

POPULATION STRUCTURE IN *PSEUDOROEGNERIA SPICATA* (POACEAE: TRITICEAE) MODELED BY BAYESIAN CLUSTERING OF AFLP GENOTYPES¹

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Pseudoroegneria spicata (Poaceae: Triticeae) is an abundant, allogamous species widely adapted to the temperate, semiarid steppe and open woodland regions of western North America. Amplified fragment length polymorphism (AFLP), model-based Bayesian clustering, and other methods of hypothesis testing were used to investigate genetic diversity and population structure among 565 *P. spicata* plants from 82 localities representing much of the species distribution. Comparisons with four Asiatic *Pseudoroegneria* species and two North American *Elymus wawawaiensis* accessions demonstrate cohesiveness in *P. spicata*. However, *P. spicata* genotypes group by locality and geographic region based on genetic distance analysis. Average DNA polymorphism among *P. spicata* localities was significantly correlated ($r = 0.58$) with geographical distance. The optimum Bayesian cluster model included 21 *P. spicata* groups, indicating that dispersal among sampling locations was not sufficient to group genotypes into one unstructured population. Approximately 18.3% of the DNA polymorphism was partitioned among the 21 regional groups, 14.9% among localities within groups, and 66.8% within accessions. Average DNA polymorphism among Bayesian groups was correlated ($r = 0.53$) with the average geographic distance among Bayesian groups, which partly reflects isolation by distance. However, conspicuous regional boundaries were discernable among several divergent genetic groups.

Key words: AFLP; bluebunch wheatgrass; genetic diversity; North America; Poaceae; population structure; *Pseudoroegneria spicata*.

Pseudoroegneria spicata (Pursh) Á. Löve is a cool-season perennial grass native to semi-arid regions of western North America. It reportedly ranges throughout inland regions, from Alaska to California in the West and from Saskatchewan to West Texas in the East (Zlatnik, 1999). Once dominant on millions of acres of semi-arid grass and sagebrush sites, this species is prized for drought tolerance and palatability to many grazing animals, including a variety of livestock and wildlife species (Daubenmire, 1942). Despite its ecological importance and widespread use in large-scale rangeland revegetation, relatively little is known about population structure in this species. Variation in awn structure and growth habit has been examined (Daubenmire, 1960; Passey and Hugie, 1963). Cultivars of *P. spicata* display relatively high rates of overall nucleotide sequence variation (within varieties), but surprisingly little sequence divergence (6.9%) was detected between varieties once thought to represent different species or subspecies (Larson et al., 2000). No true-breeding amplified fragment length polymorphisms were detected between these cultivars (Larson et al., 2000). Most forms of *P. spicata* are diploid ($2n$

$= 2x = 14$), but autotetraploid forms exist in the northern geographic range.

Pseudoroegneria (Nevski) Á. Löve is a genus of the Northern Hemisphere, with its species occurring from the Middle East and Transcaucasia, across Central Asia and Northern China to western North America (Dewey, 1984). Based on chromosome pairing during meiosis, Jensen et al. (1995) suggested that Eurasian and North American *Pseudoroegneria* taxa may form one continuous polymorphic species complex. The chloroplast DNA sequences of Eurasian and North American *Pseudoroegneria* taxa are very similar (Redinbaugh et al., 2000; Mason-Gamer, 2002). However, there is evidence from chloroplast DNA restriction site variation that North American *P. spicata* is different from Eurasian *P. strigosa* (M. Bieb.) Á. Löve and *P. libanotica* (Hack.) D. R. Dewey (Mason-Gamer, 2002). A survey of New World and Old World species of *Pseudoroegneria*, including *P. spicata*, indicates that this genus is highly self-sterile (Jensen et al., 1990). Although *P. spicata* has many close relatives, this species has no recognized congeners in North America. North American *Elymus wawawaiensis* J. R. Carlson and Barkworth was previously misidentified as *P. spicata* (Carlson and Barkworth, 1997). However, the genomically defined *Elymus* genus is derived from diploid *Pseudoroegneria* and *Hordeum* (formerly *Criticism*) progenitors (Dewey, 1984; Löve, 1984; Mason-Gamer, 2001). The chloroplast genome of *Elymus* derives from *Pseudoroegneria* (Redinbaugh et al., 2000). Thus, allotetraploid *E. wawawaiensis* is genetically distinct from diploid or autotetraploid *P. spicata*. Moreover, *E. wawawaiensis* and *P. spicata* can be distinguished by glume morphology and rachis internode length (Carlson and Barkworth, 1997).

This study used amplified fragment length polymorphism and model-based Bayesian clustering to investigate genetic diversity and population structure among 565 *P. spicata* plants from 82 localities in western North America. Bayesian clus-

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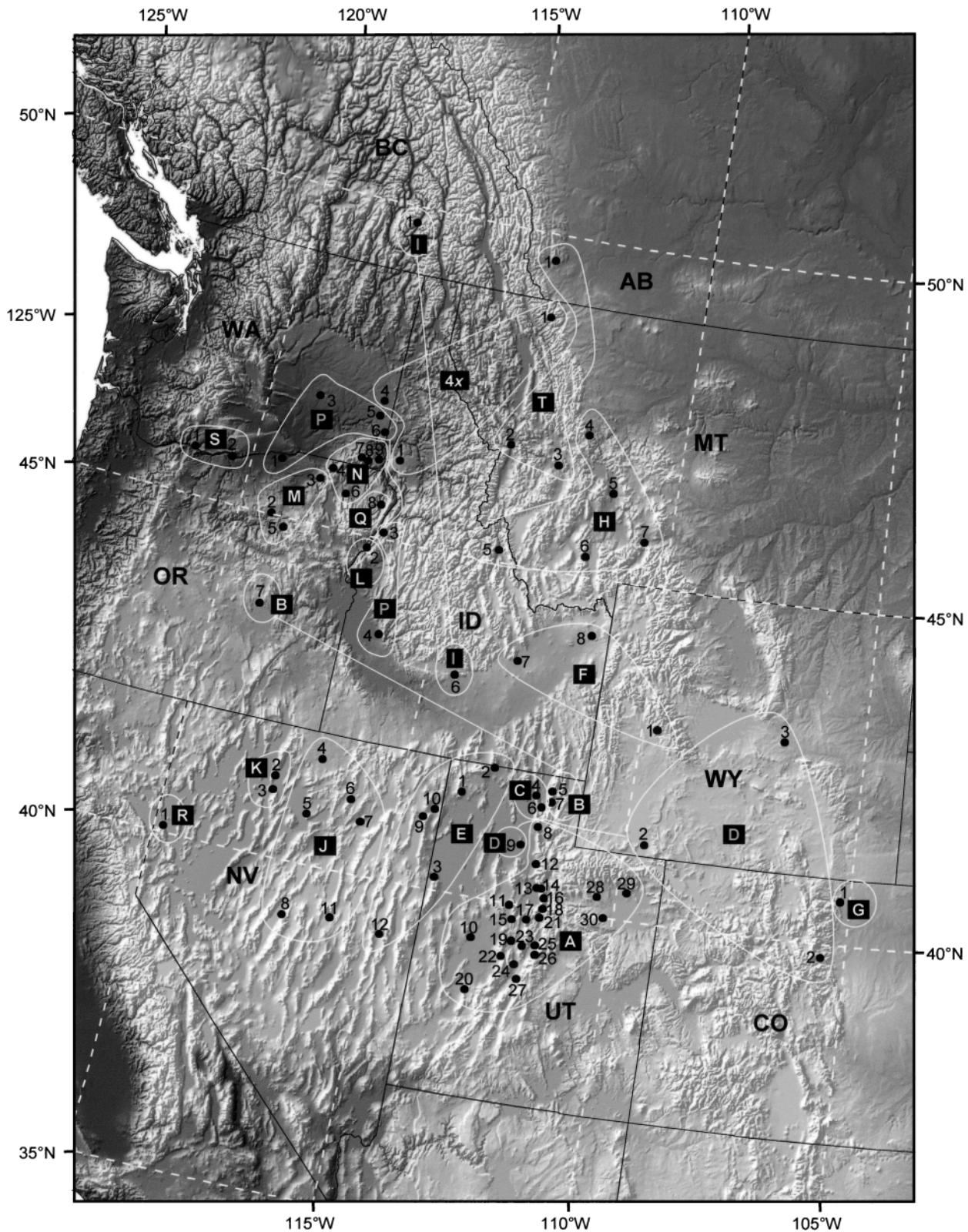


Fig. 1. Shaded relief map of 81 *Pseudoroegneria spicata* collection sites, grouped according to genotypic populations (A–T). Localities are numbered within boundaries of Alberta (AB), British Columbia (BC), Colorado (CO), Idaho (ID), Montana (MT), Nevada (NV), Oregon (OR), Utah (UT), Washington (WA), and Wyoming (WY) circumscribed by solid black lines. Genotypic groups (A–T) of diploid accessions determined using Bayesian clustering methods (Pritchard et al., 2000) are circumscribed by solid white lines. A solid white line also encircles the tetraploid (4x) group of *P. spicata* accessions. Localities for AK1 (Alaska), P1 (unknown origin), and P7 (multiple origin) *P. spicata* accessions including genotypic groups O and U are not shown.

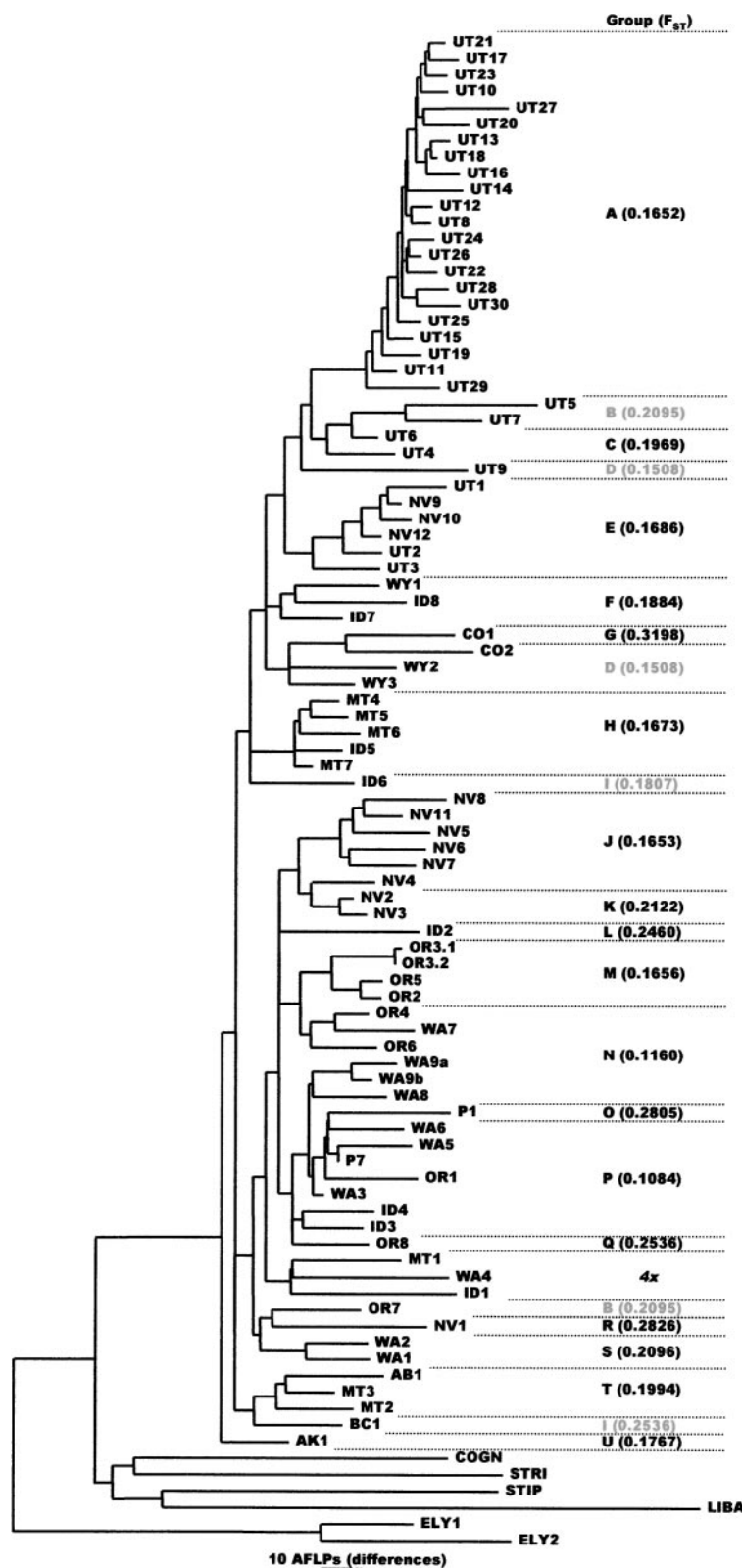


Fig. 2. Comparison of distance-based neighbor-joining tree vs. model-based Bayesian clusters for 86 *Pseudoroegneria spicata* accessions, *P. cognata* (COGN), *P. libanotica* (LIBA), *P. stipifolia* (STIP), and *P. strigosa* (STRI) and two *Elymus wawawaiensis* accessions (ELY1 and ELY2). The 86 North American *P. spicata* accessions are numbered according to localities within Alberta (AB), British Columbia (BC), Colorado (CO), Idaho (ID), Montana (MT), Nevada (NV), Oregon (OR), Utah (UT), Washington (WA), and Wyoming (WY). The neighbor-joining tree was developed and scaled based on the average number of DNA polymorphisms among accessions, corrected for the average number of DNA polymorphism within accessions. Bayesian clusters (Pritchard et al., 2000) for 83 diploid *P. spicata* accessions, excluding three tetraploid *P. spicata* accessions, were identified by letters A–U based on the optimum model of $K = 31$, which included 21 nontrivial groups. Bayesian groups separated into more than one neighbor-joining cluster are indicated by gray shaded text.

TABLE 1. Summary of AFLP profiles from 86 *Pseudoroegneria spicata* accessions, four Old World *Pseudoroegneria* accessions (*P. cognata*, *P. libanotica*, *P. stipifolia*, and *P. strigosa*), and two *Elymus wawawaiensis* accessions investigated by AFLP analysis. The 86 *P. spicata* accessions are grouped into 21 diploid Bayesian groups (A–U) and one tetraploid group (4X).

Identifier	Plants analyzed	Bayesian group	Polymorphic fragments	Fragments per plant (SE)	Similarity coefficient ^b (SE)	Polymorphisms ^b (P _s)
AB1	7	T	392 (92.5%)	242 (3.1)	0.75 (0.01)	120.9
AK1	5	U	397 (80.4%)	250 (5.3)	0.622 (0.029)	189.4
BC1	5	I	321 (73.6%)	249 (6.4)	0.693 (0.027)	152.4
CO1	7	G	357 (91.3%)	236 (3.8)	0.763 (0.023)	112
CO2	7	D	332 (89.0%)	235 (2.7)	0.784 (0.02)	101.6
ID1	7	4X	405 (90.8%)	265 (6.9)	0.757 (0.05)	127.1
ID2	6	L	412 (88.2%)	255 (6.1)	0.721 (0.016)	141.8
ID3	6	P	435 (93.5%)	245 (6.4)	0.689 (0.008)	152.1
ID4	7	P	413 (92.8%)	239 (4.6)	0.707 (0.012)	140.4
ID5	7	H	449 (94.9%)	236 (2.8)	0.713 (0.015)	135.6
ID6	7	I	405 (92.9%)	235 (6.3)	0.726 (0.013)	128.2
ID7	7	F	432 (93.1%)	243 (3.4)	0.708 (0.018)	142
ID8	5	F	212 (61.6%)	230 (3.6)	0.781 (0.011)	100.8
MT1	7	4X	400 (94.3%)	255 (5.3)	0.761 (0.008)	121.4
MT2	6	T	374 (92.3%)	232 (3.6)	0.717 (0.007)	131.5
MT3	7	T	439 (91.8%)	250 (6.1)	0.701 ^a	149.6
MT4	5	H	271 (69.0%)	228 (3.1)	0.713 (0.025)	131
MT5	7	H	423 (94.4%)	237 (2.8)	0.722 (0.016)	131.5
MT6	7	H	373 (93.3%)	225 (4.7)	0.738 (0.018)	118.1
MT7	5	H	328 (74.7%)	230 (5)	0.674 (0.017)	150
NV1	6	R	366 (89.5%)	234 (2.7)	0.715 (0.011)	133.3
NV2	6	K	417 (87.8%)	249 (3.9)	0.68 (0.004)	159.2
NV3	7	K	445 (95.1%)	244 (2.3)	0.697 (0.01)	148.2
NV4	7	J	444 (91.4%)	250 (4.3)	0.745 ^a	152.6
NV5	7	J	417 (92.3%)	251 (2)	0.722 (0.005)	139.7
NV6	7	J	443 (94.5%)	256 (5.2)	0.727 (0.016)	139.8
NV7	7	J	413 (92.8%)	255 (3.4)	0.724 (0.017)	140.5
NV8	6	J	384 (92.3%)	254 (4.4)	0.745 ^a	129.7
NV9	7	E	458 (92.3%)	245 (4.2)	0.695 (0.01)	149.9
NV10	7	E	402 (94.1%)	230 (5.1)	0.731 (0.011)	123.4
NV11	5	J	288 (69.1%)	256 (3.4)	0.697 ^a	155.4
NV12	7	E	441 (93.2%)	239 (2.9)	0.704 (0.01)	141.6
OR1	7	P	388 (91.3%)	244 (5.7)	0.734 (0.037)	129.1
OR2	7	M	454 (93.6%)	239 (2.3)	0.696 (0.002)	145.6
OR3.1	7	M	469 (94.2%)	243 (4)	0.69 (0.009)	150.5
OR3.2	7	M	449 (94.1%)	230 (6.3)	0.684 (0.01)	145
OR4	7	N	499 (95.0%)	248 (4.4)	0.675 (0.006)	160.8
OR5	6	M	435 (91.6%)	242 (1.8)	0.672 (0.006)	158.9
OR6	7	N	424 (94.0%)	232 (6.7)	0.706 (0.021)	136.9
OR7	7	B	416 (92.9%)	234 (2.5)	0.715 (0.007)	133
OR8	7	Q	385 (93.2%)	222 (3.3)	0.708 (0.017)	129.7
P1	7	O	409 (90.9%)	259 (2.3)	0.733 (0.017)	138.6
P7	7	P	489 (95.1%)	240 (5.5)	0.668 (0.01)	159.8
UT1	7	E	422 (94.2%)	244 (3)	0.74 (0.011)	127.1
UT2	7	E	435 (92.2%)	239 (3.6)	0.7 (0.008)	143.6
UT3	7	E	439 (93.4%)	248 (5)	0.706 (0.016)	145.2
UT4	7	C	489 (93.7%)	252 (3.7)	0.675 (0.011)	163.8
UT5	7	B	312 (90.4%)	230 (2.6)	0.792 (0.006)	95.5
UT6	6	C	410 (90.1%)	240 (2.8)	0.679 (0.01)	153.9
UT7	6	B	361 (89.1%)	232 (3.4)	0.727 ^a	126.5
UT8	7	A	437 (93.4%)	246 (3.6)	0.714 (0.008)	140.3
UT9	6	D	379 (91.1%)	237 (4.5)	0.73 (0.014)	127.7
UT10	7	A	430 (95.8%)	247 (3.4)	0.753 (0.008)	121.8
UT11	7	A	504 (96.6%)	255 (3.5)	0.7 (0.013)	153.2
UT12	6	A	307 (71.4%)	246 (3.5)	0.712 (0.005)	141.6
UT13	6	A	366 (90.6%)	237 (4.7)	0.752 (0.012)	117.5
UT14	6	A	371 (91.8%)	239 (4.1)	0.758 (0.01)	115.9
UT15	7	A	442 (94.6%)	242 (2.4)	0.722 (0.006)	134.5
UT16	7	A	413 (95.2%)	242 (4.8)	0.737 (0.015)	127.2
UT17	7	A	449 (96.1%)	247 (3.2)	0.723 (0.009)	136.8
UT18	7	A	442 (94.4%)	241 (4.7)	0.721 (0.01)	134.6
UT19	7	A	435 (96.0%)	242 (2.5)	0.722 (0.018)	134.3
UT20	6	A	343 (89.0%)	237 (4.2)	0.786 (0.013)	101.3
UT21	7	A	408 (94.2%)	239 (3.7)	0.751 (0.008)	119.1
UT22	7	A	403 (93.3%)	238 (1.3)	0.732 (0.01)	127.4

TABLE 1. Continued.

Identifier	Plants analyzed	Bayesian group	Polymorphic fragments	Fragments per plant (SE)	Similarity coefficient ^b (SE)	Polymorphisms ^b (P _s)
UT23	7	A	409 (93.4%)	238 (4.2)	0.727 (0.017)	129.6
UT24	6	A	397 (90.8%)	240 (3)	0.728 (0.01)	130.4
UT25	7	A	424 (94.4%)	239 (2.8)	0.734 (0.007)	127.2
UT26	6	A	402 (88.5%)	248 (3.2)	0.719 (0.018)	139.3
UT27	7	A	364 (92.6%)	245 (2.2)	0.79 (0.022)	102.9
UT28	7	A	432 (94.9%)	245 (2.2)	0.74 (0.005)	127.1
UT29	7	A	391 (92.0%)	240 (3.5)	0.733 (0.013)	128
UT30	5	A	283 (69.7%)	246 (5)	0.722 (0.003)	136.4
WA1	7	S	435 (93.5%)	239 (6.7)	0.702 (0.016)	142.4
WA2	6	S	398 (89.0%)	247 (5.1)	0.708 (0.028)	143.7
WA3	7	P	503 (94.9%)	236 (4.4)	0.653 (0.006)	164
WA4	6	4X	327 (87.9%)	256 (4.1)	0.793 (0.015)	106
WA5	7	P	429 (91.7%)	249 (4.6)	0.711 (0.021)	144.5
WA6	7	P	438 (94.8%)	245 (3.1)	0.722 (0.019)	136.6
WA7	6	N	410 (92.3%)	245 (3.4)	0.71 (0.015)	141.9
WA8	7	N	437 (92.6%)	247 (2.8)	0.692 (0.006)	152
WA9a	7	N	437 (95.0%)	237 (4.8)	0.708 (0.017)	138.5
WA9b	7	N	505 (94.4%)	246 (4.2)	0.673 (0.015)	160.8
WY1	6	F	392 (88.5%)	242 (5.6)	0.711 (0.018)	140.1
WY2	7	D	394 (91.4%)	230 (1.4)	0.702 (0.012)	137
WY3	4	D	301 (71.2%)	247 (6.8)	0.67 (0.016)	163.2
ELY1	7		442 (92.5%)	280 (3.1)	0.748 (0.011)	141
ELY2	7		415 (89.6%)	294 (3.9)	0.786 (0.017)	126
COGN	6		256 (90.1%)	194 (2.3)	0.811 (0.016)	73.4
LIBA	7		310 (92.3%)	208 (6.7)	0.773 (0.009)	94.2
STIP	7		351 (92.3%)	223 (6.7)	0.741 (0.012)	115.5
STRI	7		337 (93.9%)	223 (3.3)	0.779 (0.018)	98.8

^a Undefined negative variance (Leonard et al., 1999).^b Average pairwise comparisons.

tering (Pritchard et al., 2000) was used to evaluate models of population structure by (1) allocating individual genotypes into K populations, where K is a series of numbers and (2) determining the likelihood for each model of K populations. The best resulting model of population structure will be further evaluated using analysis of molecular variance and other methods of hypothesis testing. This study tested and compared the relative importance of genetic panmixia vs. genetic structure, in *P. spicata*, over a broad geographic region of western North American. We investigated relationships among genetic distance, geographic distance, and population structure. For comparison, *E. wawawaiensis* and Old World *P. cognata* (Hack.) Å. Löve, *P. libanotica*, *P. stipifolia* (Czern. Ex Nevski) Å. Löve, and *P. strigosa* species were also included.

MATERIALS AND METHODS

Plant materials—A total of 92 accessions were examined including 86 *P. spicata* accessions and 6 outgroups (see Supplemental Data accompanying the online version of this article). One half (43) of the 86 *P. spicata* accessions were composed of wildland collected seed (see footnotes in Supplemental Data accompanying the online version of this article), whereas the remaining accessions (including outgroups) were regenerated at least once.

The 86 *P. spicata* accessions included single-origin accessions from 81 mapped collection sites (Fig. 1), one single-origin accession from Alaska, one single-origin *P. spicata* accession from an unknown collection site, and one multiple-origin accession. The 86 *P. spicata* accessions also included the OR3.1 and OR3.2 accessions replicated from the same DNA samples (technical replicates) in addition to the WA9a and WA9b accessions collected from the same site (biological replicates). Awnless bluebunch wheatgrass (*P. spicata* [Pursh] Å. Löve ssp. *inermis* Scribn. & J. G. Sm. = *A. inermis* [Scribn. & J. G. Sm.] Rydb.) was distinguished from awned bluebunch wheatgrass (*P. spicata* ssp. *spicata* [Pursh] Å. Löve = *A. spicatum* Pursh), however no other

dispersion of characters between these taxa have been observed (Holmgren and Holmgren, 1977; Carlson and Barkworth, 1997). Hybrids of these taxa are meiotically regular (Stebbins and Pun, 1953), form fertile hybrids, and hybridize freely when found growing together (Holmgren and Holmgren, 1977). Our *P. spicata* accessions display awns as a continuously variable trait evident (including awnless forms), both within and among accessions, but we have not classified the accessions according to this characteristic.

Seeds were germinated on blotter papers and planted into single-plant cone containers. Genomic DNA was extracted from seedlings using the DNeasy 96 plant DNA extraction kit and MM300 mixer mill (Qiagen, Valencia, California, USA). Plants were subsequently transplanted to the Utah Agriculture Experiment Station Evans Research Farm in Cache County Utah in the spring of 2001 or 2002. One or two voucher specimens, from each accession (see Supplemental Data accompanying the online version of this article), were prepared from flowering plants in 2003 and deposited in the Utah State University Intermountain Herbarium, Logan, Utah.

AFLP analysis—Amplified fragment length polymorphisms (AFLPs) were assayed as described by Vos et al. (1995), except that *EcoRI* selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on the 5' nucleotide. Selective amplifications were performed using six *EcoRI* + 3/*MseI* + 3 primer pairs (e.g., E36M59, E36M62, E37M48, E37M49, E40M49, E40M59, and E41M47), described by Larson et al. (2000). The amplified DNA fragments were size fractionated using an ABI3100 instrument with 50-cm capillaries, POP-6 polymer, GeneScan 400HD ROX (rhodamine X) internal size standards, and GeneScan software (PE Applied Biosystems, Foster City, California, USA). The GeneScan sample files were visually analyzed for the presence and absence of DNA fragments, between 50 and 400 base pairs, using Genographer version 1.5 (Benham et al., 1999).

Data analyses—Neighbor-joining genetic distance analysis (Saitou and Nei, 1987), model-based Bayesian clustering (Pritchard et al., 2000), analyses of variance (Excoffier et al., 1992), and other methods of hypothesis testing

(Leonard et al., 1999) were used to investigate genetic diversity and population structure. Genetic distances between individual plants were determined by the numbers of amplified fragment length polymorphisms (Euclidean distance) and similarity coefficients computed as $2N_m/(N_x + N_y)$ where N_m is the number of pairs of bands matching between individuals, and N_x and N_y are the total number of bands amplified from the individuals (Dice, 1945; Nei and Li, 1979; Lynch, 1990). Neither of these Euclidean distances nor similarity coefficients factor null alleles as shared characteristics. Pairwise comparisons of genetic distance were computed using SAS (SAS Institute, Cary, North Carolina, USA).

The average number of differences between accessions (P_{xy}), average number of pairwise differences within accessions (P_x and P_y), corrected average pairwise difference among accessions ($P_{xy} - (P_x + P_y)/2$), and corresponding analysis of molecular variance based on Euclidean distances was computed using Arelequin (Excoffier et al., 1992). Likewise, analyses of variances based on pairwise comparisons of similarity coefficients were performed using programs described by Leonard et al. (1999).

The neighbor-joining genetic distance analysis (Saitou and Nei, 1987) was based on a user defined Euclidean distance matrix of the corrected average pairwise difference among accessions ($P_{xy} - (P_x + P_y)/2$), from Arelequin (Excoffier et al., 1992), using PAUP* version 4.0b8 (Swofford, 2000). The neighbor-joining tree (Fig. 2) was rooted a priori using allotetraploid *E. wawawaiensis* as the outgroup. A graphic display of the neighbor-joining tree was developed using TREEVIEW (Page, 1996). Bayesian cluster analysis was performed using the Structure program (Pritchard et al., 2000) on a Linux cluster at the Virginia Bioinformatics Institute (Blacksburg, Virginia, USA), using locality as prior population information as describe by Pritchard et al. (2000).

Correlations between geographical and genetic distances were evaluated by the Mantel (1967) test statistic (Z), using the MxComp procedure of NTSYS-pc (Rohlf, 1998). Significance tests for these correlations were determined by comparing observed values to values obtained by 9999 random permutations (Smouse et al., 1986). Therefore, the upper-tail probability (P) that 1000 random Mantel test statistic (Z) values are (by chance) less than observed values of Z equals 0.0002 or greater. Geographical distance (in kilometers) matrices were computed from geographical coordinates, using a SAS program developed by the first author, based on the formula: $\text{km} = \text{Arccos}[\cos(\text{LAT}_x)\cos(\text{LONG}_x)\cos(\text{LAT}_y)\cos(\text{LONG}_y) + \cos(\text{LAT}_x)\sin(\text{LONG}_x)\cos(\text{LAT}_y)\sin(\text{LONG}_y) + \sin(\text{LAT}_x)\sin(\text{LAT}_y)]r$, where LAT_x , LONG_x and LAT_y , LONG_y are the latitude, longitude (expressed in radians) for the two accessions (X and Y) and r is 6378 km, the radius of Earth.

RESULTS

A total of 146 644 DNA fragments were amplified using seven AFLP primer pairs, including 136 882 fragments from *P. spicata*. A total of 2017 fragment categories were discerned, which is an average of 288 categories per primer (scored from 50 to 400 bp). At least one *P. spicata* fragment was present in 1975 of the 2017 fragment categories. No monomorphic fragments were detected, overall. However, three amplified fragments were present in all *P. spicata* plants (i.e., monomorphic within *P. spicata*). Interestingly, 1597 AFLPs were present in <10% of the *P. spicata* plants, whereas only 83 fragments were present in >90% of the *P. spicata* plants. Thus, 295 AFLPs were present in >10% and <90% of the *P. spicata* plants. As expected, similarity coefficients between *P. spicata* and *E. wawawaiensis* (0.415), *P. spicata* and *P. cognata* (0.415), *P. spicata* and *P. strigosa* (0.397), *P. spicata* and *P. stipifolia* (0.382), or *P. spicata* and *P. libanotica* (0.280) were substantially lower than similarity coefficients within *P. spicata* (0.587). Conversely, the average number of differences between *P. spicata* and *E. wawawaiensis* (310), *P. spicata* and *P. cognata* (255), *P. spicata* and *P. strigosa* (281), *P. spicata* and *P. stipifolia* (288), or *P. spicata* and *P. liban-*

otica (324) are substantially greater than the average number of differences among *P. spicata* localities (210). The most divergent *P. spicata* accessions (P1 and UT17) displayed an average of 243 differences.

The number of polymorphic fragments, average number of fragments per plant, average similarity coefficient, and average number DNA polymorphisms within accessions varied by locality and species (Table 1). As might be expected, the average (± 1 SE) number of fragments per diploid *P. spicata* plant (242 ± 0.5) was significantly less than the average number of fragments per tetraploid plant (259 ± 3.6). Moreover, the allotetraploid *Elymus* accessions displayed substantially more fragments per plant compared with the diploid or autotetraploid *Pseudoroegneria* plants (Table 1). The average similarity coefficient within the AK1 accession (0.622) was significantly lower than for any other accession. Nevertheless, the average similarity coefficients within AK1 and other *P. spicata* accessions (Table 1) were significantly greater than the average similarity coefficient among *P. spicata* localities (0.587). Conversely the average number of differences within *P. spicata* localities (Table 1) was substantially less than the average number of differences among *P. spicata* localities (210).

Analysis of molecular variance (Excoffier et al., 1992) partitioned 31.9% of the DNA polymorphism among 80 diploid sample populations. The apportionment of DNA polymorphism among accessions was significant ($P < 0.0001$, 1023 permutations), indicating that genotypes are subdivided among the 80 sample populations. This analysis of variance excluded three tetraploid populations (MT1, ID1, and WA4), OR3.2 (technical replicate), P7 (multiple origin), and WA9b (biological replicate). Individual *P. spicata* plants group almost strictly by locality, based on neighbor-joining distance analysis (results not shown). One of the few exceptions was the multiple-origin P7 accession (Larson et al., 2000; Jones et al., 2002), which did not form a cohesive group of plant genotypes. Moreover, neighbor-joining analysis of the average number of polymorphisms among localities (corrected for polymorphism within localities) revealed a substantial number of regional (geographic) groups (Fig. 2). The neighbor-joining tree (Fig. 2) was rooted using *E. wawawaiensis* as the outgroup.

The Mantel test of the correlation between the average number of polymorphisms and geographical distances among 78 diploid populations resulted in an r value of 0.58412, significant ($P = 0.0002$) among 9999 random permutations. Likewise, correlations between geographical distance and the corrected number of polymorphisms ($r = 0.57526$) was significant among 9999 random permutations ($P = 0.0002$) as was the correlation between geographical distance and F_{ST} ($r = 0.54474$). These correlation tests excluded three tetraploid populations (MT1, ID1, and WA4), OR3.2 (replicated sample population), P1 (unknown origin), P7 (multiple origin), WA9b (duplicate sample population), and the AK1 accession from Alaska. Although AK1 appears to be the most unique *P. spicata* germplasm, genetic identity between the AK1 population and other *P. spicata* populations evidently transcends wide geographical distances, which substantially diminishes the overall correlations between DNA polymorphism and geographical distances among sample populations.

Models of population structure among the 81 diploid accessions (531 genotypes) were evaluated from $K = 1$ to $K = 40$, where K equals the number of populations (model) as determined by the investigator (Fig. 3). These evaluations excluded three tetraploid populations (MT1, ID1, and WA4),

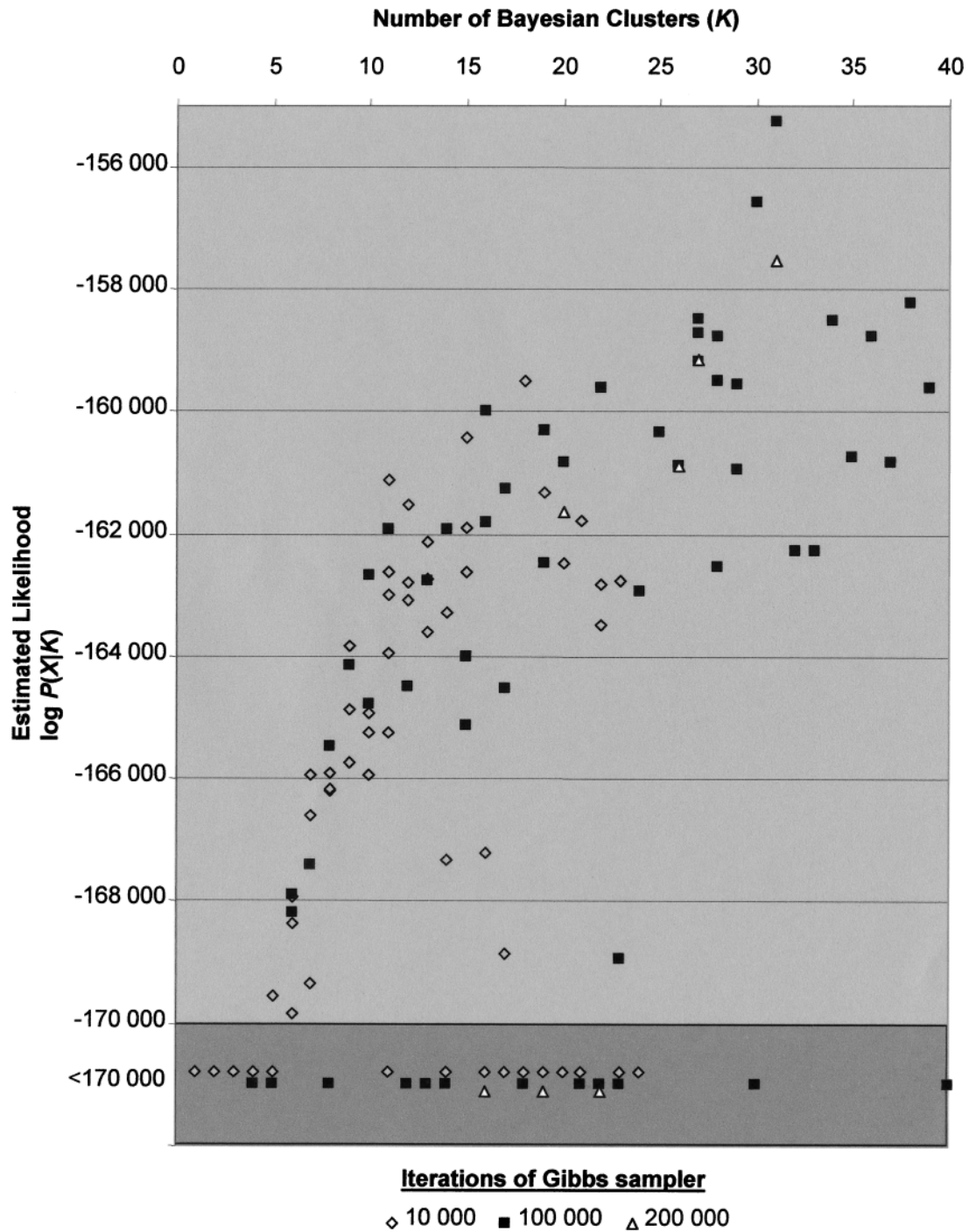


Fig. 3. Model choice criteria for grouping 81 *Pseudoroegneria spicata* accessions: Estimated likelihoods ($\log P(X|K)$) vs. the number of Bayesian clusters (K) (Pritchard et al., 2000). Estimated likelihoods from $\log P(X|K) = -310432$ to $\log P(X|K) = -170098$ were displayed as $\log P(X|K) < -170000$. The optimum solution at $K = 31$ included 21 nontrivial Bayesian groups designated A–U.

OR3.2 (replicated sample population), WA9b (duplicate sample population). We initially evaluated two or three runs of the Gibbs sampler using 10 000 iterations with a burn-in of 10 000, at each level of K from $K = 1$ to $K = 24$ (Fig. 3). The model choice criteria, $\log P(X|K)$, were relatively low and stable where $K < 5$. The estimated likelihood generally improved from $K = 1$ up to about $K = 15$. Although the model choice criteria improved beyond $K = 15$, the likelihood estimates

became highly unstable (Fig. 3). The model choice criterion is a function of the mean likelihood and variance of likelihood over multiple iterations of the Gibbs sampler (Pritchard et al., 2000). Thus, we evaluated another two runs using 100 000 iterations at each level of K from $K = 4$ to $K = 30$, plus one run at each level of K from $K = 31$ to $K = 40$ (Fig. 3). Finally, we evaluated seven additional runs with 200 000 iterations (plus 20 000 burn-in) where K equals 16, 19, 20, 22, 26, 27,

TABLE 2. Apportionment of DNA polymorphism within and among 21 Bayesian groups (A–U) of diploid *Pseudoroegneria spicata*. Above diagonal: the average number of polymorphisms among groups (P_{XY}). Diagonal: average number of polymorphisms within groups (P_X and P_Y). Below diagonal: corrected number of polymorphisms among groups ($P_{XY} - (P_X + P_Y)/2$) and the corresponding apportionment of variation among groups in italic parentheses ($\times 100$).

	A	B	C	D	E	F	G	H	I	J
A	148.6	200.6	185.9	199.8	195.9	194.0	203.1	194.3	210.4	219.1
B	42.2 (22.0)	168.2	194.0	207.4	201.5	197.3	207.0	195.9	200.5	217.1
C	26.9 (15.5)	25.2 (13.0)	169.5	207.7	200.7	202.6	213.2	199.7	209.0	222.5
D	33.9 (18.4)	31.7 (15.2)	31.3 (14.8)	183.3	200.7	192.9	188.2	192.1	203.1	220.9
E	41.4 (21.6)	37.3 (18.7)	35.8 (18.2)	28.9 (14.7)	160.3	189.0	201.0	191.0	198.9	213.2
F	40.3 (21.3)	33.8 (17.1)	38.5 (19.1)	21.8 (11.1)	29.4 (15.5)	158.8	190.5	172.5	185.0	207.9
G	72.8 (32.3)	66.9 (29.3)	72.5 (31.9)	40.6 (18.0)	64.8 (28.7)	55.1 (26.6)	112.0	186.2	199.5	219.8
H	45.5 (23.4)	37.3 (19.3)	40.4 (20.9)	26.0 (13.7)	36.3 (18.9)	18.5 (10.9)	55.7 (27.3)	149.1	182.6	202.1
I	44.9 (23.3)	34.2 (17.0)	42.0 (20.1)	29.2 (14.0)	36.5 (18.5)	23.4 (12.7)	61.3 (29.0)	25.8 (14.6)	164.5	206.4
J	57.0 (27.1)	45.1 (20.6)	49.9 (22.2)	41.5 (18.9)	45.2 (21.2)	40.7 (19.2)	75.9 (30.2)	39.7 (19.5)	36.3 (17.2)	175.7
K	56.8 (27.7)	43.4 (20.8)	47.4 (22.5)	42.4 (19.4)	46.8 (22.6)	42.5 (21.2)	74.4 (33.8)	41.1 (21.4)	37.4 (18.9)	22.5 (11.4)
L	71.6 (32.4)	63.1 (27.4)	66.0 (28.6)	55.2 (23.0)	64.6 (28.6)	54.0 (25.5)	93.8 (42.9)	57.5 (27.8)	54.4 (25.3)	46.8 (20.5)
M	60.3 (28.7)	43.9 (20.9)	54.1 (24.6)	41.5 (19.2)	50.1 (23.7)	41.3 (20.3)	75.3 (32.4)	43.8 (22.1)	39.9 (19.5)	28.9 (14.3)
N	56.2 (26.8)	42.6 (19.6)	48.1 (21.4)	35.6 (16.5)	46.2 (21.6)	34.9 (16.8)	68.8 (27.9)	33.9 (17.1)	31.0 (14.9)	21.6 (10.9)
O	87.1 (36.8)	72.9 (30.7)	80.1 (33.2)	62.8 (25.8)	72.1 (31.0)	64.7 (29.4)	101.1 (44.7)	61.5 (29.3)	59.4 (27.4)	47.3 (20.9)
P	57.7 (27.3)	43.9 (20.3)	52.7 (23.4)	35.2 (16.6)	44.7 (21.1)	34.0 (16.7)	64.4 (26.9)	29.4 (15.3)	25.8 (13.0)	23.3 (11.8)
Q	66.6 (30.7)	53.0 (24.4)	58.1 (26.6)	45.0 (19.7)	56.1 (25.8)	45.1 (22.5)	76.8 (38.9)	41.6 (21.7)	39.0 (19.9)	37.4 (17.0)
R	80.7 (34.9)	56.5 (25.3)	67.1 (29.2)	59.6 (24.4)	67.2 (29.3)	63.0 (28.7)	89.0 (42.4)	64.8 (30.3)	58.0 (26.8)	54.6 (23.2)
S	60.1 (28.8)	43.0 (20.7)	55.6 (25.3)	42.3 (19.3)	50.6 (24.0)	37.3 (19.0)	73.3 (33.4)	39.6 (20.8)	32.1 (16.6)	36.9 (17.5)
T	49.3 (24.8)	39.1 (19.3)	45.9 (22.0)	30.8 (15.1)	41.0 (20.4)	23.8 (13.0)	61.5 (28.6)	25.9 (14.5)	24.3 (13.1)	44.8 (20.8)
U	47.3 (25.5)	50.5 (23.3)	51.4 (23.2)	38.1 (17.3)	45.5 (22.8)	33.8 (18.0)	72.4 (34.5)	34.2 (19.7)	35.1 (17.6)	47.1 (21.4)

and 31 (Fig. 3). Altogether, these evaluations required approximately 15 000 h using Pentium III and Pentium IV processors (>2 Ghz). Thus we were practically limited in the number of iterations and models that could be tested. However, judging from Fig. 3, one seemingly good model among these computationally intensive searches was $K = 31$. With 100 000 iterations of the Gibbs sampler, the estimated likelihood of the $K = 31$ model was substantially greater than any other model (Fig. 3). The $K = 31$ model was also evaluated in another run with 200 000 iterations of the Gibbs sampler, which also produced one of the highest estimated likelihoods (Fig. 3). The $K = 31$ model included 21 nontrivial groups (Figs. 1 and 2) and 10 trivial groups composed of three individual plants, including one from WY3, one from ID7, and one from OR6. These three plants group with other *P. spicata* plants by locality based neighbor-joining and UPGMA searches (data not shown). It is not clear why these three plants formed 10 trivial groups (i.e., groups with less than one plant), especially under the no-admixture model (Pritchard, 2000). Nevertheless, sam-

ples from a given locality were usually allocated within Bayesian clusters, which show reasonable congruence with the distance-based neighbor-joining tree (Fig. 2).

The geographic significance of 19 regional (Bayesian) groups, excluding group O (one accession of unknown origin) and group U (AK1 accession), was evaluated using a Mantel correlation test. The correlation between the average total number of polymorphisms (between individual plants) and the average geographical distances between localities among regional groups resulted in an r value of 0.44, which was significant ($P = 0.0003$) among 9999 random permutations. Likewise, the correlation between the F_{ST} and the average geographical distances between localities among regional groups resulted in an r value of 0.50, which was significant ($P = 0.0002$) among 9999 random permutations.

The significance of population structure among the 21 regional (Bayesian) groups was reexamined using several well-recognized methods. Analysis of molecular variance (AMOVA) partitioned 18.3% of the DNA polymorphism among the

TABLE 2. Extended.

K	L	M	N	O	P	Q	R	S	T	U
210.2	216.8	216.7	219.6	230.6	218.6	205.6	221.1	213.7	203.0	215.5
206.5	218.1	210.1	215.8	226.3	214.6	201.8	206.7	206.4	202.6	228.4
211.2	221.6	221.0	222.0	234.2	224.0	207.6	218.0	219.7	210.1	230.0
213.0	217.7	215.3	216.4	223.7	213.5	201.4	217.4	213.3	201.9	223.6
206.0	215.7	212.4	215.5	221.5	211.4	201.0	213.5	210.0	200.6	219.5
201.0	204.2	202.8	203.4	213.4	200.0	189.2	208.5	195.9	182.6	207.1
209.4	220.7	213.4	213.9	226.4	207.1	197.5	211.1	208.6	196.9	222.3
194.6	203.0	200.5	197.6	205.3	190.6	180.8	205.5	193.4	179.9	202.7
198.7	207.6	204.3	202.3	210.9	194.7	186.0	206.4	193.6	185.9	211.3
189.4	205.5	198.9	198.6	204.4	197.8	189.9	208.6	204.1	212.1	228.9
158.1	199.7	194.0	193.6	199.2	193.4	182.8	202.7	197.0	202.2	221.3
49.7 (24.2)	141.8	203.4	202.1	214.7	201.9	193.7	216.9	206.2	210.5	229.8
32.8 (16.8)	50.4 (23.5)	164.3	190.7	205.9	196.5	189.1	200.2	199.6	207.3	225.5
25.4 (12.6)	42.1 (18.6)	19.4 (10.0)	178.2	195.7	190.3	182.3	212.6	203.2	207.4	225.5
50.9 (24.9)	74.6 (34.8)	54.5 (25.2)	37.3 (16.9)	138.6	191.5	197.5	222.6	214.3	218.6	234.0
27.7 (13.8)	44.4 (19.9)	27.7 (13.9)	14.6 (7.7)	35.6 (16.6)	173.3	175.5	212.2	195.1		218.2
39.1 (20.4)	58.1 (30.1)	42.3 (20.6)	28.5 (13.1)	63.5 (32.2)	24.2 (11.4)	129.4	204.1	185.0	198.9 190.2	211.6
57.5 (27.2)	79.9 (36.8)	51.9 (24.1)	57.4 (23.9)	87.2 (39.1)	59.4 (25.1)	73.2 (35.9)	132.3	203.2	209.6	228.3
38.7 (19.6)	56.0 (26.4)	38.2 (19.0)	34.8 (16.5)	65.7 (30.0)	29.2 (14.5)	41.0 (21.2)	57.7 (27.2)	158.6	197.7	217.8
43.7 (21.6)	60.1 (27.6)	45.7 (22.1)	38.8 (18.4)	69.9 (30.9)	32.8 (16.2)	46.1 (22.7)	64.0 (28.9)	39.0 (19.7)	158.9	205.5
48.3 (23.3)	65.0 (28.9)	49.5 (23.3)	42.5 (19.5)	70.8 (31.4)	37.6 (18.2)	53.0 (26.4)	68.2 (30.6)	44.6 (21.9)	32.1 (17.3)	187.8

21 regional groups, 14.9% among localities within regional groups, and 66.8% within localities. Irrespective of localities, AMOVA partitioned 21.9% of the DNA polymorphism among regional groups and 78.1% of the DNA polymorphism within regional groups (Table 2). All of these regional and locality factors were highly significant ($P < 0.0001$, 1023 permutations). Diversity within and among Bayesian groups was also evaluated based on variances of the similarity index (Table 3). The average similarity coefficients within groups ranged from 0.622 for group U (composed of the AK1 accession) and 0.733 for group O (composed of the P1 accession, unknown locality). The average similarity coefficients among groups ranged from 0.524 between groups B and U to 0.633 between groups H and F (Table 3). Permutation tests (Leonard et al., 1999) indicate that most comparisons of similarity coefficients among groups were significantly less ($P < 0.05$) than similarity coefficients within groups (Table 3). However, in some comparisons, the number of genotypes allocated in one and/or the other group limits the power of the permutation test. In

any case, significant differences among the 21 groups were detectable in pair-wise comparisons of DNA polymorphism and similarity matching coefficients, based on permutations of individuals within and among groups. Although DNA polymorphism and similarity matching coefficients, between individual plants, are very much related (inversely), the permutation test based on similarity matching coefficients (Leonard et al., 1999) was more conservative than the permutation test based on Euclidean distances (Excoffier et al., 1992).

DISCUSSION

A large number of polymorphic and homologous fragments were detected within and among species, providing a highly informative measure of genetic identity within and among species. The five outgroup taxa (*E. wawawaiensis*, *P. cognata*, *P. strigosa*, *P. stipifolia*, and *P. libanotica*) displayed relatively few unique fragment categories (i.e., unique alleles); most categories contained at least one *P. spicata* fragment. Some of

TABLE 3. Average similarity coefficients within (diagonal) and between (below diagonal) 21 Bayesian groups (A–U) of diploid *Pseudoroegneria spicata*. Standard errors for the average similarity coefficients within groups (Leonard et al., 1999) are shown in bold italic font (diagonal). The rankings of the average similarity coefficients between groups relative to 100 random permutations of the respective individuals (Leonard et al., 1999) are shown in parentheses.

	A	B	C	D	E	F	G	H	I	J
A	0.694 0.003									
B	0.576 (101)	0.637 0.010								
C	0.620 (101)	0.594 (101)	0.656 0.006							
D	0.582 (101)	0.556 (99)	0.570 (98)	0.612 0.008						
E	0.595 (101)	0.573 (101)	0.588 (101)	0.579 (101)	0.667 0.005					
F	0.597 (101)	0.581 (99)	0.583 (101)	0.594 (99)	0.606 (101)	0.668 0.008				
G	0.576 (101)	0.557 (99)	0.558 (99)	0.601 (100)	0.579 (99)	0.599 (93)	0.763 0.023			
H	0.590 (101)	0.576 (101)	0.582 (101)	0.588 (101)	0.595 (101)	0.633 (99)	0.602 (101)	0.678 0.006		
I	0.583 (101)	0.575 (97)	0.571 (99)	0.574 (101)	0.587 (100)	0.614 (97)	0.582 (99)	0.613 (101)	0.658 0.010	
J	0.558 (101)	0.552 (101)	0.555 (99)	0.549 (101)	0.569 (101)	0.578 (101)	0.551 (101)	0.583 (101)	0.582 (101)	0.654 0.005
K	0.570 (101)	0.567 (101)	0.571 (101)	0.558 (99)	0.557 (101)	0.586 (101)	0.546 (95)	0.592 (101)	0.591 (100)	0.621 (101)
L	0.564 (101)	0.551 (100)	0.558 (93)	0.556 (100)	0.564 (101)	0.586 (100)	0.550 (93)	0.582 (101)	0.580 (94)	0.596 (99)
M	0.546 (101)	0.549 (101)	0.512 (100)	0.542 (101)	0.553 (100)	0.570 (100)	0.547 (100)	0.568 (101)	0.569 (101)	0.592 (101)
N	0.546 (101)	0.544 (101)	0.545 (101)	0.548 (101)	0.554 (101)	0.577 (101)	0.555 (101)	0.585 (101)	0.581 (100)	0.600 (101)
O	0.541 (101)	0.538 (100)	0.537 (100)	0.548 (101)	0.557 (101)	0.571 (99)	0.543 (93)	0.581 (98)	0.577 (95)	0.601 (100)
P	0.550 (101)	0.548 (101)	0.542 (101)	0.555 (101)	0.563 (101)	0.586 (101)	0.568 (101)	0.599 (101)	0.597 (101)	0.601 (101)
Q	0.557 (101)	0.555 (94)	0.557 (100)	0.560 (101)	0.565 (101)	0.589 (99)	0.569 (93)	0.601 (99)	0.598 (96)	0.600 (101)
R	0.535 (101)	0.555 (97)	0.545 (96)	0.536 (101)	0.549 (99)	0.558 (98)	0.550 (91)	0.557 (101)	0.564 (93)	0.571 (101)
S	0.559 (101)	0.564 (101)	0.551 (99)	0.554 (101)	0.565 (99)	0.593 (100)	0.564 (96)	0.591 (99)	0.599 (99)	0.589 (101)
T	0.580 (101)	0.571 (101)	0.569 (101)	0.577 (101)	0.584 (101)	0.620 (101)	0.587 (100)	0.619 (100)	0.614 (101)	0.571 (101)
U	0.561 (101)	0.524 (98)	0.536 (95)	0.539 (101)	0.552 (99)	0.575 (96)	0.542 (91)	0.578 (98)	0.568 (100)	0.545 (101)

these categories probably contained nonhomologous fragments, especially among the interspecific comparisons. With <40 fragments per plant per primer (Table 1), we estimate that <2% of the fragments from two completely unrelated DNA profiles would migrate into the same fragment category by random association, assuming one bp resolution (or better) between 50 and 400 bp (i.e., a 40/350 chance that each plant would randomly draw a fragment in any single fragment category, squared). In our judgment, the resolution of the capillary electrophoresis technique has been under 1 bp because the relative mobility of DNA fragments is also influenced by nucleotide sequence (i.e., composition) in addition to length (i.e., number of bp). Although a high density of fragments has often been observed between approximately in the lower size ranges, the sizing of smaller fragments is generally more precise. We believe that interspecific and intraspecific similarity coefficients were much greater than expected by chance association.

As expected, *P. spicata* plants show a relatively high degree

of intraspecific identity (i.e., greater than interspecific comparisons). As shown in the neighbor-joining tree (Fig. 2) *P. cognata*, *P. strigosa*, and *P. stipifolia* displayed more genetic relatedness than *E. wawawaiensis* to *P. spicata* on the basis of the absolute number of differences (amplified length fragment polymorphisms). In particular, *P. cognata* displayed a relatively strong genetic similarity to *P. spicata* as determined by amplified fragment length polymorphism. Although chromosome pairing in *P. cognata* (syn = *Agropyron ferganense* Drobow) $\times$ *P. spicata* hybrids averaged 6.21 bivalents per cell, compared to 6.98 in *P. cognata*, these hybrids failed to produce seed (Dewey, 1981). Hybrids of *P. spicata* $\times$ *E. wawawaiensis* are sterile and display irregular pairing (Dewey, 1982; Carlson and Barkworth, 1997). The neighbor-joining tree (Fig. 2) was rooted a priori using allotetraploid *E. wawawaiensis* as the outgroup. However, *P. libanotica* and *P. spicata* actually displayed the greatest number of interspecific differences and the lowest interspecific similarity coefficients. Thus, interpretation of interspecific relationships as depicted

TABLE 3. Extended.

K	L	M	N	O	P	Q	R	S	T	U
0.678										
0.007										
0.601	0.721									
(95)	0.016									
0.596	0.585	0.658								
(101)	(101)	0.005								
0.604	0.592	0.601	0.632							
(101)	(99)	(101)	0.004							
0.605	0.582	0.585	0.610	0.733						
(95)	(94)	(101)	(101)	0.017						
0.605	0.593	0.590	0.613	0.620	0.664					
(101)	(101)	(100)	(101)	(99)	0.004					
0.609	0.593	0.587	0.610	0.589	0.623	0.708				
(98)	(93)	(101)	(101)	(89)	(99)	0.017				
0.577	0.555	0.569	0.554	0.547	0.554	0.551	0.715			
(100)	(87)	(98)	(101)	(93)	(101)	(94)	0.011			
0.596	0.585	0.580	0.583	0.573	0.598	0.601	0.573	0.673		
(100)	(97)	(101)	(99)	(100)	(101)	(93)	(100)	0.005		
0.585	0.575	0.533	0.574	0.563	0.590	0.589	0.558	0.591	0.671	
(101)	(93)	(101)	(101)	(101)	(101)	(101)	(99)	(98)	0.004	
0.553	0.544	0.534	0.542	0.540	0.558	0.551	0.526	0.557	0.581	0.622
(99)	(95)	(101)	(96)	(90)	(101)	(96)	(97)	(99)	(100)	0.029

by the neighbor-joining tree (Fig. 2) should be reviewed with caution. Allotetraploid *E. wawawaiensis* displayed many differences from *P. spicata*, but relatively strong similarity coefficients with *P. spicata*. It is quite conceivable that North American *E. wawawaiensis* and *P. spicata* have introgressed, or that *P. spicata* is a relatively recent diploid progenitor of *E. wawawaiensis*. Morphological similarities between *E. wawawaiensis* and *P. spicata* also suggest a very close relationship. In any case, comparisons of similarity coefficients and the absolute number of differences within and between species, demonstrate a relatively high degree of genetic relatedness and cohesiveness in North American *P. spicata* (Fig. 2).

Although our sampling strategy emphasized sampling of localities rather than individual plants, DNA profiles from 4–7 plants per locality were sufficient to demonstrate a relatively high degree of genetic identity within localities and significant differences in the average similarity coefficients within accessions (Table 1). Of particular interest, the P7 accession was the third most polymorphic accession in terms of similarity

coefficients and the absolute number of differences (Table 1). The P7 germplasm was developed as a multiple-origin synthetic, which may be appropriate for large-scale revegetation uses in western North America (Larson et al., 2000; Jones et al., 2002). Only two other accessions, AK1 and WA3, displayed more DNA polymorphism than P7. The AK1 accession, in particular, displayed substantially more variation than any other accession. The explanation for this is not certain, but it may have cross-pollinated with other *P. spicata* accessions. The AK1 seed accession was collected in 1971 and probably regenerated two or three times, whereas most of the other *P. spicata* seed accessions were collected from wildland plants or regenerated once with reasonable pollen isolation (see Supplemental Data accompanying the online version of this article). The AK1 collection site is dislocated from other *P. spicata* collection sites examined in this study. Thus, we might expect to find highly elevated levels of DNA variation in AK1 if it was outcrossed with *P. spicata* from the lower latitudes. Moreover, we would also expect to find reduced

DNA polymorphism between AK1 and other *P. spicata* accessions from lower latitudes, had AK1 been outcrossed. For example, divergence among *Pascopyrum smithii* (Rydb.) Barkworth & D. R. Dewey accessions from the Northern Great Plains was reduced by intentional cross breeding (Larson et al., 2003). Likewise, it is possible that many of our *P. spicata* seed collections, some of which have been regenerated (see Supplemental Data accompanying the online version of this article), were inadvertently biased toward sibling relationships that may or may not fully reflect genetic diversity within localities. Therefore, sibling relationships and the spatial limitations of most seed collections (usually <1 ha) probably affect the apportionment of genetic variation observed within and among *P. spicata* sample localities. Nevertheless, the apportionment of DNA polymorphism among *P. spicata* sample locations (approximately 32%) compares very closely to the apportionment of *P. smithii* DNA polymorphism among natural germplasm sources from the Northern and Central Rocky Mountain regions (28 and 33%, respectively) (Larson et al., 2003). Moreover, the correlation of DNA polymorphism and geographic distance among *P. smithii* localities ($r = 0.66$) (Larson et al., 2003) is very similar to *P. spicata* (0.58). Thus, significant effects of geographic provenance can be detected within and among localities of allogamous range grasses in western North America.

A primary objective of this study was to determine whether broad regional population structure exists in North American *P. spicata*. Distance-based neighbor-joining, model-based Bayesian clustering, analysis of molecular variance, Mantel correlation tests, and other methods of hypothesis testing based on amplified fragment length polymorphism and associated similarity coefficients provide substantial evidence of population structure and geographic origin in *P. spicata*. In particular, Bayesian clustering (Pritchard et al., 2000) separated the *P. spicata* samples from 81 localities into 21 groups, which display significant geographic dispersion. Thus, dispersal among sampling locations was not sufficient to group genotypes into one unstructured population. The apportionment of DNA polymorphism among the 21 regional groups of *P. spicata* (approximately 18%) was threefold greater than the apportionment of DNA polymorphism among the Northern Great Plains, Northern Rocky Mountain, and Central Rocky Mountain groups of *P. smithii* (approximately 6%) (Larson et al., 2003). However, the separation of 21 regional *P. spicata* groups may result from insufficient sampling across the species distribution. For example, group U was strictly composed of samples from one locality (AK1) in Alaska, which is dislocated from other collection sites examined in this study. In actuality, genetic similarity between the AK1 and other *P. spicata* accessions transcends this large geographic distance (see Results above). Conversely, wide gaps in the geographic sampling of *P. spicata* (Fig. 1) exist where one might expect to find new sources of genetic variation such as Oregon, Arizona, Colorado, eastern Montana, western North Dakota, western South Dakota, and vast areas of western Canada. Thus, other geographically significant populations may have been overlooked as a result of insufficient sampling. In many instances, conspicuous geographic boundaries exist between several genetically distinct lineages (compare Figs. 1, 2, and 4). In particular, two distinct lineages converge between the Central Great Basin and Bonneville Basin Floristic sections of the Great Basin (Holmgren, 1972). A lineage of related groups A, B, C, and E (Bonneville Basin) interface with a distinct lineage

of groups K and J (Central Great Basin) along the Nevada-Utah border. The overall correspondence of population structure in *P. spicata* with floristic sections of the Intermountain region is tenuous. Nevertheless, we postulate that distinct lineages of *P. spicata* have admixed between the Central Great Basin and Bonneville Basin Floristic sections of the Great Basin. The Structure program can detect admixture (Rosenberg et al., 2002), however the procedure is not recommended for dominant marker data (Pritchard et al., 2000). In any case, more continuous and expansive geographic collections of *P. spicata* genotypes are needed to fully elucidate the population structure of this North American species.

Most of the *P. spicata* AFLPs examined in this study were nuclear DNA sequences. The nuclear DNA content of *P. spicata* is virtually identical to *Hordeum vulgare* (Vogel et al., 1999), which has an approximate DNA content of 4873 Mbp (Arumuganathan and Earle, 1991) compared to the 134 545 bp chloroplast genome of *Triticum aestivum* (Ogihara et al., 2002) and 490 520 bp mitochondrial genome of *Oryza sativa* (Notsu et al., 2002). Although plastid genome sizes vary among grasses, the combined plastid genomes of wheat and rice are nearly four orders of magnitude smaller than the nuclear genome of *P. spicata*. Moreover, the vast majority of polymorphic AFLP markers in full-sib plant families display Mendelian segregation and map into well-defined linkage groups that correspond to nuclear chromosomes (Wu et al., 2003). Thus, AFLPs provide a good overall measure of genetic identity among plants that should reflect patterns of seed and pollen dispersal. The Bayesian cluster analysis of AFLP genotypes provide a useful starting point and framework to investigate population structure and adaptive variation in *P. spicata*.

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