenotypic evaluations in the greenhouse and at a single common-garden site, we cannot estimate the importance of genotype-by-environment interaction in influencing the measured plant traits. In addition, because we did not have reciprocal common gardens, we refrain from presenting seed-transfer guidelines.

Despite this limitation, these data did allow us to distinguish three phenotypic clusters (A, B, and C) that correspond to three phenotypic zones (PZ-A, PZ-B, and PZ-C). Phenotypic Zone-A encompasses the Blue Mountains (western part), Columbia Plateau, and Eastern Cascades Slopes and Foothills Level III ecoregions, whereas PZ-B encompasses the Sierra Nevada, Snake River Plain, and Northern Basin and Range Level III ecoregions. Phenotypic Zone-C encompasses the eastern portion of the Blue Mountains Level III ecoregion, and its populations are quite divergent phenotypically from PZ-A and PZ-B populations. This information complements previous work that identifies elevation as an important character for matching plant material to restoration site in C (Parsons et al., 2011).

It is not surprising to find genetically distinct plants, like the four biotypes reported here, within populations of an inbreeding species. Similar findings have been reported with other inbreeding species like Indian ricegrass (*Achnatherum hymenoides* [Roem. & Schult.] Barkworth; Jones et al. 2007). In this case, sympatric combinations of biotypes appear to have resulted through independent migration to a common site.

The positive correlations between each pair of the four distance matrices suggest that both environmental heterogeneity and isolation by distance have shaped genetic and phenotypic divergence in this group. Specifically, the positive relationship between environmental and phenotypic distances indicates 1) that race C populations are indeed exhibiting ecotypic divergence and 2) that natural selection is the dominant force driving phenotypic variation among populations. Of the physiographic and environmental variables we considered, the most important for shaping adaptation appear to be elevation and, to a lesser extent, T_{max} and T_{min}.

Studies with two native, autogamous perennial grasses, purple needlegrass (*Nassella pulchra* [Hitchc.] Barkworth; Knapp and Rice 1998) and blue wildrye (*Elymus glaucus* Buckley; Erickson et al. 2004), have found quantitative-trait variation to be strongly associated with climatic variation. These findings are consistent with the hypothesis that selection associated with climate has an important influence on patterns of quantitative-trait variation. On the other hand, selection is not always the dominant force shaping population differentiation. For the neotropical savanna C4 grass, *Trachypogon plumosus* (Humb. & Bonpl. ex Willd.) Nees, Baruch et al. (2004) attributed the existence of locally differentiated ecotypes to neutral processes (genetic drift, founder effect, or migration) rather than to local adaptation.

The results of the AFLP analysis provide important insight into the phylogenetic relationships between C and the other squirreltail taxa. Previous research based on AFLP markers (Larson et al. 2003) found that C was genetically dissimilar from races A, B, and D and was more similar to subsp. *elymoides* than other squirreltail taxa included therein. However, subsp. *californicus* was not included in that study, unlike here.

Our data are consistent with the Larson et al. (2003) finding that race C is an anomaly within subsp. *brevifolius*. In fact, our results (Fig. 3) suggest that the so-called race C is a legitimate subspecies on par with subsp. *elymoides*, subsp. *californicus*, and subsp. *brevifolius* (encompassing races A, B, and D only), despite the fact that C, like A, B, and D, keys to subsp. *brevifolius* (Wilson 1963). In hindsight, this may not be surprising, based on the disjunct geographical distribution of C to the west relative to A, B, and D to the east. In addition, restoration practitioners should be aware that subsp. *elymoides* and subsp. *californicus*, as well as *E. multisetus*, are also prevalent in the northern Intermountain West. In fact, the second author has often found multiple squirreltail species or subspecies within a site in the region.

IMPLICATIONS

Our DNA data clearly show that the northern Intermountain material identified as *E. elymoides* subsp. *brevifolius* represents a new and distinct subspecies. Therefore, plant materials of subsp. *brevifolius* that originate in the Rocky Mountains or western Great Plains, e.g., Tusas, Pueblo, and Wapiti germplasms, do not genetically represent the northern Intermountain material (or vice versa). Previous research has shown that elevation is a useful variable to match seed source to restoration site for the new subspecies in the northern Intermountain West. Here we present three phenotypic zones that are useful to characterize C populations initially and to provide preliminary guidance to future research toward establishing seed-transfer guidelines.

LITERATURE CITED

Allard, R. W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. *Journal of Heredity* 79:225.

Allard, R. W. 1999. History of plant population genetics. *Annual Review of Genetics* 33:1–27.

Barkworth, M. E. 1997. Taxonomic and nomenclatural comments on the Triticeae in North America. *Phytologia* 83:302–311.

Baruch, Z., J. M. Nassar, and J. Bubis. 2004. Quantitative trait, genetic, environmental, and geographical distances among populations of the C_4 grass *Trachypogon plumosus* in neotropical savannas. *Diversity and Distributions* 10:283–292.

Benham, J., J. U. Jeung, M. Jasieniuk, V. Kanazin, and T. Blake. 1999. Genographer: a graphical tool for automated AFLP and microsatellite analysis. *Journal*

of Agricultural Genomics 4. Available at: http://wheat.pw.usda.gov/jag/papers99/paper399/indexp399.html. Accessed 30 August 2010.

Clary, W. P. 1975. Ecotypic adaptation in *Sitanion hystrix*. *Ecology* 56:1407–1415.

Cronquist, A., A. H. Holmgren, N. H. Holmgren, J. L. Reveal, and P. K. Holmgren. 1977. Intermountain flora, vascular plants of the Intermountain West, U.S.A. Volume 6. New York, NY, USA: Columbia University Press. 584 p.

Dice, L. R. 1945. Measures of the amount of ecologic association between species. *Ecology* 26:297–302.

Dunne, R. A., and C. G. Dunne. 2002. Potential for expanded production of native rangeland seeds in western North America. *Native Plants Journal* 3:34–37.

Dunne, R. A., and C. G. Dunne. 2003. Trends in the native plant seed industry since 1990. *Native Plants Journal* 4:88–94.

Erickson, V. J., N. L. Mandel, and F. C. Sorenson. 2004. Landscape patterns of phenotypic variation and population structuring in a selfing grass, *Elymus glaucus* (blue wildrye). *Canadian Journal of Botany* 82:1776–1789.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.

Forage and Range Research Laboratory and Utah State University RS/GIS Lab. 2008. ARS data extraction tool. Available at: http://earth.gis.usu.edu/ars/ index.phtml. Accessed 30 August 2010.

Hair, J. F., W. C. Black, B. J. Babin, R. E. Anderson, and T. L. Tatham. 2006. Multivariate data analysis. 6th ed. Upper Saddle River, NJ, USA: Pearson. 899 p.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. *In:* D. A. Falk and K. E. Holsinger [eds.]. Genetics and conservation of rare plants. New York, NY, USA: Oxford University Press. p. 75–86.

Hufford, K. M., and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* 18:147–155.

Jensen, K. B., Y. F. Zhang, and D. R. Dewey. 1990. Mode of pollination of perennial species of the Triticeae in relation to genomically defined genera. *Canadian Journal of Plant Science* 70:215–225.

Johnson, R., L. Strich, P. Olwell, S. Lambert, M. E. Horning, and R. Cronn. 2010. What are the best seed sources for ecosystem restoration on BLM and USFS lands? *Native Plants Journal* 11:117–131.

Jones, T. A. 1998. Viewpoint: the present status and future prospects of squirreltail research. *Journal of Range Management* 51:326–331.

Jones, T. A. 2010. Notice of release of Rattlesnake Germplasm bottlebrush squirreltail. *Native Plants Journal* 11:45–50.

Jones, T. A., and T. A. Monaco. 2009. A role for assisted evolution in designing native plant materials for domesticated landscapes. *Frontiers in Ecology and the Environment* 7:541–547.

Jones, T. A., D. C. Nielson, J. T. Arredondo, and M. G. Redinbaugh. 2003. Characterization of diversity among three squirreltail taxa. *Journal of Range Management* 56:474–482.

Jones, T. A., D. C. Nielson, S. R. Larson, D. A. Johnson, T. A. Monaco, S. L. Caicco, D. G. Ogle, S. A. Young, and J. R. Carlson. 2004a. Registration of Fish Creek bottlebrush squirreltail germplasm. *Crop Science* 44:1879–1880.

Jones, T. A., D. C. Nielson, S. R. Larson, D. A. Johnson, T. A. Monaco, S. L. Caicco, D. G. Ogle, S. A. Young, and J. R. Carlson. 2004b. Registration of Toe Jam Creek bottlebrush squirreltail germplasm. *Crop Science* 44:1880–1881.

Jones, T. A., D. C. Nielson, D. G. Ogle, D. A. Johnson, and S. A. Young. 1998. Registration of Sand Hollow squirreltail germplasm. *Crop Science* 38:286.

Jones, T. A., M. G. Redinbaugh, S. R. Larson, Y. Zhang, and B. D. Dow. 2007. Polymorphic Indian ricegrass populations result from overlapping waves of migration. *Western North American Naturalist* 67:338–346.

Karron, J. D. 1991. Patterns of genetic variation and breeding systems in rare plant species. *In:* D. A. Falk and K. E. Holsinger [eds.]. Genetics and conservation of rare plants. New York, NY, USA: Oxford University Press. p. 87–98.

Keller, M., J. Kollmann, and P. J. Edwards. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology* 37:647–659.

Knapp, E. E., and K. J. Rice. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nassella pulchra*). *Conservation Biology* 12:1031–1041.

Larson, S. R., T. A. Jones, and K. B. Jensen. 2004. Population structure in *Pseudoroegneria spicata* (Poaceae: Triticeae) modeled by Bayesian clustering of AFLP genotypes. *American Journal of Botany* 91:1789–1801.

Larson, S. R., T. A. Jones, C. L. McCracken, and K. B. Jensen. 2003. Amplified fragment length polymorphism in *Elymus elymoides*, *Elymus multisetus*, and other *Elymus* taxa. *Canadian Journal of Botany* 81:789–804.

Lynch, M. 1990. The similarity index and DNA fingerprinting. *Molecular Biology and Evolution* 7:478–484.

Mantel, N. A. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209–220.

McKay, J. M., C. E. Christian, S. Harrison, and K. J. Rice. 2005. "How local is local?"—a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13:432–440.

Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* 76:5269–5273.

Page, R. D. 1996. Treeview: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12:357–358.

Parsons, M. C., T. A. Jones, and T. A. Monaco. 2011. Genetic variation for adaptive traits in bottlebrush squirreltail in the northern Intermountain West, USA. *Restoration Ecology* 19:460–469.

Rohlf, F. J. 1998. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0. Setauket, NY, USA: Exeter Software.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406–425.

SAS Institute Inc. 1999. SAS/STAT User's Guide, Version 8. Cary, NC, USA: SAS Institute. 3884 p.

Smouse, P. E., J. C. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* 35:627–632.

Tilley, D. J., D. Ogle, L. St. John, L. Holzworth, T. A. Jones, and S. R. Winslow. 2006. Plant guide—bottlebrush squirreltail and big squirreltail. Washington, DC, USA: USDA–NRCS. Available at: http://plants.usda.gov/plantguide/pdf/pg_elmu3.pdf. Accessed 7 April 2010.

Vos, P. R., M. Hogers, M. Bleeker, T. Reijans, T. Van de Lee, M. Hornes, L. Frijters, J. Pot, M. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407–4414.

Waser, N. M., and M. V. Price. 1994. Crossing-distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness. *Evolution* 48:842–852.

Western Ecology Division, United States Environmental Protection Agency. 2007. Level III ecoregions. Available at: http://www.epa.gov/wed/pages/ ecoregions/level_iii.htm. Accessed 22 October 2010.

Wilson, F. D. 1963. Revision of *Sitanion* (Triticeae, Gramineae). *Brittonia* 15:303–323.