Phylogeography of North American mountain bromes

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ABSTRACT

Although native grasses are often desired and used for revegetation of disturbed areas, genetic differences may exist within and among natural and cultivated germplasm sources. This phylogeographic study compares geographic origin and genealogical linkages of 25 natural and cultivated germplasm sources of mountain brome (*Bromus carinatus* Hook. & Arn. [Poaceae]) from western North America. Significant variation among accessions (F_{ST} = 0.70) was detected by analysis of molecular variance (AMOVA), based on the number of amplified fragment length polymorphisms (AFLPs) between individual plants. Likewise, significant differences among 4 hierarchical genotypic groups, encompassing all but 5 unique accessions, were also detected (F_{CT} = 0.47). This study identified at least one well-defined genealogical lineage, comprising 8 accessions, distributed over a broad geographic region and different ecosystems of western North America. Two other hierarchical groups, comprising 6 accessions and 3 accessions, were located within or near specific ecoregions. Results of this study indicate that natural genealogical lineages of cultivars, such as Garnet mountain brome, have dispersed and succeeded over broad geographical regions. However, more research and plant material work are needed before specific recommendations can be made over the entire species distribution.

KEY WORDS

AFLP, genetic diversity, *Bromus carinatus*

NOMENCLATURE

ITIS (2001)

Bromus research plots in Utah. Photo by Steven R Larson

Phylogeography is a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages (Avise 2000). Morphological variability within the *Bromus carinatus* complex has been documented (Hitchcock and others 1969; Harlan 1945), however, the relationship between genetic identity and geographic provenance (phylogeography) in the *B. carinatus* complex has not been examined. Our objective was to compare the geographic origin and genetic identity of 25 natural germplasm sources of North American mountain brome using the amplified fragment length polymorphism (AFLP) method of DNA fingerprinting. The geographic distribution of genealogical lineages was also compared by ecological provinces accepted and used by the USDA Forest Service (Bailey 1996). Phylogeographic documentation for these North American mountain bromes can be used to facilitate the development and selection of new plant materials used for fire rehabilitation and other revegetation projects.

Bromus sect. *Ceratochloa* includes some of the most abundant native bromegrasses of the Rocky Mountain and Pacific Coast regions of western North America. They are predominantly perennial bunch grasses and constitute an excellent source of forage (Hitchcock and Chase 1951). The most common *Ceratochloa* bromegrasses of western North America are California brome (*B. carinatus* Hook. & Arn.) and mountain brome (*B. marginatus* Nees ex Steud.). Both are valued for their high forage quality and ability to reestablish naturally on disturbed sites (May and others 1998, 1999). Other *Bromus* sect. *Ceratochloa* taxa include Aleut brome (*B. aleutensis* Trin. ex Griseb.), seaside brome (*B. maritimus* (Piper) AS. Hitchc.), Colorado brome (*B. polyanthus* Scribn. ex Shear), Alaska brome (*B. sitchensis* Trin.), and hoary brome (*B. luzonensis* J. Presl. Shear) (Pavlick 1995).

Cytogenetic and morphological evidence indicates that the North American *Bromus* sect. *Ceratochloa* species are very closely related. All are octoploids ($2n = 8x = 56$), predominantly self-pollinated (autogamous), and have keeled lemmas with long awns (Stebbins and Togby 1944). Cytogenetic affinities among North American octoploids are also evident in F_1 hybrids between individuals representing different morphological types (Stebbins and Togby 1944; Harlan 1945). F_1 hybrids show normal chromosome pairing and 28 bivalents in meiosis, indicating that they have homologous chromosomes of allopolyploid origin (Stebbins and Togby 1944). Based on morphological and cytogenetic affinities, members of this group are frequently referred to as the *B. carinatus* complex (in the broadest sense) (Harlan 1938, 1945). Whether individuals of this complex make up different species or are diverse forms of a single polymorphic species is poorly understood.

Grasses are an extremely important part of fire rehabilitation and other large-scale revegetation efforts. Commercial grass seed producers can provide an abundant and readily available source of high quality seeds, however, significant differences may exist among cultivated and natural germplasm sources. Although there is considerable concern regarding the genetic identity and adaptation of native plant materials used for large-scale revegetation, there is a general lack of relevant scientific information in this area pertaining to the native grasses of western North America. Based on a relatively new DNA fingerprinting technique specifically developed for the complex and diverse genomes of vascular plants, amplified fragment length polymorphisms (AFLPs) provide highly informative and reliable measures of genetic identity and genetic variation (Vos and others 1995). High-throughput fluorescent DNA analyzers have been utilized to detect and compare amplified fragment polymorphism within and among natural and/or cultivated germplasm sources of key North American range grasses (Larson and others 2000, 2001a, 2001b, 2003a, 2003b, 2004). Likewise, Massa and others (2001) used these techniques to characterize genetic diversity within and among *Bromus* sect. *Ceratochloa* species of South America. In all of our examinations (Larson and others 2001a, 2003a, 2003b, 2004; Massa and others 2001), significant correlations were detected between DNA polymorphism and geographic distance among natural germplasm sources. Moreover, our examinations consistently demonstrate significant correlations between DNA polymorphism and morphological variation (Jones and others 2003; Larson and others 2003b; Massa and others 2001, 2004).

MATERIALS AND METHODS

This study examined 30 accessions totaling 55 genotypes of *Bromus* sect. *Ceratochloa* taxa, including 46 North American octoploids ($2n = 8x = 56$), 5 South American hexaploids, and 4 South American octoploids (Table 1). Our strategy of sampling 2 genotypes per accession was primarily designed to detect hierarchical (geographic) groups rather than to compare diversity within accessions. Similar studies (Larson and others 2001a, 2003b; Massa and others 2001) often detect little or no variation within site-specific collections (accessions) of autogamous plant species.

Accessions from North America were originally collected in the Rocky Mountains and Pacific Coast areas between 38° N and 51° N latitude (Figure 1). Most of the seed samples were obtained from the USDA Plant Introduction Station, Pullman, Washington. One accession (CAR47) is a cultivated variety ('Garnet'), released by the USDA Natural Resources Conservation Service, Bridger Plant Materials Center (Bridger, Montana), intended for general use (Table 1). Conversely, the CAR69 and CAR70 seed accessions were obtained as cultivated seed increases of local ecotypes contracted by the USDA Forest Service (Table 1). We identified these grasses *Bromus carinatus* sensu lato regardless of the original taxonomic identification. *Bromus* sect. *Ceratochloa* accessions from South America (Massa and others 1997), including 2 octoploids and 4 hexaploids ($2n = 8x = 56$), were utilized as reference genotypes (Table 1).

Genomic DNA was isolated from young leaves of single plants using the DNeasy Plant system (QIAGEN, Valencia, California). Seeds were germinated in the greenhouse at Logan, Utah, and seedlings outplanted into a field nursery during May 2001 at the Utah Agriculture Experiment Station (Evans Farm), approximately 2 km (1.2 mi) south of Logan, Utah (41° 41' N, 111° 50' W, 1350 m [4400 ft] above sea level). Voucher specimens collected from flowering plants at the Evans Farm in 2003 were submitted to the Intermountain Herbarium at Utah State University in Logan, Utah.

AFLP analysis was performed using 5 selective amplification primer pairs (Massa and others 2001) according to the methods of Vos and others (1995), except that *EcoR* I selective amplification primers were labeled with fluorescent 6-FAM (6-carboxy fluorescein) on the 5' nucleotide. Fluorescent amplified fragments were fractionated on an ABI 3100 instrument using 50-cm capillaries and POP-6 polymers (Applied Biosystems, Foster City, California). The AFLP profiles from each sample were subsequently aligned, using Genographer (Benham and others 1999), to form one gel file for each primer pair. Scoring of the AFLP phenotypes, as present (1) or absent (0), was also performed with Genographer (Benham and others 1999).

Estimates of genetic similarity between individual genotypes (plants) were based on the proportion of shared AFLP fragments within (F_W) and among (F_A) accessions: $F = 2n_{XY}/(n_X + n_Y)$, where n_{XY} is the number of shared fragments between 2 genotypes, whereas n_X and n_Y are the number of fragments in genotype *X* and *Y*, respectively (Nei and Li 1979). A dendrogram of genealogical lineages was developed using the Neighbor Joining analysis, in PAUP 4.0 (Swofford 1998), of the Nei's genetic similarity (F)

TABLE 1

Description of 30 Bromus *sect.* Ceratochloa *accessions examined by AFLP analysis .*

ID	N	Voucher ID [z]	Accession [y]	Collection Site [x]	Latitude	Longitude
***Bromus carinatus* senus lato (2n=8x=56)**						
CAR03	2	UTC 236009	PI 232202	Sir Francis Drake Road, Inverness Park, Marin County, California	38.07 N	122.82 W
CAR04	2	UTC 236010	PI 232203	Point Ryes Road, 6.4 km west of Inverness Park, Marin County, California	38.12 N	122.87 W
CAR05	2	UTC 236011	PI 232204	Beckwourth School, Plumas County, California	39.82 N	122.38 W
CAR06	2	UTC 236012	PI 232205	4.8 km south of Fort Klamath, Klamath County, Oregon	42.70 N	122.00 W
CAR08	2	UTC 236013	PI 236755	Route 1; 128.7 km north of Golden, British Columbia	51.30 N	117.00 W
CAR12	2	UTC 236014	PI 232220	Route 40 near Donner Lake, Nevada County, California	39.33 N	120.27 W
CAR13	2	UTC 236015	PI 232221	Lake Tahoe shoreline, Placer County, California	39.10 N	120.15 W
CAR15	2	UTC 236016	PI 232223	Route 62 at southern border Roger River National Forest, Klamath County, Oregon	42.80 N	120.80 W
CAR16	2	UTC 236017	PI 232224	Whitebird, Idaho County, Idaho	45.76 N	116.30 W
CAR18	1	UTC 236018	PI 232227	9.7 km south of Pollock, Idaho County, Idaho	45.30 N	116.36 W
CAR19	2	UTC 236019	PI 232229	Lamoille Canyon, Elko County, Nevada	40.67 N	115.42 W
CAR20	2	UTC 236020	PI 232230	Huntsville, Weber County, Utah	41.26 N	111.77 W
CAR25	1	UTC 236021	PI 232236	Route 191; 11.3 km north of Warm River, Fremont County, Idaho	44.18 N	111.31 W
CAR26	2	UTC 236022	PI 232237	Stemple Pass, Lewis and Clark County, Montana	46.90 N	112.50 W
CAR27	1	UTC 236023	PI 232238	Stemple Pass, Lewis and Clark County Montana	46.90 N	112.50 W
CAR31	2	UTC 236024	PI 236767	Zincton mine dump, British Columbia	50.00 N	117.40 W
CAR32	2	UTC 236025	PI 236768	9.7 km southeast of Moyie, British Columbia	49.30 N	115.90 W
CAR37	2	UTC 236026	PI 241048	9.7 km north of Union Creek, Route 62, Klamath County, Oregon	42.89 N	122.22 W
CAR43	2	UTC 236027		Cave Falls Road, Fremont County, Idaho	44.12 N	111.28 W
CAR44	2	UTC 236028		Card Canyon, Cache County, Utah	41.75 N	111.66 W
CAR47	2	UTC 236029	'Garnet'	Garnet Mountain, Gallatin County, Montana	45.44 N	111.20 W
CAR48	2	UTC 236030	9019117	Clearwater Junction, Missoula County, Montana	47.00 N	113.38 W
CAR49	1	UTC 236031	9039066	Dearborn River, Lewis and Clark County, Montana	47.10 N	112.30 W
CAR69	2	UTC 236032	9070979	Routt National Forest near Steamboat Springs, Routt County, Colorado	40.48 N	106.80 W
CAR70	2	UTC 236033	9070980	Meadow Lake, White River National Forest, Garfield County, Colorado	39.80 N	107.50 W
***Bromus coloratus* (2n=8x=56)**						
COL512		ARC 1265	BG232	Termas de Epulafquen, Neuquen, Argentina	39.88 S	71.25 W
COL52	2	UTC 235178	BG390	103 km south of Coyhaique, XI Región, Coyhaique, Chile	46.50 S	72.44 W
***Bromus catharticus* (2n=6x=42)**						
CAT04	1	ARC 1629	BG239	30.6 km east of Paso Hau-Hum, Neuquén, Argentina	40.53 S	71.39 W
CAT35	2	ARC1599	BG387	25.7 km south La Tapera, XI Región, Coyhaique, Chile	44.70 S	72.11 W
CAT30	2	UTC235187	BG379	9.7 km Paso Coyhaique Alto, Chubut, Argentina	45.53 S	71.33 W

[z] UTC = Utah State Intermountain Herbarium, Logan, Utah. ARC = Herbarium Facultad de Ciencias Agrarias, UN Comahue, Rio Negro, Argentina.

[y] PI = USDA Plant Introduction Station, Pullman, Washington.

[x] 1 km = 0.6 mi.

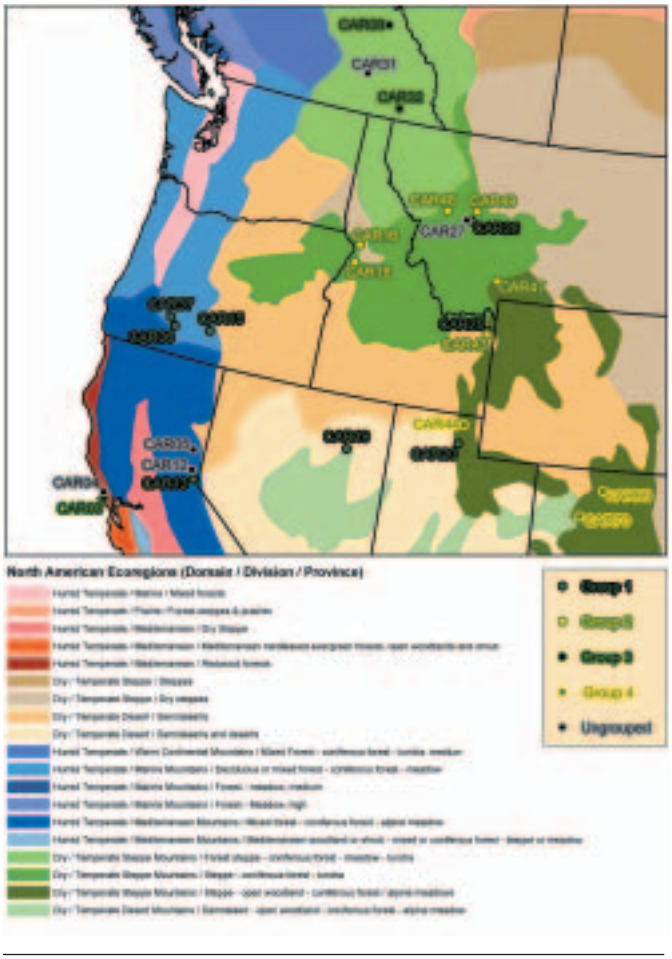

Figure 1. Geographic distribution of collection sites for 25 North American *Bromus carinatus* sensu lato accessions overlaid on the map of North American ecoregions (Bailey 1996). All but 5 accessions were classified into genetic groups by DNA fingerprinting.

matrix. Bootstrap significance levels were also obtained using PAUP 4.0, with 1000 replications. However, the corresponding bootstrap trees were based strictly on the total number of amplified fragment length polymorphisms rather than Nei's genetic similarity coefficients as shown in Figure 2. The significance of genetic variation within and among accessions and hierarchical groups was also evaluated by analysis of molecular variance (AMOVA), based on the number of amplified fragment length polymorphisms between individual genotypes, with corresponding estimates of genetic fixation among accessions (F_{ST}) and hierarchical groups (F_{CT}) computed using Arlequin (Excoffier and others 1992).

Correlations between matrices of geographical distance and genetic similarity, strictly among the 25 North American *B. carinatus* accessions, were evaluated by the Mantel (1967) test statistic (Z), using the MxComp procedure of NTSYS-pc (Rohlf 1993). Geographical distance (km) matrices were computed from geographical coordinates, using the formula described in Math Forum (1997): km = Arccos [cos(LAT_x) cos($LONG_x$) cos(LAT_y) cos($LONG_y$) + cos(LAT_x) sin($LONG_x$) cos(LAT_y) sin($LONG_y$) + sin(LAT_x) sin(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. The genetic similarity matrix was developed using the average proportion of shared fragments (Nei's similarity coefficients) among accessions (that is, averages of pairwise comparisons between individual plants). Significance tests for these correlations were determined by comparing observed values to values obtained by 1000 random permutations. Therefore, the upper tail probability (*p*) that 1000 random Mantel test statistic (Z) values are (by chance) less than observed values of Z equals 0.002 or greater. A geographical map of collection sites for the 25 mountain brome accessions was developed using ArcMap™ 8.2 (ESRI®, Redlands, California), including the Ecoregions of North America shapefile downloaded from http://www.fs.fed.us/institute/ecoregions/eco_download.html (Bailey 1996).

RESULTS AND DISCUSSION

A total of 432 bands were amplified by 5 primer combinations, including 381 (88%) polymorphic fragments and 51 (12%) monomorphic fragments (Table 2). Thus, each primer combination generated an average of 86 bands per primer combination, with about 45 fragments per primer pair per genotype. These AFLP profiles were suitable for comparisons of genetic identity and genealogical lineages within and among North and South American *Bromus* sect. *Ceratochloa* accessions.

On average, octoploids displayed nearly 30% more AFLP fragments per plant as compared with the South American hexaploid accessions (Table 2). Thus, octoploid *Ceratochloa* genomes are more complex than hexaploid *Ceratochloa* genomes. Of the 209 fragments that were unique to the octoploid accessions, a total of 52 (25%) were common to both North and South American accessions. Moreover, the average proportion of shared bands between the North and the South American octoploids was higher than the average proportion of shared bands between the South American octoploid and South American hexaploid accessions (Figure 2). As expected, the 55 *Bromus* genotypes were separated into 3 distinct lineages corresponding to North American *B. carinatus* octoploids ($2n = 8x = 56$), South American *B. catharticus* hexaploids ($2n = 6x = 42$), and South American *B. coloratus* octoploids ($2n = 8x = 56$) based on Neighbor-Joining analysis of Nei's genetic similarity coefficients (Figure 2). The genealogical tree (Figure 2) was rooted using hexaploid South American *B. catharticus* as the outgroup, the most distinct of the 3 taxons based on pairwise comparisons of the Nei's genetic similarity coefficients (proportion of shared bands). These genetic relationships, including the affinity of North and South American octoploids, are supported by the bootstrap confidence values

TABLE 2

Summary of AFLP variation in accessions of Bromus *sect.* Ceratochloa*, with estimates of similarity (F) and diversity within (F_W) and among (F_A) accessions of groups.*

Taxa	2*n*	*N*	Average number of fragments per plant (range)	Total number of fragments (polymorphic)	Proportion of shared fragments (F) Average F_W (range)	Average F_A (range)
Octoploids	56	50	229 (188–248)	416 (351)	0.918 (0.86–0.99)	0.780 (0.68–0.97)
B. carinatus sensu lato		46	231 (206–248)	374 (286)	0.928 (0.86–0.99)	0.856 (0.81–0.97)
B. coloratus		4	205 (188–215)	242 (78)	0.879 (0.82–0.98)	0.840 (0.82–0.86)
Hexaploids (*B. cartharticus*)	42	5	161 (150–166)	223 (119)	0.820 (0.75–0.91)	0.783 (0.68–0.81)
Total		55	223 (150–248)	432 (381)	0.902 (0.82–0.99)	0.741 (0.65–0.86)

(Figure 2). These findings support the assertion that the South and the North American octoploids are closely related (Massa and others 2001).

Genetic similarity coefficients within and among the *B. carinatus* sensu lato accessions ranged from 0.81 to 0.99 with an average of 0.86. The average proportion of shared AFLP fragments within accessions was greater than the average proportion of shared fragments among accessions (Table 2). As a group, significant fixation ($F_{ST} = 0.70$, $P < 0.0001$) among the 25 North American accessions was detected by AMOVA, based on the number of amplified fragment length polymorphisms. Thus, our sampling strategy was sufficient to demonstrate that the average genetic distance was greater among accessions relative to the average genetic distance within accessions. Our sampling of 2 genotypes per accession, however, was insufficient to compare diversity within one accession to diversity within another accession or to test for pairwise differences between accessions. Nevertheless, a relatively high degree of genetic identity was observed, overall, within accessions. In all but 3 cases (CAR48, CAR26, and CAR69), the 2 individual plants group together by accessions (Figure 2). These results are consistent with morphological uniformity within naturally self-fertilizing *B. carinatus* populations (Harlan 1938, 1945; Stebbins 1947). Similar patterns of DNA polymorphism were detected in autogamous purple needlegrass (*Nassella pulchra* (A.S. Hitchc.) Barkworth [Poaceae]) (Larson and others 2001a), autogamous bottlebrush squirreltail (*Elymus elymoides* (Raf.) Swezey [Poaceae]) of western North America (Larson and others 2003b), and autogamous South American *Bromus* sect. *Ceratochloa* species (Massa and others 2001).

All but five of the North American *B. carinatus* accessions were classified into 4 well-defined genealogical groups based on genetic similarity, supported by the bootstrap confidence values (Figure 2). Significant differences among 4 hierarchical genotypic groups ($F_{CT} = 0.47$, $P < 0.0001$), encompassing all but 5 unique accessions, were also detected by AMOVA. The 5 remaining accessions, CAR04, CAR05, CAR12, CAR27, and CAR31, displayed relatively unique AFLP profiles (Figure 2). Excluding comparisons within accessions, a weak ($r = -0.257$) but significant ($P \leq 0.01$) correlation was detected between the geographic distance matrix (illustrated in Figure 1) and genetic similarity matrix (summarized in Figure 2) strictly among the 25 North American *B. carinatus* accessions. Much of this correlation can be attributed to groups 2 and 4 (Figure 2), which evidently have restricted geographic range (Figure 1). Conversely, genetic similarities within groups 1 and group 3 (Figure 2) transcend large geographic ranges (Figure 1). Likewise, geographic proximity between: 1) CAR44 and CAR20; 2) CAR26 and CAR27; 3) CAR25 and CAR43; 4) CAR4 and CAR3; and 5) CAR12 and CAR13 (Figure 1) belie relatively large genetic differences among corresponding comparisons (Figure 2). Although significant correlation has been detected between DNA polymorphism and geographic distance has been detected in other grasses (Larson and others 2001a, 2003a, 2003b, 2004; Massa and others 2001), these associations are not predictive here. Seemingly local seed sources may not match other local populations (Figure 1). Conversely, similar or nearly identical genotypes may be naturally abundant and widely dispersed over broad geographic regions (Figure 1). Anomalies between genetic distance and geographic distance have also been noted in other autogamous North American grasses including purple needlegrass ($r = 0.55$) (Larson and others 2001a) squirreltail ($r = 0.55$) (Larson and others 2003b), and *Bromus* sect. *Ceratochloa* species of South America ($r = 0.243$) (Massa and others 2001). Although ecotypic genetic structure is often apparent and readily detected in autogamous plants, geographic patterns of genetic variation may often reflect history of dispersal and colonization. Indeed, self-fertilization may be an essential attribute of colonizing species (Larson and others 2001a; 2003b). In contrast to self-pollinating plants, genetic structure can be difficult to detect in cross-pollinating species (Larson and others 2003a). Neverthe-

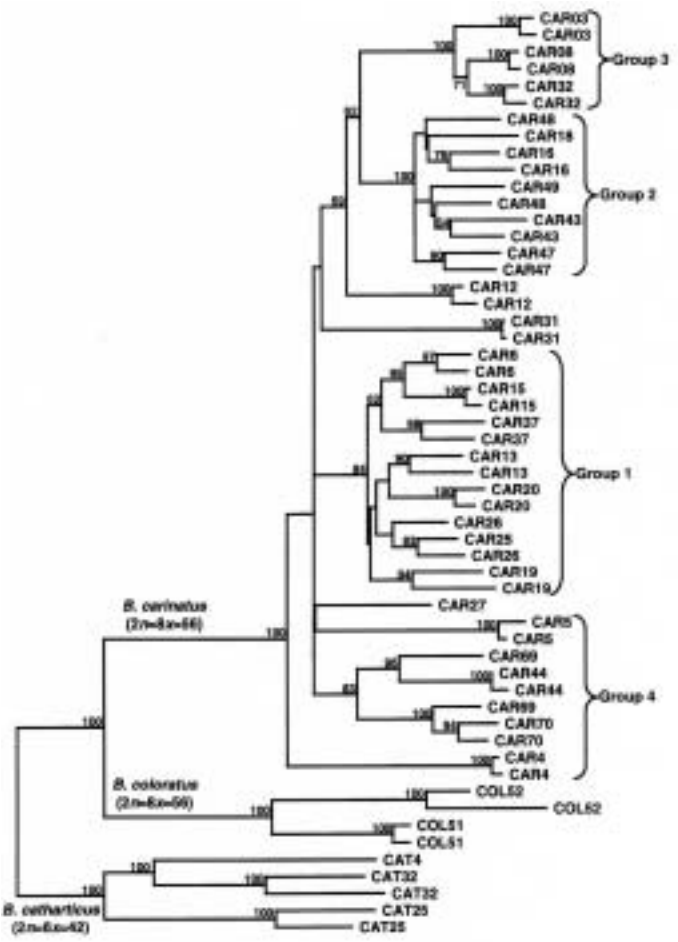

Figure 2. Unrooted neighbor joining tree based on Nei's genetic similarity coefficients among 30 *Bromus* sect. *Ceratochloa* accessions.

less, correlations between genetic distance and geographic distance ($r = 0.66$ and $r = 0.58$) have been detected in allogamous grasses including western wheatgrass (*Pasocoyrum smithii* (Rydb.) A. Love [Poaceae]) (Larson and others 2003a) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Love [Poaceae]) (Larson and others 2004).

These AFLP profiles elucidated new phylogeographic information for the mountain bromes of western North America. Interestingly, ecoregions of California displayed the greatest diversity of genotypes. It is tempting to speculate that these ecoregions may have been glacial refugia, thus harboring a hot spot of intraspecific biodiversity. However, glaciated regions, such as dry (Rocky) mountain regions (Figure 1), may also harbor a melting pot of intraspecific diversity, a possible consequence of the admixture of divergent lineages colonizing the continent from separate refugia (Petit and others 2003).

We believe that phylogeographic information will facilitate the development and utilization of native plant materials. One of the accessions in this study, CAR47 (Garnet mountain brome) was released by the USDA Natural Resources Conservation Service (Noller and others 2000) as a commercial seed source intended for general use in western Montana and northern Idaho (see Figures 1 and 2). Although Garnet was a single source collection, the DNA profiles of this cultivar (CAR47) closely match other natural germplasm sources in genetic group 2 (Figure 2) from the same dry temperate steppe mountain ecoregion (Figure 2). Therefore, not only was adaptation good based on field testing (Noller and others 2000) but also this molecular genetic analysis confirms that Garnet is similar to natural germplasm sources from the same region of origin (Figure 2). With somewhat different intentions, USDA Forest Service contracted isolated seed increases of CAR69 and CAR70 for site-specific use in the Routte National Forest and White River National Forests, respectively. Although CAR69 and CAR70 are relatively distinct from most other accessions examined in this study, these 2 seedlots have a mixture of related genotypes in that one of the CAR69 plants (from Routte National Forest) was most similar to collections from Utah (CAR44), whereas the other CAR69 plant was most similar to the White River National Forest germplasm (CAR70) (Figure 2). The most naturally abundant and widely distributed group of accessions, group 1 (Figure 1), was not represented by any of the commercial germplasm sources examined, which may be considered in future plant material efforts. In any case, molecular analyses allow confirmation that an accession is similar (or different) from natural ecotypes within a geographic region or ecological province, which helps plant breeders and land managers select and utilize natural germplasm sources.

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