Genetic Diversity of Bluebunch Wheatgrass Cultivars and a Multiple-Origin Polycross

S.R. Larson,* T. A. Jones, Z-M. Hu, C. L. McCracken, and A. Palazzo

ABSTRACT

Bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve = *Agropyron spicatum* Pursh: Poaceae) is a cross-pollinating perennial grass native to western North America. Two bluebunch wheatgrass cultivars, Goldar and Whitmar, are currently available for large-scale rangeland seeding. However, cultivars may lack the genetic diversity and adaptation necessary for dynamic non-local environments. The objective of this study was to quantify and compare genomic DNA variation within and between Goldar, Whitmar, and Generation 2 of P-7, a multiple-origin polycross ($MOPX_2$) of 25 naturally diverse bluebunch wheatgrass collections. We assayed 1043 polymorphic amplified fragment length polymorphism (AFLP) products and 88 monomorphic AFLP products from three sample populations of 22 plants. The number of polymorphic loci (and unique alleles) within sample populations of P-7, Goldar, and Whitmar was 898 (99), 813 (49), and 746 (59), respectively. Conversely, the number of fixed AFLP loci within sample populations of P-7, Goldar, and Whitmar was 233, 318, and 385, respectively. The overall nucleotide-sequence diversity [$\pi \pm$ SE ($\times$1000)] estimated for P-7, Goldar, and Whitmar was 100.2 $\pm$ 7.1, 80.1 $\pm$ 6.6, and 79.4 $\pm$ 6.7, respectively. By all measures, genetic variation within P-7 is significantly higher than genetic variation within cultivars. However, the estimated number of inter-population nucleotide differences per site [$d_X \pm$ SE ($\times$1000)] between Goldar and Whitmar, e.g., 36.6 $\pm$ 1.6, is only slightly higher than π within these cultivars, therefore the net nucleotide-sequence divergence [$d_A \pm$ SE ($\times$ 1000)] between these cultivars is relatively small, e.g. 2.5 $\pm$ 0.3. These results indicate that selectively neutral genetic diversity has not been dramatically reduced or inadvertently lost via genetic drift that may have occurred since the divergence of Goldar and Whitmar. No AFLP markers completely distinguish Goldar and Whitmar, therefore discrete morphological differences between these cultivars (e.g., the presence and absence of awns) most likely result from natural or artificial selection.

Bluebunch wheatgrass is a cool-season perennial grass native to semi-arid regions of western North America. Once dominant on millions of acres of semi-arid grass and sagebrush sites, this species is still prized for drought tolerance and palatability to many grazing animals, including a variety of livestock and wildlife species (Daubenmire, 1942). A survey of New World and Old World species of *Pseudoroegneria* indicates that this genus is highly self-sterile (Jensen et al., 1990). Most forms of bluebunch wheatgrass are diploid ($2n = 2x = 14$) and display a caespitose growth habit, although autotetraploid and/or rhizomatous populations are also observed in nature (Carlson and Barkworth, 1997; Daubenmire, 1960; Passey and Hugie, 1963). Beardless wheatgrass (*P. spicata* [Pursh] A. Löve ssp. *inermis* Scribn. & J.G. Sm. = *A. inerme* [Scribn. & J.G. Sm.] Rydb.) has been distinguished from typical bluebunch wheatgrass (*P. spicata* ssp. *spicata* [Pursh] A. Löve = *A. spicatum* Pursh) on the basis of the absence of awns in the *inermis* form and the presence of awns in the *spicata* form. However, no other dispersion of characters between these forms have been observed (Holmgren and Holmgren, 1977; Carlson and Barkworth, 1997) and hybrids of these grasses are meiotically regular (Stebbins and Pun, 1953). Therefore, awned and awnless forms can be considered one species, *A. spicatum* (Holmgren and Holmgren, 1977), now recognized as *P. spicata* (Löve, 1980; Dewey, 1984; Carlson and Barkworth, 1997).

Only two bluebunch wheatgrass cultivars, Goldar and Whitmar, are currently available despite the increasing standards and demand for native plant materials that will be used for many rangeland seedings in the western USA (Shaw and Roundy, 1997; Richards et al., 1998; Holzworth and Brown, 1999). These diploid cultivars were selected from an evaluation of approximately 500 natural bluebunch wheatgrass collections representing six putative ecotypes from the Pacific Northwest. A third cultivar, Secar (Morrison, 1981), was also released from this collection. However, Secar actually belongs to a distinct allotetraploid species known as Snake River wheatgrass (*Elymus wawawaiensis* J. Carlson and Barkw.) (Jones et al., 1991; Carlson and Barkworth, 1997). Whitmar, released in 1946 (Hein, 1958), was derived from an awnless collection originating from a prairie grassland near Colton, WA, in an area of 500 mm annual precipitation on a Palouse silt-loam soil. Goldar, released in 1989 (Gibbs et al., 1991), was derived from a collection with strongly divergent awns originating from an open *Pinus ponderosa* Douglas ex Lawson & C. Lawson woodland on Mallory Ridge, Umatilla National Forest near Anatone, WA. Therefore, Goldar and Whitmar are morphologically distinct (i.e., awned versus awnless) and originate from different plant communities (e.g., mountain woodland versus Palouse grassland) less than 83 km apart.

Supplemental funding and free-market seed availability are factors that limit implementation of native plant species in large-scale fire rehabilitation efforts, the overwhelming revegetation effort on public rangelands in

USDA-ARS, Forage and Range Research Laboratory, Utah State University, Logan, UT 84322-6300; A. Palazzo, USDOD-ACE, Cold Regions Research and Engineering Laboratory, 72 Lyme Road, Hanover, NH 03755-1290. This work was supported by joint contributions of the USDA-Agriculture Research Service, Utah Agriculture Experiment Station, and US Army Cold Regions Research and Engineering Laboratory. Trade names are included for the benefit of the reader, and imply no endorsement or preferential treatment of the products listed by the USDA. *Corresponding author (stlarson@cc.usu.edu).

Published in Crop Sci. 40:1142–1147 (2000).

Abbreviations: AFLP, amplified fragment length polymorphism; bp, (nucleotide) base pair; B, intrapopulation nucleotide-sequence diversity; d_A, interpopulation net nucleotide-sequence divergence; d_{XY}, interpopulation nucleotide-sequence differences; *F*, proportion of shared AFLP bands for a pair of haploid genomes; MOPX, multiple-origin polycross; PCR, polymerase chain reaction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; OTUs, operational taxonomic units; UPGMA, unweighted pair-group method by arithmetic average.

Table 1. Sources of bluebunch wheatgrass germplasm used to synthesize P-7.

Component	Collection location	Awnless seeds	Seed contribution	Cumulative seed contribution
			%	
T-17	Colfax, WA	0	10.6	10.6
K-36	Colton, WA	78	7.5	18.1
DS134	Almota Road, Whitman Co., WA	86	7.0	25.1
T-9	Riggins, ID	0	6.9	32.0
K-26	New Meadows, ID	97	6.2	38.2
K-68	Lind, WA	93	5.6	43.8
Goldar	Mallory Ridge, WA	0	5.3	49.1
Whitmar	Colton, WA	100	5.0	54.1
T-7	Pollock, ID	0	4.8	58.9
DS120	Wawawai Road, Whitman Co., WA	100	4.2	63.1
P-3	Grande Ronde River, OR	0	4.2	67.3
T-22	Durkee, OR	0	3.5	70.8
K-67	Connell, WA	100	3.4	74.2
T-655	Green Canyon, Cache Co., UT	99	3.4	77.6
P-5	unknown	0	3.3	80.9
T-578	Dayton, WA	0	3.1	84.0
K-48	Wawawai Park, Whitman Co., WA	0	2.9	86.9
T-15	Wawawai Park, Whitman Co., WA	96	2.8	89.7
PI236670	Slocan, BC, Canada	0	2.4	92.1
K-44	Wawawai Park, Whitman Co., WA	0	2.2	94.3
K-43	Wawawai Road, Whitman Co., WA	0	2.2	96.5
B101	Darby, MT	0	1.0	97.5
KJ-10	Salina Canyon, Sevier Co., UT	0	0.9	98.4
T-36	Lone Mountain Junction, NV	0	0.8	99.2
T-420	Seneca, OR	0	0.8	100.0

the western USA (Richards et al., 1998). Cultivars have the potential to provide a readily available, abundant source of high-quality native grass seed. However, some ecologists are concerned that the agronomic approach typically used in cultivar development has been to select or breed plants with specific characteristics, which reduces genetic diversity (Roundy et al., 1997; Roundy, 1999). They assert that such plant materials may lack the genetic diversity to maintain adaptation in dynamic environments, non-local seed sources may not have adapted genomes, or that non-local sources may result in genetic pollution (Roundy et al., 1997; Roundy, 1999). However, gene flow in cross-pollinated species may reduce the capacity of natural selection to create locally adapted populations even across strong environmental gradients (Rice and Knapp, 1997). A better scientific understanding of how genetic variation is partitioned and maintained through this process of cultivar development in cross-pollinating native grasses will help plant breeders and natural resource managers develop and implement these plant materials.

The AFLP technique (Vos et al., 1995), a relatively new method of genomic DNA fingerprinting, is highly informative in biosystematic studies of closely related grass species (Aggarwal, 1999; Massa et al., 1999) or cultivars (Pakniyat et al., 1997; Pejic et al., 1998; Zhu, 1998; Burkhamer et al., 1998). This technique also offers the opportunity to measure analytically selectively neutral genetic variation within and among heterogeneous populations of cross-pollinated grasses. The extent of DNA polymorphism within a heterogeneous population is often measured by π, defined as the average number of either nucleotide differences or substitutions per site for a group of DNA sequences (alleles) sampled (Nei, 1987). Likewise, a standard measure of divergence between two heterogeneous populations is d_A, where the average π within populations has been subtracted from d_{XY}, the average number of nucleotide differences or substitutions per site between populations (Nei, 1987). Together, these three parameters (i.e., π, d_{XY}, and d_A) can be used to dissect interpopulation and intrapopulation variation at the DNA level (Nei, 1987). One possible method of estimating π and d_A is by DNA sequencing. However, Nei and Li (1979) developed methods for estimating π and d_A from restriction fragment length polymorphism (RFLP) data and Clark and Lanigan (1993) adapted similar methods for estimating π and d_A from random amplified polymorphic DNA (RAPD) data. More recently, Innan et al. (1999) developed a method for estimating π from AFLP data. These methods enable researchers to estimate and directly compare the same intrinsic parameters of genetic diversity (e.g., π, d_{XY}, and d_A) within and between species by different methods.

This study employs the AFLP method to quantify and compare π, d_{XY}, d_A, and other measures of genetic variation within and between Goldar, Whitmar, and Generation 2 of P-7, a multiple-origin polycross ($MOPX_2$) developed by intermating collections from 25 geographically dispersed source populations. A primary objective of this study was to quantify and test the pertinent hypothesis that P-7 is genetically more diverse than Goldar or Whitmar. Another major objective of this study is to test the hypothesis that genetic variation has been lost via genetic drift since the divergence of Goldar and Whitmar. One reference value for the upper limit of genetic diversity in the natural source population(s) of these cultivars is π within P-7, a highly diverse reference population. A second reference value for genetic diversity in the natural source population(s) of these cultivars is d_{XY} between Goldar and Whitmar. The value of d_{XY} between these cultivars is unaffected by bottlenecks that may have occurred since the divergence of these cultivars. Assuming that relatively few novel muta-

Table 2. DNA sequences of AFLP primers and adapters.

Type	Name	Sequence
*Eco*RI adapter		5′-CTCGTAGACTGCGTACC-3′ 3′-CATCTGACGCATGGTTAA-5′
*Mse*I adapter		5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′
*Eco*RI+1 primer	E01	5′-GACTGCGTACCAATTCA-3′
*Eco*RI+3 primers	E36	5′-GACTGCGTACCAATTCACC-3′
	E37	5′-GACTGCGTACCAATTCACG-3′
	E40	5′-GACTGCGTACCAATTCAGC-3′
	E41	5′-GACTGCGTACCAATTCAGG-3′
*Mse*I+1 primer	M02	5′-GATGAGTCCTGAGTAAC-3′
*Mse*I+3 primers	M47	5′-GATGAGTCCTGAGTAACAA-3′
	M48	5′-GATGAGTCCTGAGTAACAC-3′
	M49	5′-GATGAGTCCTGAGTAACAG-3′
	M59	5′-GATGAGTCCTGAGTAACTA-3′
	M61	5′-GATGAGTCCTGAGTAACTG-3′
	M62	5′-GATGAGTCCTGAGTAACTT-3′

tions have accrued since the divergence of Goldar and Whitmar, d_A between cultivars is an accurate, if not liberal, measure of genetic diversity lost via allele frequency drift since the divergence of Goldar and Whitmar populations.

In the context of this study, we assume that AFLP variation, for the most part, is a selectively neutral measure of genetic drift within and between populations. By examining allele frequency differences between Goldar and Whitmar across a large number of AFLP loci we will test the hypothesis that natural or artificial selection may have contributed to the true-breeding morphological differences between cultivars (e.g., the presence and absence of awns). If allele frequency differences for these AFLP loci are not sufficient to account for true-breeding polymorphisms between Goldar and Whitmar, then we may conclude that diaspore selection may have occurred during source population divergence and/or cultivar development. We do not believe that π, d_{XY}, d_A, or any other estimates of genetic diversity within or between Goldar and Whitmar were inadvertently "broadened" in the process of cultivar development, since these cultivars remain very uniform with regard to the presence or absence of awns, respectively.

MATERIALS AND METHODS

Foundation seed for Goldar and Whitmar was obtained from the USDA-NRCS Plant Materials Centers at Aberdeen, ID and Pullman, WA, respectively. $MOPX_2$ P-7 seed was obtained from a $MOPX_1$ population grown in isolation at the Utah State University Greenville Research Farm in North Logan, UT. The initial polycross ($MOPX_0$) was made at the Utah State University Blue Creek Farm approximately 2.5 km east of Snowville, UT. Table 1 describes the sources and relative contributions of collections used to synthesize P-7. A total of 66 genomic DNA samples were extracted from 22 individual seedlings from each population.

The AFLP technique was conducted according to the methods of Vos et al. (1995). The AFLP adapter sequences, preamplification primer sequences, and selective amplification primer sequences are described in Table 2. Polymerase chain reaction (PCR) was performed with a GeneAmp 9700 (PE Applied Biosystems, Foster City, CA) and *Taq* DNA polymerase licensed for PCR (Life Technologies, Gaithersburg, MD). The *Eco*R I selective amplification primers (Vos et al., 1995) were 5′ labeled with 6-FAM. The AFLP products and GS500-ROX (PE Applied Biosystems) internal lane size standards were fractionated and analyzed by 6% (w/v) denaturing PAGE with an ABI373XL (PE Applied Biosystems). AFLP products between 35 and 500 bp were called by GeneScan 3.1 software (PE Applied Biosystems). The GeneScan sample (trace) files were subsequently aligned and analyzed for the presence and absence of AFLP products, in ~1-bp intervals by Genographer (Benham et al., 1999).

A computer program (Innan et al., 1999) for estimating π and d_{XY} from F, the estimated proportion of shared AFLP products for a pair of haploid genomes, was kindly provided by Hideki Innan (University of Tokyo, Japan). This method is based on the fact that each AFLP product represents a 16-bp sequence assay when using the *Eco*RI and *Mse*I restriction enzymes, which have 6 and 4 bp recognition sequences, respectively, and three selective nucleotides on each of the two AFLP selective amplification primers. Therefore, each shared AFLP product indicates 0/16 nucleotide differences, whereas polymorphisms indicate >1/16 nucleotide differences. The actual number of differences that contribute to each polymorphism is a function of F, which can be used to determine the overall number of nucleotide substitutions per site as describe by Innan et al. (1999). We used equations 25, 25, and 27 (Innan et al., 1999) to estimate the F values used in calculating π within a diploid population in Hardy-Weinberg equilibrium. To estimate F for d_{XY}, we summed over loci (i) the expected frequency of the xth AFLP product in Population 1 ($f1_x$) multiplied by the expected frequency of the xth AFLP product in Population 2 ($f2_x$) to estimate h_x, the probability that the AFLP product is shared by two haploid genomes as the numerator of F, and (ii) the average frequency of each AFLP product per haploid individual, $(f1_x+f2_x)/2$, as the denominator of F (H. Innan, 1999, personal communication). Finally, d_A is determined as the average diversity, i.e., $(\pi_X+\pi_Y)/2$, subtracted from d_{XY} (Nei, 1987). These methods for estimating the number of nucleotide differences based on the proportion of shared AFLP products are good when GC content is near 0.5 and the when number of differences are not too large (Innan et al., 1999). This assumption seems acceptable for bluebunch wheatgrass because the GC contents of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), two other divergent Poaceae

Table 3. Distribution of AFLP variation across populations (including combined cultivar populations), the proportion of shared AFLP bands (F) within each population, and intra-population nucleotide-sequence diversity (π).

		Polymorphic loci		Fixed loci			
Pop.	n†	Total	Unique polymorphism	Product present‡	Product absent	F	$\pi \pm$ SE ($\times$1000)
P-7	21.0	898	99	109	124	0.563	38.7 ± 1.6
Goldar	21.6	813	49	124	194	0.597	34.2 ± 1.5
Whitmar	19.7	746	59	127	258	0.600	33.9 ± 1.5
Cultivars	41.3	942	143	98	91	–	–

† Average number of non-missing genotypes per AFLP bin.
‡ Includes 88 loci that were monomorphic across all populations.

species, are estimated to be 45.5 and 46.0%, respectively (Salinas et al., 1988; Montero et al., 1992). Nei and Miller (1990) described how to use the jackknife method (Efron, 1982) to compute variances of π, d_{XY}, and d_A. Phenograms developed by unweighted pair-group method by arithmetic average (UPGMA) analyses, based on Jaccard's similarity coefficients (the proportion of matching AFLP products between pairwise comparisons of genotypes), were developed with NTSYS-pc, Version 2.0 (Rohlf, 1992).

RESULTS AND DISCUSSION

Three populations of 22 samples were evaluated by means of eight selective amplification primer combinations of E36M59, E36M62, E37M48, E37M49, E40M49, E40M59, E41M47, and E41M61 (Table 2). A total of 1043 polymorphic AFLP loci (2086 alleles) and 88 different monomorphic AFLP loci (88 alleles) were scored across all sample populations. Therefore, we assayed 1131 different AFLP loci (2174 alleles), an effective survey of 18 096 nucleotide bp per plant (16 bp per AFLP product). However, we actually observed a total of 25 239 AFLP products in the 66 genotypes examined, which represents an average sample of 6119 nucleotide bp per plant. By either account, eight AFLP primer combinations provided a highly efficient means of examining DNA sequence variation among 66 different genotypes.

By all measures, the P-7 $MOPX_2$ population displayed more genetic variation than Goldar or Whitmar. Compared with the averages for Goldar and Whitmar, P-7 displayed about 15% more polymorphic loci (nearly twice as many unique polymorphisms) and about 34% fewer fixed loci (Table 3). Moreover, π within P-7 was about 13.5% greater than π within either cultivar (Table 3). The UPGMA phenograms, based on Jaccard's similarity coefficients, also suggest that cultivars (Fig. 1A) are less diverse than P-7 (Fig. 1B). However, the magnitude of π within cultivars is actually quite large (Table 3) and is only about 7% less than d_{XY} between Goldar and Whitmar (Table 4), a useful reference value for the upper limits of genetic diversity in the source populations of these cultivars. Therefore, d_A between Goldar and Whitmar, a liberal estimate of genetic diversity lost via genetic drift since the divergence of these cultivars, is correspondingly small (Table 4), and the UPGMA analysis does not even completely separate Goldar and Whitmar (Fig. 1A). Only six loci showed allele frequency differences greater than 0.5 (maximum difference = 0.65) between Goldar and Whitmar. Two thirds of the AFLP loci (743 out of 1131) showed allele frequency differences less than 0.1 and nearly 95% of the loci (1072 out of 1131) displayed allele frequency differences less than 0.3. Therefore, the magnitude of these allele frequency differences is not likely to account for true-breeding differences between Goldar and Whitmar. Although P-7 displayed more genetic variation than Goldar or Whitmar, these results suggest that genetic drift has been minimal and high levels of genetic diversity have been maintained during source population divergence and cultivar development of Goldar and Whitmar.

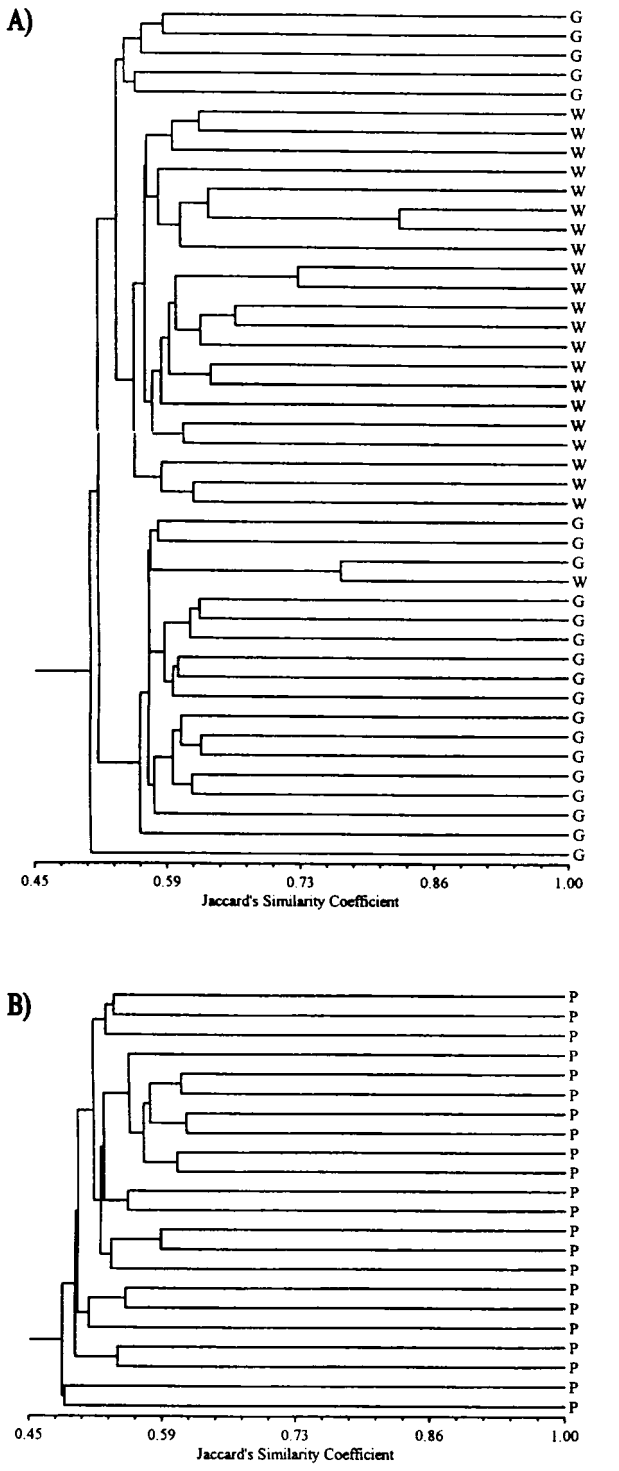

Fig. 1. Comparison of UPGMA phenograms for (A) cultivars Goldar (G) and Whimar (W), and (B) the second-generation of P-7 (P) multiple-origin polycross ($MOPX_2$), a broad-based reference population of bluebunch wheatgrass diversity. Analyzed together, operational taxonomic units (OTUs) from P-7 intercalate within the cultivar populations (results not shown). The Jaccard's similarity coefficients were derived from pairwise comparisons of AFLP profiles, including a total of 1131 polymorphic or monomorphic bands, from individual plants.

Table 4. Pair-wise comparisons of average nucleotide-sequence divergence (d_{XY}) and an average net nucleotide-sequence divergence (d_A) among three bluebunch wheatgrass populations.

Population X	Population Y	$d_{XY} \pm$ SE (×1000)	$d_A \pm$ SE (×1000)
P-7	Goldar	36.7 ± 1.5	0.3 ± 0.2
P-7	Whitmar	37.5 ± 1.6	1.3 ± 0.2
Goldar	Whitmar	36.6 ± 1.6	2.6 ± 0.3

The combined diversity of Goldar and Whitmar, collected from source populations less than 83 km apart, is sufficient to account for much of the variation observed within P-7, a highly diverse reference population. The overall d_{XY} between Goldar and Whitmar (Table 4) was only about 7% less than π within P-7 (Table 3) and d_A between P-7 and either cultivar was nearly negligible (Table 4). Less than 5% (99 out of 2174) of the alleles were unique to P-7 (Table 3), whereas over 6% (143) of the alleles were unique to cultivars (Table 3).

Goldar displayed more polymorphic loci and fewer fixed loci, compared to Whitmar, even though F and π were similar within these cultivars (Table 3). Allele frequencies within each population also contribute to F and π, which may explain why the numbers of polymorphic or fixed alleles generally show relatively more variability than π (Table 3). Careful examination of the cultivar phenogram (Fig. 1A) also suggests that that awnless Whitmar may be a sub-population of the more typical awned forms of bluebunch wheatgrass. Therefore, Goldar may be slightly more diverse than Whitmar even though F and π were similar within these cultivars.

CONCLUSIONS

To date, few studies have examined π, d_{XY}, or d_A among cross-pollinating, heterogeneous grass populations. In part, this may be attributed to a lack of efficient methods. Eight AFLP primer pairs produced 1131 different products (i.e., loci) in this bluebunch wheatgrass study, an effective survey of 18 096 nucleotide bp from each of 66 plants using eight electrophoresis gels. By a more conservative account, this study assayed an average of 6119 nucleotide bp from each plant, as determined by the number of AFLP products actually amplified. Although the latter amount of information could also be obtained from eight fluorescent DNA sequencing gels, this approach requires two series of reactions (PCR and sequencing) and is conditional on the availability of conserved PCR primer-annealing sites. Moreover, DNA sequencing results would be confounded by heterozygosity in cross-pollinated grasses unless each template was cloned and reamplified. A comparable amount of information would also be difficult to obtain with RFLP or RAPD methods. Moreover, RFLP methods require DNA clones that are not readily available in most plant species and molecular sizing of RFLP or RAPD products are typically not performed with single-bp resolution. The AFLP technique provides a robust method for estimating π, d_{XY}, or d_A within and between heterogeneous plant populations. With single base resolution and appropriate DNA size standards, it should be possible for researchers to compare AFLP profiles from different studies of similar germplasm groups.

Results of this study provide limited support for the hypothesis that selectively neutral genetic diversity has been reduced or inadvertently lost via genetic drift that may have occurred since the divergence of Goldar and Whitmar. Our results indicated that π within Goldar and Whitmar was only (i) 7% less than d_{XY} between individuals of Goldar and Whitmar, an accurate if not liberal estimate of genetic diversity in the source populations of these cultivars, and (ii) only 13.5% less than π within P-7, a highly diverse reference population of bluebunch wheatgrass. Although RFLP and AFLP methods for estimating genomic nucleotide differences may be somewhat biased, our estimates of π remaining within Goldar or Whitmar are actually higher than d_{XY} among some tetraploid wheat species, 69.8, estimated by RFLP analysis. The d_{XY} values between 13 wheat species ranged from 6.3 (×1000) between *Triticum abyssinicum* Vav. and *T. pyramidale* Perc. to 90.4 (×1000) between *T. orientale* Perc. and *T. araraticum* Jakubz, with an average of 69.8 (×1000) (Mori et al., 1997).

No true breeding AFLP differences between Goldar and Whitmar were detected, and the vast majority of AFLP loci showed subtle changes in allele frequencies since the divergence of these cultivars. Therefore, genetic drift is not sufficient to account for any true-breeding differences between Goldar and Whitmar or source populations of these cultivars. Natural or artificial selection is a more likely explanation for the presence of awns in Goldar and the absence of awns in Whitmar. However, it is difficult to discern how much selection and genetic drift occurred through source population divergence versus cultivar development, without directly comparing these cultivars to their source populations. This may be difficult considering that these collections were made more than 50 yr ago. Nevertheless, results of this study support the hypothesis that natural or artificial selection may have contributed to the true-breeding morphological differences between cultivars (e.g., the presence and absence of awns).

Results of this study support our hypothesis that P-7 is more genetically diverse than Goldar or Whitmar. Moreover, allele frequencies have remained remarkably stable since the divergence of Goldar and Whitmar, indicating that it should also be possible to maintain genetic diversity, in P-7, over the typical lifetime of cross-pollinating cultivars. A more extensive survey of trait differences and nucleotide variation among widely dispersed natural bluebunch wheatgrass populations may help ecologists better understand the impact of gene flow, genetic drift, and local adaptations among natural grass populations. This, in turn, will enable plant breeders and natural resource managers develop and implement the most appropriate plant materials for these areas.

REFERENCES

Aggarwal, R.K., D.S. Brar, S. Nandi, N. Huang, and G.S. Khush. 1999. Phylogenetic relationships among Oryza species revealed by AFLP markers. Theor. Appl. Genet. 98:1320–1328.

Benham, J., J-U. Jeung, M. Jasieniuk, V. Kanazin, and T. Blake. 1999. Genographer: A graphical tool for automated AFLP and microsatellite analysis. J. Agric. Genomics. 4:article 3.

Burkhamer, R.L., S.P. Lanning, R.J. Martens, J.M. Martin, and L.E. Talbert. 1998. Predicting progeny variance from parental divergence in hard red spring wheat. Crop Sci. 38:243–248.

Carlson, J.R., and M.E. Barkworth. 1997. *Elymus wawawaiensis*: A species hitherto confused with *Psuedoroegneria spicata* (Triticeae, Poaceae). Phytologia 83:312–330.

Clark, A.G., and C.M.S. Lanigan. 1993. Prospects for estimating nucleotide divergence with RAPDs. Mol. Biol. Evol. 10:1096–1111.

Daubenmire, R.F. 1942. An ecological study of the vegetation of southeastern Washington and adjacent Idaho. Ecol. Mongr. 12: 53–79.

Daubenmire, R.F. 1960. An experimental study of variation in the *Agropyron spicatum-A. interme* complex. Bot. Gaz. (Chicago) 122:104–108.

Dewey, D. 1984. The genomic system of classification as a guide to intergeneric hybridization within the perennial Triticeae. p. 209–279. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement. Plenum Publishing Corp., New York.

Efron, B. 1982. The jacknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia, PA.

Gibbs, J.L., G. Young, and J.R. Carlson. 1991. Registration of 'Goldar' bluebunch wheatgrass. Crop Sci. 31:1708.

Hein, M.A. 1958. Registration of varieties and strains of grasses. Agron. J. 50:685–686.

Holmgren, A.H., and N.H. Holmgren. 1977. Poaceae. p. 175–464. *In* A. Cronquist et al. (ed.) Intermountain flora, v. 6. New York Botanical Garden, New York.

Holzworth, L.K., and R.W. Brown, comps. 1999. Revegetation with native species: Proceedings. RMRS-P-8. USDA, Forest Service, Rocky Mountain Research Station. Ogden, UT.

Innan, H., R. Terauchi, G. Kahl, and F. Tajima. 1999. A method for estimating nucleotide diversity from AFLP data. Genetics 151: 1157–1164.

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. Can. J. Plant Sci. 70:215–225.

Jones, T.A., D.C. Nielson, and J.R. Carlson. 1991. Developing a grazing-tolerant native grass for bluebunch wheatgrass sites. Rangelands 13:147–150.

Löve, A. 1980. IOPB chromosome number reprots. LXVI. Poaceae-Triticeae-Americanae. Taxon 29:163–169.

Massa, A.N., S.R. Larson, D.J. Hole, and K.B. Jensen. 1999. AFLP variation in the perennial bromegrasses of Patagonia. p. 163. *In* Agronomy abstracts. ASA, Madison, WI.

Montero, L.M., J. Filipski, P. Gil, J. Capel, J.M. Martinez-Zapater, and J. Salinas. 1992. The distribution of 5-methylcytosine in the nuclear genome of plants. Nucleic Acids Res. 20:3207–3210.

Mori, N., T. Moriguchi, and C. Nakamura. 1997. RFLP analysis of nuclear DNA for study of phylogeny and domestication of tetraploid wheat. Genes Genet. Syst. 72:153–161

Morrison, K.J. 1981. Secar bluebunch wheatgrass. Extension bulletin – Washington State University, Pullman. Cooperative Extension Service. Dec. (1991).

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. (USA) 76:5296–5273.

Nei, M., and J.C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction site data. Genetics 125:873–879.

Pakniyat, H., W. Powell, E. Baird, L.L. Handley, D. Robinson, C.M. Scrimgeour, E. Nevo, C.A. Hackett, P.D.S. Caligari, and B.P. Foster. 1997. AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. Genome 40:332–341.

Passey, H.B., and V.K. Hugie. 1963. Variation in bluebunch wheatgrass in relation to environment and geographic location. Ecology 44:158–161.

Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglione, G. Taramino, and M. Motto. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor. Appl. Genet. 97:1248–1255.

Rice, K.J., and E.E. Knapp. 1997. Genes on the range: Ecological genetics of restoration on rangelands. p. 21. *In* N.L. Shaw and B.A. Roundy (comps.) Proceedings: Using seeds of native species on rangelands. Gen. Tech. Rep. INT-GTR-372. U.S. Department of Agriculture, Forest Service, Intermountain Research Station. Ogden, UT.

Richards, R.T., J.C. Chambers, and C. Ross. 1998. Use of native plants on federal lands: policy and practice. J. Range Manage. 51:625–632.

Rohlf, F.J. 1992. NTSYS-pc. Numerical taxonomy and multivariate analysis system (version 2.02). Exeter Software, Setauket, NY.

Roundy, B.A., N.L. Shaw, and D.T. Booth. 1997. Using native seeds on rangelands. p. 1–8. *In* N.L. Shaw and B.A. Roundy (comps.) Proceedings: Using seeds of native species on rangelands. Gen. Tech. Rep. INT-GTR-372. U.S. Department of Agriculture, Forest Service, Intermountain Research Station. Ogden, UT.

Roundy, B.A. 1999. Lessons from historical rangeland revetation for todays restoration. p. 33–38. *In* L.K. Holzworth and R.W. Ray (comps.) Revegetation with native species: proceedings. RMRS-P-8. USDA, Forest Service, Rocky Mountain Research Station. Ogden, UT.

Salinas, J., G. Matassi, L.M. Montero, and G. Bernardi. 1988. Compositional compartmentalization and compositional patterns in the nuclear genomes of plants. Nucleic Acids Res. 16:4269–4285.

Shaw, N.L., and B.A. Roundy, comps. 1997. Proceedings: Using seeds of native species on rangelands. Gen. Tech. Rep. INT-GTR-372. USDA, Forest Service, Intermountain Research Station. Ogden, UT.

Stebbins, G.L., and F.T. Pun. 1953. Artificial and natural hybrids in the Gramineae tribe Hordeae. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the Tritceae. Genetics 38:600–608.

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

Zhu, J., M.D. Gale, S. Quarrie, M.T. Jackson, and G.J. Bryan. 1998. AFLP markers for the study of rice biodiversity. Theor. Appl. Genet. 96:602–611.