# Amplified fragment length polymorphism in *Elymus elymoides*, *Elymus multisetus*, and other *Elymus* taxa

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**Abstract:** The geographic and phylogenetic significance of amplified fragment length polymorphism within and among 22 *Elymus elymoides* (Raf.) Swezey subsp. *elymoides*, 24 *E. elymoides* subsp. *brevifolius* (J.G. Sm.) Barkworth, and 13 *Elymus multisetus* (J.G. Sm.) Burtt-Davy squirreltail accessions was assessed relative to six other North American and three Eurasian *Elymus* taxa. *Elymus elymoides* and *E. multisetus*, comprising *Elymus* sect. *Sitanion* (Raf.) Á. Löve, were both monophyletic and closely related compared with other congeners. The monophyly of subsp. *elymoides* was also supported; subsp. *brevifolius*, however, was paraphyletic and separated into four genetically distinct groups. Estimates of nucleotide divergence among the five *E. elymoides* groups range from 0.0194 to 0.0288, with approximately 0.0329 differences per site between *E. elymoides* and *E. multisetus*. Corresponding estimates of nucleotide divergence range from 0.0243 to 0.0387 among North American taxa and from 0.0337 to 0.0455 between North American and Eurasian taxa. DNA polymorphism among *E. elymoides* accessions was correlated with geographic provenance and previously reported quantitative traits. Distinct genetic groups of *E. elymoides* generally correspond to different geographic regions, whereas divergent *E. multisetus* and *E. elymoides* accessions are sympatric. Thus, taxonomic ranks of *E. multisetus* and *E. elymoides* were supported and geographic groups within *E. elymoides* were distinguished.

*Key words:* AFLP, *Elymus*, nucleotide diversity, squirreltail.

**Résumé :** Les auteurs ont évalué la signification géographique et phylogénétique du polymorphisme de la longueur des fragments de restriction, à l'intérieur et entre 22 accessions d'*Elymus elymoides* (Raf.) Swzey subsp. *elymoides*, 24 d'*E. elymoides* subsp. *brevifolia* (J. G. Sm.) Barkworth, et 13 d'*Elymus multisetus* (J. G. Sm.) Burtt-Davy, comparativement à six autres taxons nord-américains et trois taxons eurasiens d'*Elymus*. L'*Elymus elymoides* et l'*Elymus multisetus*, comprenant l'*Elymus* sect. *Sitanion* (Raf.) A. Löve, sont tous deux monophylétiques et étroitement reliés comparativement à d'autres congénères. Les données supportent également la monophylie de la subsp. *elymoides*; la subsp. *brevifolius* est cependant paraphylétique et se sépare en quatre groupes génétiquement distincts. L'estimation de la divergence des nucléotides parmi les cinq groupes de l'*E. elymoides* se situe entre 0,0194 et 0,0288, avec une différence d' environ 0,0329 par site entre les *E. elymoides* et *E. multisetus*. Les estimés correspondants des divergences des nucléotides vont de 0,0243 à 0,0387 entre les taxons nord-américains et 0,0337 à 0,0455 entre les taxons nord-américains et eurasiens. Le polymorphisme de l'ADN au sein des accessions de l'*E. elymoides* est corrélé avec la provenance géographique et les caractères quantitatifs déjà rapportés. Les groupes distincts de l'*E. elymoides* correspondent généralement à différentes régions géographiques, alors que les accessions divergentes de l'*E. multisetus* et de l'*E. elymoides* sont sympatriques. Ainsi, les rangs taxonomiques de l'*E. multisetus* et de l'*E. elymoides* trouvent un support et on distingue des groupes géographiques au sein de l'*E. elymoides*.

*Mots clés :* AFPL, *Elymus*, diversité des nucléotides, élyme.

[Traduit par la Rédaction]

## Introduction

Large-scale seedings of perennial grasses, shrubs, and forbs on North American rangelands have had a major impact on fire cycles, weed suppression, soil stabilization, forage production, and habitat qualities. These plantings have also impacted rangeland species composition, as evidenced by the widespread abundance of introduced crested wheatgrasses (*Agropyron cristatum* and *Agropyron desertorum*). Likewise, large-scale seedings of native plants may also change the natural abundance, distribution, and variability of the respective natural flora. However, the latter effects are not easily discerned and the geographic significance of natural genetic variability has not been well documented in native range grasses of western North America. A better appreciation of genetic diversity in these rangeland grasses will help researchers and land managers develop and select seed sources needed for large-scale revegetation.

*Elymus* sect. *Sitanion* (Raf.) Á. Löve comprises *Elymus elymoides* (Raf.) Swezey (*Sitanion hystrix* (Nutt.) J.G. Sm.)

Received 28 October 2002. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 4 September 2003.

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 doi: 10.1139/B03-077 

bottlebrush squirreltail and *Elymus multisetus* (J.G. Sm.) Burtt Davy (*Sitanion jubatum* J.G. Sm.) big squirreltail grasses of western North America (Barkworth 1997; Holmgren and Holmgren 1977). Taxonomists (Barkworth 1997; Holmgren and Holmgren 1977) also recognized four subspecific taxa of bottlebrush squirreltail: *Elymus elymoides* subsp. *elymoides* (*Sitanion hystrix* var. *hystrix*), *Elymus elymoides* subsp. *brevifolius* (J.G. Sm.) Barkworth (*Sitanion hystrix* var. *brevifolium* (J.G. Smith) C.L. Hitchc.), *Elymus elymoides* subsp. *californicus* (J.G. Sm.) Barkworth (*Sitanion hystrix* var. *californicum* (J.G. Sm.) F.D. Wilson), and *Elymus elymoides* subsp. *hordeoides* (Suksd.) Barkworth (*Sitanion hystrix* var. *hordeoides* (Suksd.) C.L. Hitchc.). These squirreltail taxa were transferred from genus *Sitanion* Raf. to genus *Elymus* L. (Barkworth et al. 1983; Barkworth and Dewey 1985) on the basis of hybridization and cytogenetic studies (Brown and Pratt 1960; Church 1967*a*, 1967*b*; Dewey 1967, 1969; Stebbins et al. 1946; Stebbins and Snyder 1956; Stebbins and Vaarama 1954). Chromosome-pairing data suggest that virtually all of the native North American species of *Elymus* are **StStHH** allotetraploids ($2n = 4x = 28$), derived from *Pseudoroegneria* (**St** genome) and *Hordeum* (**H** genome) (Dewey 1982, 1983*a*, 1983*b*, 1984). Little or no significant variation in nuclear DNA content has been detected among divergent allotetraploid *Elymus* species (Vogel et al. 1999). Likewise, the chloroplast *ndh*F DNA sequences of *E. elymoides* and other allotetraploid *Elymus* species are virtually identical (Redinbaugh et al. 2000; Mason-Gamer et al. 2002). The placement of *E. elymoides* in *Elymus* was also supported by the phylogeny of granule-bound starch synthase nuclear DNA sequences (Mason-Gamer 2001). Yet, bottlebrush squirreltail (*E. elymoides*) and big squirreltail (*E. multisetus*) taxa are distinguished from other *Elymus* species by having a brittle rachis and subulate glumes extending into long awns (Wilson 1963), suggesting a sister relationship of these two taxa. Consequently, *E. elymoides* and *E. multisetus* were retained together as sect. *Sitanion* of genus *Elymus* (Löve 1984). However, these explicitly phylogenetic studies (Redinbaugh et al. 2000; Mason-Gamer 2001; Mason-Gamer et al. 2002) did not detect sufficient DNA polymorphism to evaluate genetic diversity or relationships within and between *E. elymoides* and *E. multisetus*.

Squirreltail grasses are widely distributed across diverse elevation and precipitation zones of western North America (Wilson 1963). The disarticulating rachis and long, arcuately diverging glume and lemma awns promote wind dispersal across open ground (Barkworth 1997). Like most *Elymus* species, squirreltail grasses are relatively short-lived, prolific seed producers. With the exception of several species (including *E. lanceolatus* and *E. wawawaiensis* examined here), most allotetraploid *Elymus* species are self-fertilizing (Keller 1948; Jensen et al. 1990; Smith 1944). Although squirreltail grasses have not been regarded as important forages, these perennial grasses are gaining increasing attention in rangeland revegetation partly because they are naturally adapted to colonize disturbed areas and may help suppress the invasion of weeds (Jones 1998). Consequently, Jones et al. (2002) evaluated 12 quantitative traits among five *E. elymoides* subsp. *brevifolius*, 17 *E. elymoides* subsp. *elymoides*, and four *E. multisetus* accessions and nine quantitative traits among 21 *E. elymoides* subsp. *brevifolius*, 10 *E. elymoides* subsp. *elymoides*, and 16 *E. multisetus* accessions grown under uniform field conditions. Quantitative traits measured in both evaluations (Jones et al. 2002) included days to emergence, heading date, leaf length, plant height, root length, root to shoot ratio, seed mass, specific root length, and total plant dry matter. Geographically diverse collections evaluated by Jones et al. (2002) represent the three most abundant and widely distributed squirreltail taxa, particularly in the Rocky Mountain and Intermountain Floristic provinces. In both germplasm comparisons, these three squirreltail taxa were effectively separated into different groups by multivariate principle components analysis. Moreover, the subsp. *brevifolius* accessions also separated into three well-defined subgroups designated A, B, and C. These squirreltail groups evidently display different adaptations and recognition of these groups may be important. However, the phylogenetic significance of these squirreltail groups was uncertain (Jones et al. 2002).

The amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) is a robust and highly effective method of DNA fingerprinting that can be used to measure and estimate nucleotide diversity (Innan et al. 1999), as demonstrated in cross-pollinating (Larson et al. 2000) and self-pollinating (Larson et al. 2001) plant species. Nucleotide diversity and divergence are standard measures of DNA variation used to investigate population dynamics, evolutionary relationships, and other biological phenomena (Nei 1987). In the context of natural populations within plant species, amplified fragment length polymorphism may be correlated with geographic origin (Larson et al. 2001; Massa et al. 2001). Moreover, the AFLP technique can also resolve phylogenetic relationships among closely related (i.e., congeneric) grass species (Aggarwal et al. 1999; Massa et al. 2001).

This study investigates the geographic and phylogenetic significance of amplified fragment length polymorphism within and among squirreltail taxa and subtaxonomic groups previously distinguished by quantitative trait variation (Jones et al. 2002). Specific objectives of this investigation were to (*i*) test genetic relationships and compare rates of nucleotide variation within and among *E. elymoides* subsp. *elymoides* and *E. elymoides* subsp. *brevifolius* groups A, B, C, and D that were distinguished by quantitative trait evaluations (Jones et al. 2002), (*ii*) test genetic relationships and compare rates of nucleotide variation between *E. elymoides* and *E. multisetus* (sect. *Sitanion*) relative to nine other allotetraploid *Elymus* species, including six North American taxa (*Elymus canadensis* L., *Elymus glaucus* Buckl., *Elymus hystrix* (Moench) Á. Löve, *Elymus lanceolatus* (Scribner & Smith) Gould, *Elymus trachycaulus* (Link) Gould ex Shinners, and *Elymus wawawaiensis* J.R. Carlson J.R. Carlson and Barkworth) and three Eurasian taxa (*Elymus caninus* L., *Elymus mutabilis* (Drobov) Tzvelev, and *Elymus sibiricus* L.) representing five other sections of *Elymus* (Löve 1984), and (*iii*) test overall correlations among quantitative traits (Jones et al. 2002), geographic provenance, and amplified fragment length polymorphism. This research will help identify natural germplasm sources that represent genetic diversity in squirreltail, particularly among the Intermountain and Rocky Mountain floristic provinces, and elucidate phylogenetic relationships among *Elymus* species.

## Methods

### Plant materials

Seeds of each accession were originally collected and defined by their native site origin (Table 1) and reproduced at least one generation. The 46 *E. elymoides* accessions and 13 *E. multisetus* accessions were grown near Logan, Utah, and classified to species or subspecies (Table 1) using a dichotomous key (Wilson 1963) as described by Jones et al. (2002). Accessions with D-, DJ-, or T- collection numbers and the Sand Hollow germplasm release were maintained in the USDA-ARS Forage and Range Research Laboratory (Logan, Utah) germplasm collection along with detailed descriptions for each collection site (Jensen et al. 1997). Most of these accessions were submitted for preservation and distribution by the USDA-ARS National Plant Germplasm System (NPGS) Western Regional Plant Introduction Station (Pullman, Wash.). Accessions with NRCS collection numbers were obtained directly from the USDA-NRCS or via NPGS. The MULT-13 accession (Table 1) was provided by the United States Department of Interior (USDOI) Bureau of Land Management. Similarly, the ELYMb-01 accession (Table 1) Oregon State University (Corvallis, Oreg.). Seed for the ELYMe-41, ELYMe-42, ELYMe-43, and ELYMe-44 accessions (Table 1) was provided by the Maughan & Barton, Granite, Rainier, and Wind River seed companies, respectively. The remaining accessions (Table 1) were obtained directly from NPGS. The latitude and longitude coordinates of collection sites for commercial seed sources, NRCS accessions, and other NPGS accessions were approximated for purposes of this study.

The *E. canadensis* (CANA), *E. caninus* (CANI), *E. glaucus* (GLAU), *E. hystrix* (HYST), *E. lanceolatus* (LANC), *E. mutabilis* (MUTA), *E. sibiricus* (SIBI), *E. trachycaulus* (TRAC), and *E. wawawaiensis* (WAWA) accessions (Table 1) were obtained directly from the USDA Forage and Range Research Laboratory (Jensen et al. 1997). All taxa and accessions are allotetraploid ($2n = 4x = 28$).

Seeds were germinated on moist blotter paper. Seven seedlings of each accession were grown in single-plant containers in a greenhouse. Although morphological variation is readily apparent among most of the squirreltail accessions, individual plants are generally very uniform within accessions (Jones et al. 2002). For the purposes of this study, two seedlings were randomly sampled from each accession. Voucher specimens (listed in Table 1) were submitted to the Intermountain Herbarium at Utah State University (Logan, Utah).

### DNA analyses

Samples of 100 mg of leaf tissue were collected from each seedling and placed in 2-mL microcentrifuge tubes containing two steel bearings (5 mm in diameter). These samples were subsequently frozen under liquid nitrogen and vortexed into a fine powder. One millilitre of extraction buffer (2% hexadecyltrimethyl-ammonium bromide (CTAB), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 100 mM Tris–HCl (pH 8.0), 0.2% β-mercaptoethanol, and 0.1 RNAase mg/mL, 65 °C) was added to the frozen leaf powder and incubated at 65 °C for at least 1 h. A 24:1 (v/v) solution of chloroform – isoamyl alcohol was added and mixed vigorously prior to phase separation by centrifugation (14 000*g* for 5 min). The upper aqueous phase (containing nucleic acids) was transferred to a 1.5-mL microcentrifuge tube and mixed with 0.7 mL of cold isopropanol. Nucleic acids were hooked out with a glass pipette and washed in a solution of 70% ethanol and 10 mM ammonium acetate, air dried, and dissolved in TE buffer (10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (pH 8.0)). Genomic DNA quantity and quality were evaluated by agarose gel electrophoresis.

DNA fingerprinting was conducted using the AFLP technique according to the methods of Vos et al. (1995), except that *Eco*RI selective amplification primers included a fluorescent 6-carboxyfluorescein label on the 5′ nucleotide. Selective amplifications were performed using six *Eco*RI +3 – *Mse*I +3 primer pairs (e.g., E.AGC//M.CAG, E.AGC//M.CAT, E.AGC//M.CTG, E.AGG//M.CAA, E.AGG/M.CAC, E.AGG/M.CAG), where E and M designate the *Eco*RI and *Mse*I adapters with three selective nucleotides as described by Vos et al. (1995). The amplified DNA fragments were size fractionated using an ABI3100 instrument with 50-cm capillaries, POP-6 polymer, GeneScan 400HD (rhodamine X) internal size standards, and Genescan software (PE Applied Biosystems, Foster City, Calif). The GeneScan sample files were subsequently analyzed for the presence and absence of DNA fragments, between 50 and 400 bp, using Genographer version 1.5 (Benham et al. 1999). Although subjective, the first author attempted to score virtually all fragments into allelic categories based on comparisons of fragments with similar relative migration coefficients (determined primarily by the number of nucleotide base pairs). Categories were separated by obvious or seemingly discrete differences in relative migration. Thus, some categories were more or less variable than others in terms of relative migration units (estimated in nucleotide base pairs). However, most categories were at least 0.5 relative migration unit apart. Virtually all fragments that showed a smooth fluorescent trace signal (i.e., clearly above stochastic background signals) were considered. However, possible fragment categories that did not show discrete differences from stochastic background signals were ignored.

### Data analyses

The total number of fragments per plant ($M$) and total number of differences between plants ($P$) were computed directly from binary data sets of fragment present (1) and fragment absent (0). The proportion of shared fragments between plants ($F$) was computed using the formula $F = (M_X + M_Y - P)/(M_X + M_Y)$, where $M_X$ and $M_Y$ denote the total number of fragments for each of the two plants being compared. This formula for $F$ is equivalent to that reported by Nei and Li (1979). Estimates of total nucleotide diversity within taxa ($\pi_t$), nucleotide diversity within accessions ($\pi$), and nucleotide divergence among subspecific groups or taxa ($D$) were estimated based on corresponding $F$ and $M$ values using methods and software described by Innan et al. (1999). The corrected nucleotide divergence ($D_A$) was calculated using the formula $D_A = D - (\pi_X - \pi_Y)/2$, where $\pi_X$ and $\pi_Y$ denote the total nucleotide diversity ($\pi_t$) within the two taxa or groups being compared. Differentiation ($g_{st}$) among accessions within taxa or subspecific groups was calculated using the formula $g_{st} = (\pi_t - \pi)/\pi_t$. Similarly, differentiation among taxa or subspecific groups ($G_{st}$) was calculated using the for-

**Table 1.** Description of *Elymus* taxa and accessions evaluated for amplified fragment length polymorphism.

| Label | Taxon | Herbarium voucher[c] | Seed accession identifier(s) | Origin (°N, °W) |
|---|---|---|---|---|
| **Section *Sitanion* with type species *E. elymoides*** | | | | |
| ELYMb-01 | *E. elymoides* subsp. *brevifolius* | 235878 | Grandview | Grandview, Jefferson co., Oreg. (44.5, 121.6) |
| ELYMb-02 | *E. elymoides* subsp. *brevifolius* | 235879 | PI 611151,[d] T-920 | Turin, Alta. (50, 112.6) |
| ELYMb-03 | *E. elymoides* subsp. *brevifolius* | 235880 | PI 611152,[d] T-926 | Buffalo, Alta. (50.8, 110.7) |
| ELYMb-04[a] | *E. elymoides* subsp. *brevifolius* | 235881 | PI 531605,[d] D-3345, Acc1105 | Gardner, Huerfano co., Colo. (37.62, 105.19) |
| ELYMb-05[a] | *E. elymoides* subsp. *brevifolius* | 235882 | NRCS 9040187, Acc1122 | Wet Mountains, Custer co., Colo. (38.05, 104.8) |
| ELYMb-06[a] | *E. elymoides* subsp. *brevifolius* | 235883 | NRCS 9040189, Acc1123 | Buford, Rio Blanco co., Colo. (39.98, 107.63) |
| ELYMb-07[a] | *E. elymoides* subsp. *brevifolius* | 235884 | PI 628688,[d] NRCS 9026083, Acc1130 | Savageton, Campbell co., Wyo. (43.82, 105.8) |
| ELYMb-08 | *E. elymoides* subsp. *brevifolius* | 235885 | W6 20997,[d] Acc1139 | Ft. Carson, El Paso co., Colo. (38.5, 104.8) |
| ELYMb-09[b] | *E. elymoides* subsp. *brevifolius* | 235886 | W6 20998,[d] T-1180 | Wagon Mound, Mora co., N.M. (36.054, 104.795) |
| ELYMb-10[b] | *E. elymoides* subsp. *brevifolius* | 235887 | W6 20999,[d] T-1202 | Hwy 75 × 20, Blaine co., Idaho (43.3, 114.29) |
| ELYMb-11[b] | *E. elymoides* subsp. *brevifolius* | 235888 | W6 21004,[d] T-1228 | Colton, Utah co., Utah (39.83, 110.95) |
| ELYMb-12[b] | *E. elymoides* subsp. *brevifolius* | 235889 | W6 21005,[d] T-1233 | Hermosa, LaPlata co., Colo. (37.43, 107.81) |
| ELYMb-13[b] | *E. elymoides* subsp. *brevifolius* | 235890 | W6 21007,[d] T-1239 | Pagosa Springs, Archuleta co., Colo. (37.38, 106.9) |
| ELYMb-14[b] | *E. elymoides* subsp. *brevifolius* | 235891 | W6 21009,[d] T-1243 | Powderhorn, Gunnison co., Colo. (38.34, 107.1) |
| ELYMb-15 | *E. elymoides* subsp. *brevifolius* | 235892 | W6 21010,[d] T-1245 | Almont, Gunnison co., Colo. (38.7, 106.85) |
| ELYMb-16[b] | *E. elymoides* subsp. *brevifolius* | 235893 | W6 21011,[d] T-1249 | Sargents, Saguache co., Colo. (38.4, 106.47) |
| ELYMb-17[b] | *E. elymoides* subsp. *brevifolius* | 235894 | W6 21012,[d] T-1260 | Westcliffe, Custer co., Colo. (38.11, 105.46) |
| ELYMb-18[b] | *E. elymoides* subsp. *brevifolius* | 235895 | W6 21013,[d] T-1264 | Colmor, Colfax co., N.M. (36.265, 104.642) |
| ELYMb-19[b] | *E. elymoides* subsp. *brevifolius* | 235896 | W6 21016,[d] T-1272 | La Cueva, Santa Fe co., N.M. (35.944, 105.253) |
| ELYMb-20[b] | *E. elymoides* subsp. *brevifolius* | 235897 | W6 21017,[d] T-1277 | Tres Piedras, Rio Arriba co., N.M. (36.641, 105.968) |
| ELYMb-21[b] | *E. elymoides* subsp. *brevifolius* | 235898 | W6 21018,[d] T-1299 | Flagstaff, Coconino co., Ariz. (35.339, 111.557) |
| ELYMb-22[b] | *E. elymoides* subsp. *brevifolius* | 235899 | W6 21003,[d] T-1206 | Dixie, Elmore co., Idaho (43.32, 115.35) |
| ELYMb-23[b] | *E. elymoides* subsp. *brevifolius* | 235900 | W6 23104,[d] T-1308 | Almont, Gunnison co., Colo. (38.7, 106.9) |
| ELYMb-24 | E. elymoides subsp. brevifolius | 235901 | PI 2323534, Acc1315 | Daggett co., Utah (40.9, 109.5) |
| ELYMe-25 | E. elymoides subsp. elymoides | 235902 | W6 220334, NRCS 9041720, Acc1134 | Brooks Spring, Lander co., Nev. (40.74, 117.31) |
| ELYMe-26 | E. elymoides subsp. elymoides | | PI 6109784, T-1047 | Leamington Canyon, Juab co., Utah (39.5, 112.2) |
| ELYMe-271 | E. elymoides subsp. elymoides | 235903 | W6 220184, NRCS 9045926, Acc1108 | Butte co., Idaho (43.63, 113.31) |
| ELYMe-281 | E. elymoides subsp. elymoides | 235904 | W6 220264, NRCS 9045937, Acc1116 | Bradbury Flat, Custer co., Idaho (44.41, 114.16) |
| ELYMe-29 | E. elymoides subsp. elymoides | 235905 | W6 220284, NRCS 9046458, Acc1119 | Power co., Idaho (42.74, 112.91) |
| ELYMe-301 | E. elymoides subsp. elymoides | 235906 | PI 6194894, NRCS 9019224, Acc1126 | Whitehall, Jefferson co., Mont. (46.13, 111.98) |
| ELYMe-31[a] | *E. elymoides* subsp. *elymoides* | 235907 | PI 619555,[d] NRCS 9005549, Acc1127 | Warren, Carbon co., Mont. (45.01, 108.63) |
| ELYMe-32[a] | *E. elymoides* subsp. *elymoides* | 235908 | PI 619561,[d] NRCS 9019218, Acc1128 | Big Piney, Sublette co., Wyo. (42.8, 110.4) |
| ELYMe-33[a] | *E. elymoides* subsp. *elymoides* | 235909 | NRCS 9019219, Acc1129 | Ten Sleep, Washakie co., Wyo. (44.03, 107.53) |
| ELYMe-34[b] | *E. elymoides* subsp. *elymoides* | | PI 628747,[d] T-1173 | Mountain Home, Elmore co., Idaho (43.03, 115.56) |
| ELYMe-35[b] | *E. elymoides* subsp. *elymoides* | 235910 | PI 619553,[d] T-1171 | Shoshone, Lincoln co., Idaho (42.97, 114.29) |
| ELYMe-36[b] | *E. elymoides* subsp. *elymoides* | 235911 | W6 20989,[d] T-1175 | Ditto Crk. Rd., Elmore co., Idaho (43.29, 115.84) |
| ELYMe-37[b] | *E. elymoides* subsp. *elymoides* | 235912 | PI 628685,[d] T-1191 | Moffat co., Colo. (40.94, 108.77) |
| ELYMe-38[b] | *E. elymoides* subsp. *elymoides* | 235913 | PI 628686,[d] T-1193 | Superior, Sweetwater co., Wyo. (41.78, 108) |
| ELYMe-39[b] | *E. elymoides* subsp. *elymoides* | 235914 | PI 628687,[d] T-1198 | Sage Junction, Rich co., Utah (41.78, 111.19) |
| ELYMe-40 | *E. elymoides* subsp. *elymoides* | 235915 | W6 20994,[d] T-1223 | Fish Creek, Blaine co., Idaho (43.34, 113.86) |
| ELYMe-41 | *E. elymoides* subsp. *elymoides* | 235916 | 19 (1999) Maughn and Barton Seed | Sanpete co., Utah (39.15, 111.85) |

**Table 1 (***continued*).

| Label | Taxon | Herbarium voucher[c] | Seed accession identifier(s) | Origin (°N, °W) |
|---|---|---|---|---|
| ELYMe-42 | *E. elymoides* subsp. *elymoides* | 235917 | 21171 Granite Seed | Sanpete co., Utah (39.25, 111.7) |
| ELYMe-43 | *E. elymoides* subsp. *elymoides* | 235918 | SIHY-18326 Rainier Seed | Oasis, Elko co., Nev. (6000) (41.05, 114.5) |
| ELYMe-44 | *E. elymoides* subsp. *elymoides* | 235919 | SIHY-1341 Wind River Seed | Jim Bridger Trail, Washakie Co, Wyo. (44, 107.5) |
| ELYMe-45 | *E. elymoides* subsp. *elymoides* | 235920 | W6 23099,[d] T-1303 | Dietrich, Lincoln co., Idaho (42.9, 114.3) |
| ELYMe-46 | *E. elymoides* subsp. *elymoides* | 235921 | W6 22032,[d] NRCS 9041713, Acc1133 | Pershing co., Nev. (40.09, 118.86) |
| MULT-01[a] | *E. multisetus* | 235922 | PI 531603,[d] D-2857, Acc1103 | Bodie Flat, Douglas co., Nev. (38.85, 119.7) |
| MULT-02[a] | *E. multisetus* | 235923 | PI 531606,[d] D-3546, Acc1106 | Central Ferry, Whitman co., Wash. (46.6, 117.8) |
| MULT-03 | *E. multisetus* | 235924 | PI 595899,[d] Acc1118, 'Sand Hollow' | Gem co., Idaho (43.9, 116.5) |
| MULT-04[a] | *E. multisetus* | 235925 | W6 20962,[d] NRCS 9034042, Acc1132 | Paradise Valley, Humboldt co., Nev. (41.47, 117.58) |
| MULT-05[b] | *E. multisetus* | 235926 | T-1165 | King Hill, Elmore co., Idaho (42.98, 115.27) |
| MULT-06[b] | *E. multisetus* | 235927 | PI 619457,[d] W6 20965, T-1177 | Little Ranch, Canyon co., Idaho (43.78, 116.53) |
| MULT-07 | *E. multisetus* | 235928 | W6 20970,[d] T-1201 | Dietrich, Lincoln co., Idaho (42.9, 114.31) |
| MULT-08 | *E. multisetus* | 235929 | PI 619552,[d] T-1207 | Dixie, Elmore co., Idaho (43.31, 115.44) |
| MULT-09[b] | *E. multisetus* | 235930 | PI 619463,[d] T-1209 | Ditto Creek Rd., Elmore co., Idaho (43.29, 115.84) |
| MULT-10[b] | *E. multisetus* | 235931 | PI 619460,[d] T-1216 | Bogus Basin Rd., Ada co., Idaho (43.66, 116.19) |
| MULT-11[b] | *E. multisetus* | 235932 | PI 619465,[d] T-1219 | Seaman Gulch Rd., Ada co., Idaho (43.71, 116.26) |
| MULT-12[b] | *E. multisetus* | 235933 | PI 619454,[d] T-1268 | A-line canal, Gem co., Idaho (43.85, 116.6) |
| MULT-13 | *E. multisetus* | 235934 | Acc1314 | Mosier, Wasco co., Oreg. (45.68, 121.35) |
| **Section *Macrolepis* with type species *E. canadensis*** | | | | |
| CANA-01 | *E. canadensis* | 235938 | PI 531565[d] | Colorado |
| CANA-02 | *E. canadensis* | | D-3364 | Utah |
| **Section *Hystrix* with type species *E. hystrix*** | | | | |
| HYST-01 | *E. hystrix* | | PI 531615,[d] D-3479 | Missouri |
| HYST-02 | *E. hystrix* | 235939 | D-3605 | Missouri |
| **Section *Goulardia* with type species *E. caninus*** | | | | |
| TRAC-01 | *E. trachycaulus* | 235951 | PI 372650[d] | Alaska |
| TRAC-02 | *E. trachycaulus* | 235952 | PI 442444[d] | via Belgium |
| TRAC-03 | *E. trachycaulus* | 235941 | D-3270 | Utah |
| MUTA-01 | *E. mutabilis* | 235940 | PI 564954,[d] DJ-4149 | Kazakhstan |
| CANI-01 | *E. caninus* | 235947 | PI 564912,[d] DJ-4005 | Russia |
| CANI-02 | *E. caninus* | | PI 564915,[d] DJ-3975 | Russia |
| CANI-03 | *E. caninus* | 235948 | PI 252044[d] | Italy |
| **Section *Elymus* with type species *E. sibiricus*** | | | | |
| SIBI-01 | E. sibiricus | | W6 14340,[d] AJC268 | Russia |
| SIBI-02 | *E. sibiricus* | 235953 | PI 499464[d] | PRC |
| GLAU-01 | *E. glaucus* | 235942 | PI 387917[d] | Canada |
| GLAU-02 | *E. glaucus* | 235943 | D-3268 | Colorado |
| GLAU-03 | *E. glaucus* | 235944 | PI 232281[d] | California |
| **Section *Dasystachyae* with type species *E. lanceolatus*** | | | | |
| LANC-01 | *E. lanceolatus* | 235950 | D-3354 | |
| LANC-02 | *E. lanceolatus* | 235935 | D-3627 | North Dakota |
| LANC-03 | *E. lanceolatus* | 235936 | PI 531623[d] | Nevada |

**Table 1** (*concluded*).

| Label | Taxon | Herbarium voucher[c] | Seed accession identifier(s) | Origin (°N, °W) |
|---|---|---|---|---|
| LANC-04 | *E. lanceolatus* | 235937 | D-3626 | Canada |
| LANC-05 | *E. lanceolatus* | | PI 387883[d] | Alberta |
| LANC-06 | *E. lanceolatus* | | PI 387886[d] | Alberta |
| WAWA-01 | *E. wawawaiensis* | 235945 | PI 285272[d] | Washington |
| WAWA-02 | *E. wawawaiensis* | 235946 | PI 440921,[d] 'Secar' | Idaho |

[a]Accessions from Jones et al. (2002) assemblage 1.
[b]Accessions from Jones et al. (2002) assemblage 2.
[c]Utah State University Intermountain Herbarium (serial 235878–235953).
[d]USDA National Plant Germplasm System identifiers.

mula $G_{st} = D_A/D$. Neighbor-joining analyses (Saitou and Nei 1987) of genetic relationships were based on a user-defined distance matrix ($1 - F$) in PAUP* version 4.0b8 (Swofford 2000). Bootstrap confidence levels (Efron and Tibshirani 1991; Felsenstein 1985) were recovered from the 70% majority-rule consensus of 1000 neighbor-joining searches of restriction-site distance (Nei and Li 1979) computed from the binary allele data set using PAUP*. Although the topologies of the restriction-site distance tree and $1 - F$ distance tree are identical, the restriction-site distance scale is meaningless for the AFLP data. Thus, neighbor-joining trees were constructed using the user-defined distance matrix of $1 - F$ with bootstrap confidence levels obtained from the analyses of restriction-site distances. An unrooted neighbor-joining tree was also constructed based on the total nucleotide divergence ($D$) within and among taxa or groups. Entries for each taxon were essentially duplicated except that each pair of duplicated entries was distinguished by estimates of total nucleotide variation ($\pi_t$) within taxa or groups. Graphic displays of these neighbor-joining trees were developed using TREEVIEW (Page 1996).

Correlations between matrices of geographical distance, quantitative trait variation (Jones et al. 2002), average $P$ between accessions, and corrected number of DNA polymorphism between accessions ($P_A$) 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 with values obtained by 1000 random permutations (Smouse et al. 1986). Therefore, the upper-tail probability ($p$) that 1000 random $Z$ values are (by chance) less than observed values of $Z$ is 0.002 or greater. Geographical distance (kilometres) matrices were computed from geographical coordinates using the formula described in Math Forum (1997): kilometres = $\arccos[\cos(LAT_X)\cos(LONG_X)\cos(LAT_Y)\cos(LONG_Y) + \cos(LAT_X)\sin(LONG_X)\cos(LAT_Y)\sin(LONG_Y) + \sin(LAT_X)\sin(LAT_Y)]r$, where $LAT_X$, $LONG_X$, $LAT_Y$, and $LONG_Y$ are the latitude and longitude (expressed in radians) for the two accessions (X and Y) and $r$ is 6378 km, the radius of Earth. Quantitative trait variation within two assemblages of accessions evaluated by Jones et al. (2002) was analyzed using standardized normal deviates for each trait. Taxonomic distances based on these standardized normal deviates were computed using the SimInt procedure of NTSYS-pc (Rohlf 1998). The corrected number of DNA polymorphisms among accessions ($P_A$) was computed using the formula $[P_{XY} - (P_X + P_Y)/2]$, where $P_X + P_Y$ are the average numbers of differences within accessions (X and Y) and $P_{XY}$ is the average number of differences between accessions. Metric parameters of genetic distance ($P$ and $P_A$) were used primarily because corresponding estimates of the number of nucleotide differences and corrected number of nucleotide differences among all pairwise comparisons of accessions would be tedious.

A geographical map of collection sites for the 46 *E. elymoides* and 13 *E. multisetus* squirreltail accessions was developed using ArcMap™ 8.2 (ESRI®, Redlands, Calif.).

## Results

Complete AFLP profiles produced by six *Eco*RI +3 –

*Mse*I +3 selective primer pairs were obtained from 161 plants representing 83 accessions (Table 2). In some instances, weak or failed polymerase chain reaction amplifications were successfully repeated. However, five individual DNA samples repeatedly displayed weak or failed polymerase chain reaction amplifications, which can probably be attributed to one or more steps of the template DNA preparations. Therefore, only one useable plant genotype was obtained from five accessions: ELYMb-24, ELYMb-28, ELYMb-29, MULT-13, and SIBI-01. The total number of DNA fragments amplified from each plant was very consistent within these 11 allotetraploid species (Table 2). Among the six primer pairs analyzed, the average size of the smallest fragment scored was 55 bp and the average size of the largest fragment scored was 396 bp. From Table 2, it can be deduced that the average number of fragments per plant (*M*) per primer pair (i.e., six) ranged from approximately 52 for *E. mutabilis* to 61.3 for *E. multisetus* (Table 2). A total of 1265 different DNA fragments were resolved among the 161 *Elymus* plants, with only 27 monomorphic bands. However, the proportion of shared fragments between individual plants was much higher than would be expected by chance alone, even among the most divergent comparisons such as *E. mutabilis* versus *E. multisetus* (Table 3). Methods used in this study can resolve at least 341 different fragment categories per primer pair in the size range of 55–396 bp. We detected approximately 0.18 fragment per category (61 fragments/341 categories) for *E. mutabilis* and 0.15 fragment per category (52 fragments/341 categories) for *E. mutabilis*. Thus, the deduced probability that heterologous fragments cofractionate into the same category is approximately 0.165. The proportion of shared fragments among *Elymus* species, ranging from 0.535 to 0.794 (Table 3), is much higher than expected by chance alone. These observations indicate that the aforementioned *Elymus* taxa share many homologous DNA fragments detected using the AFLP technique.

A high degree of genetic identity was apparent within accessions. With the exceptions of ELYMe-31, MULT-10, and MULT-11, individual plants group strictly by accession (Fig. 1). Genetic differentiation ($g_{st}$) among accessions ranged from 0.506 to 0.923 within the self-fertile *E. elymoides*, *E. multisetus*, *E. canadensis*, *E. hystrix*, *E. mutabalis*, *E. trachycaulus*, *E. glaucus*, *E. caninus*, and *E. sibiricus* species (Table 2). Compared with most of the self-fertile taxa, *E. multisetus* displayed relatively less DNA variation among accessions and (or) greater DNA variation within accessions (Table 2; Fig. 1). Therefore, $g_{st}$ among *E. multisetus* accessions was substantially lower than among the other eight self-fertile taxa (Table 2). The self-incompatible *E. lanceolatus* and *E. wawawaiensis* species displayed considerably more DNA variation within accessions and lower $g_{st}$ values compared with the self-fertile taxa (Table 2).

Estimates of total nucleotide divergence (*D*) among the eight North American taxa range from 24.3 and 38.7 differences per 1000 nucleotides (Table 4). Similarly, pairwise comparisons of *D* among the Eurasian taxa *E. caninus*, *E. mutabilis*, and *E. sibiricus* range from 15.2 to 36.0 differences per 1000. *Elymus caninus* and *E. mutabilis* were the two most similar taxa (i.e., *D* = 15.2 differences per 1000). Slightly greater nucleotide divergence, 33.7–45.5 per 1000, was detected between North American and Eurasian taxa. Thus, Eurasian species seemingly form a natural outgroup (Fig. 2). Based on these empirical observations, the phylogenetic tree (Fig. 1) was rooted using Eurasian *E. caninus*, *E. mutabilis*, and *E. sibiricus* as an outgroup. The apportionment of amplified fragment length polymorphism among taxa or subspecific groups (Fig. 1) is somewhat less pronounced than the apportionment of nucleotide variation among taxa or subtaxonomic groups (Fig. 2). In particular, the proportion of polymorphic DNA fragments ($1 - F$) among accessions within taxa ranges from 0.081 to 0.253 (Table 2) with corresponding estimates of 5.4–20.2 nucleotide differences per 1000 (Table 2), whereas the proportion of polymorphic DNA fragments among taxa ranges from 0.206 to 0.465 (Table 3) with corresponding estimates of 15.2–44.4 nucleotide differences per 1000 (Table 4). Likewise, the proportion of polymorphic DNA fragments among accessions within subspecific groups ranges from 0.082 to 0.183 (Table 5) with corresponding estimates of 5.8–11 (Table 5), whereas the proportion of polymorphic DNA fragments among subspecific groups ranges from 0.244 to 0.331 (Table 6) with corresponding estimates of 19.4–28.8 nucleotide differences per 1000 (Table 7). Thus, estimates of nucleotide variation slightly accentuate divergence among groups relative to diversity within groups. Estimates of nucleotide differences are corrected for the probability that heterologous DNA fragments cofractionate, by chance alone, as described by Innan et al. (1999). Moreover, the number of nucleotide differences corresponding to each amplified fragment length polymorphism increases as a function of overall genetic divergence among the genotypes being compared. In any case, the topographies of phylogenetic relationships inferred from amplified fragment length polymorphism per se (Fig. 1) and corresponding estimates of nucleotide variation (Fig. 2) are similar.

The 10 *Elymus* species examined with more than one accession were strictly monophyletic and strongly supported by high bootstrap values for each respective group (Fig. 1). Moreover, four interspecific groups were also well supported by phylogenetic analyses based on the proportion of amplified fragment length polymorphism among individual plants (Fig. 1) and the average nucleotide divergence among taxa (Fig. 2): (*i*) *E. elymoides* and *E. multisetus*, (*ii*) *E. canadensis* and *E. hystrix*, (*iii*) *E. lanceolatus* and *E. wawawaiensis*, and (*iv*) the Eurasian *E. caninus*, *E. mutabilis*, and *E. sibiricus* accessions. The *E. caninus* and *E. sibiricus* accessions were most similar (Fig. 1). Phylogenies based on the proportion of amplified fragment length polymorphism among individual plants (Fig. 1) and the average nucleotide divergence among taxa (Fig. 2) are consistent with a monophyletic origin of *Elymus* sect. *Sitanion* (i.e., the *E. elymoides* and *E. multisetus* group). However, estimates of nucleotide divergence between *E. elymoides* and *E. multisetus* are similar to or greater than *D* among the other North American *Elymus* taxa examined (Table 4). The monophyly of *E. elymoides* subsp. *elymoides* was also supported. *Elymus elymoides* subsp. *brevifolius*, on the other hand, was paraphyletic and separated into four genetically distinct groups supported by high bootstrap confidence levels (Fig. 1). Estimates of nucleotide divergence among these

**Fig. 1.** Neighbor-joining phylogeny based on proportions of amplified fragment length polymorphism (1 – *F*) among individual plants of *Elymus elymoides* subsp. *elymoides* (ELYMe), *E. elymoides* subsp. *brevifolius* (ELYMb), *E. multisetus* (MULT), *E. canadensis* (CANA), *E. hystrix* (HYST), *E. glaucus* (GLAU), *E. lanceolatus* (LANC), *E. wawawaiensis* (WAWA), *E. trachycaulus* (TRAC), *E. caninus* (CANI), *E. mutabilis* (MUTA), and *E. sibiricus* (SIBI). Bootstrap confidence levels are indicated for clades present in the 50% majority-rule consensus tree.

**Table 2.** Summary of amplified fragment length polymorphism within *Elymus* taxa detected using six *Eco*RI +3 – *Mse*I +3 selective primer pairs including average values for the number of fragments per plant (*M*), number of differences between plants (*P*), proportion of shared fragments between plants (*F*), total nucleotide diversity within taxa ($\pi_t$), nucleotide diversity within accessions ($\pi$), and genetic differentiation among accessions within taxa ($g_{st}$).

| | | | Among accessions | | | Within accessions | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Accessions (plants) | *M* (SD) | *P* (SD) | *F* (SD) | $\pi_t \times 1000$ | *P* (SD) | *F* (SD) | $\pi \times 1000$ | $g_{st}$ |
| *E. elymoides* | 46 (89) | 366.0 (14.4) | 185.6 (51.0) | 0.747 (0.068) | 20.2 | 32.0 (21.5) | 0.953 (0.039) | 3.2 | 0.842 |
| *E. multisetus* | 13 (25) | 368.2 (8.6) | 87.2 (20.9) | 0.881 (0.030) | 8.5 | 44.8 (16.0) | 0.939 (0.022) | 4.2 | 0.506 |
| *E. canadensis* | 2 (4) | 341.3 (2.3) | 76.5 (2.9) | 0.888 (0.005) | 7.8 | 9.5 (2.12) | 0.986 (0.003) | 0.9 | 0.885 |
| *E. hystrix* | 2 (4) | 329.8 (4.5) | 80.5 (6.5) | 0.878 (0.010) | 8.5 | 17.5 (0.7) | 0.973 (0.001) | 1.8 | 0.788 |
| *E. mutabilis* | 1 (2) | 311.5 (0.8) | — | — | — | 9 (—) | 0.986 (—) | 0.9 | — |
| *E. trachycaulus* | 3 (6) | 330.9 (4.3) | 118.5 (29.2) | 0.821 (0.044) | 13.0 | 10.3 (1.5) | 0.984 (0.002) | 1.0 | 0.923 |
| *E. glaucus* | 3 (6) | 306.2 (6.7) | 132.7 (45.7) | 0.784 (0.073) | 15.9 | 13.7 (9.0) | 0.977 (0.015) | 1.5 | 0.906 |
| *E. wawawaiensis* | 2 (4) | 327.5 (11.4) | 109.0 (10.5) | 0.833 (0.020) | 12.0 | 100.0 (2.8) | 0.847 (0.007) | 10.9 | 0.092 |
| *E. caninus* | 3 (6) | 331.9 (9.6) | 100.3 (25.2) | 0.849 (0.037) | 10.7 | 19.7 (18.6) | 0.970 (0.028) | 2.0 | 0.813 |
| *E. sibiricus* | 2 (3) | 318.7 (5.1) | 52.0 (0) | 0.919 (0.001) | 5.4 | 10 (—) | 0.984 (—) | 1.0 | 0.815 |
| *E. lanceolatus* | 6 (12) | 353.3 (8.7) | 159.4 (19.9) | 0.774 (0.028) | 17.4 | 94.5 (30.8) | 0.867 (0.042) | 9.5 | 0.454 |

five subspecific groups range from 19.4 to 28.8 differences per 1000 bases (Table 7), values that are less than most interspecific comparisons (Table 4).

Associations among geographic provenance, quantitative trait variation, and DNA polymorphism among accessions were detectable in two assemblages of squirreltail accessions that were evaluated by Jones et al. (2002) and this study. The 12 quantitative traits measured by Jones et al. (2002) for germplasm assemblage 1 (Table 8) included days to emergence, leaf length, total plant dry matter, root to shoot ratio, leaf area, specific leaf area, root length, heading date, seed mass, emergence index from 20 mm, emergence index from 60 mm, and nitrate reductase activity. The nine quantitative traits measured by Jones et al. (2002) for germplasm assemblage 2 (Table 9) included days to emergence, leaf length, total plant dry matter, root to shoot ratio, root length, specific root length, heading date, plant height, and seed mass. Correlation between DNA polymorphism (*P* and $P_A$) and quantitative trait variation was greater when *E. elymoides* and *E. multisetus* accessions were compared collectively (Tables 8 and 9). However, associations between DNA polymorphism (*P* and $P_A$) and geographic origin were diminished when *E. elymoides* and *E. multisetus* accessions were included together (Tables 8 and 9). Thus, genetically distinct *E. multisetus* and *E. elymoides* accessions were collected from the same general region, whereas genetically distinguishable groups within *E. elymoides* generally originate from different geographic regions (Fig. 3). Within *E. elymoides*, correlations of amplified fragment length polymorphism (*P* and $P_A$) and quantitative trait variation (Tables 8 and 9) were slightly better than correlations of DNA polymorphism and geographic distance (Tables 8 and 9). Within *E. elymoides* germplasm assemblage 1 (Table 8), the correlation of geographic provenance and quantitative trait variation was better than correlations of DNA polymorphism (*P* and $P_A$) and quantitative trait variation. Conversely, in *E. elymoides* germplasm assemblage 2 (Table 9), the correlations of DNA polymorphism (*P* and $P_A$) and quantitative trait variation were better than the correlation of geographic provenance and quantitative trait variation. Correlation of quantitative trait variation and DNA polymorphism is evident by the fact that the morphological groups described by Jones et al. (2002) precisely correspond to four genetically distinct *E. elymoides* groups (subsp. *brevifolius* groups A, B, and C and subsp. *elymoides*) (Figs. 1 and 2). Only one accession from *E. elymoides* subsp. *brevifolius* group D (Fig. 1) was examined by Jones et al. (2002). Therefore, this latter *E. elymoides* subsp. *brevifolius* group was not recognized by Jones et al. (2002).

The correlation between geographic provenance and average DNA polymorphism among all 59 squirreltail accessions was 0.42, or 0.45 corrected for DNA polymorphism within accessions. The correlation between geographic provenance and average DNA polymorphism strictly among the 46 *E. elymoides* accessions was 0.55, or 0.54 corrected for DNA polymorphism within accessions. The overall correlation between geographic provenance and DNA polymorphism among pairwise comparisons of individual plants, within and among the 46 *E. elymoides* accessions, was 0.57. Thus, a high degree of genetic identity within accessions (Fig. 1) contributed slightly to the overall correlation of DNA polymorphism and geographic provenance among the 46 *E. elymoides* accessions (Fig. 3). All of these correlations are significant ($p \leq 0.002$).

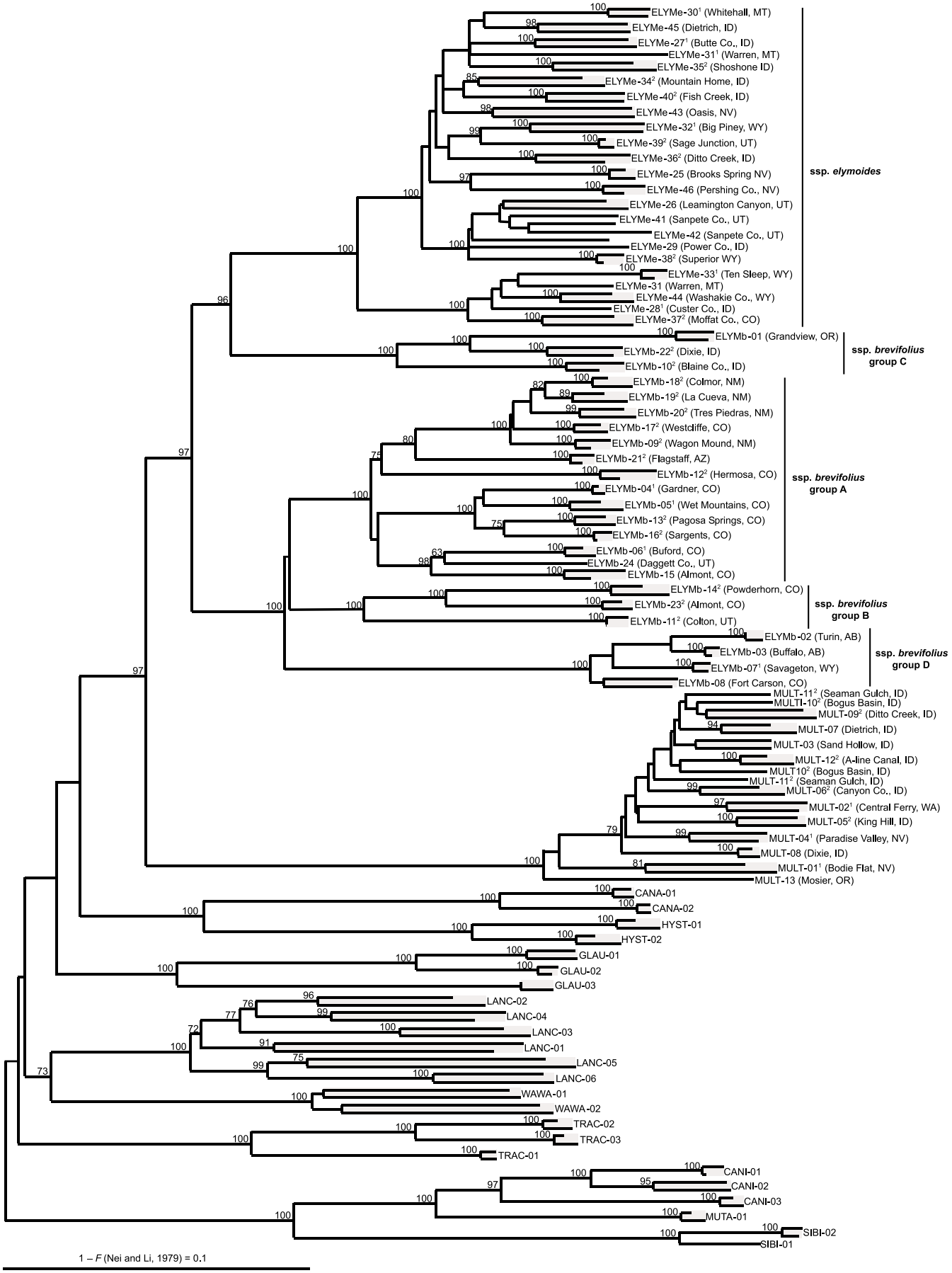


**Table 3.** Amplified fragment length polymorphism among 11 *Elymus* taxa detected using six *Eco*RI +3 – *Mse*I +3 selective amplification primer pairs.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *E. elymoides* | — | 269.7 (12.3) | 263.3 (12.1) | 256.5 (11.2) | 280.0 (10.4) | 258.7 (14.6) | 243.1 (11.5) | 254.6 (13.7) | 295.0 (13.7) | 299.0 (12.4) | 259.6 (14.2) |
| 2. *E. multisetus* | 0.632 (0.014) | — | 275.9 (6.2) | 270.7 (6.2) | 309.0 (8.2) | 290.8 (7.2) | 257.2 (9.0) | 280.8 (5.7) | 318.3 (8.4) | 317.9 (6.7) | 295.0 (9.3) |
| 3. *E. canadensis* | 0.627 (0.016) | 0.611 (0.009) | — | 201.5 (1.6) | 268.3 (13.7) | 252.0 (4.2) | 229.7 (6.9) | 250.3 (3.2) | 285.8 (4.7) | 298.0 (5.4) | 257.3 (8.1) |
| 4. *E. hystrix* | 0.631 (0.014) | 0.611 (0.009) | 0.700 (0.007) | — | 254.0 (0.7) | 255.4 (11.9) | 221.0 (12.2) | 235.4 (3.2) | 283.0 (4.6) | 273.7 (3.2) | 248.1 (13.8) |
| 5. *E. mutabilis* | 0.586 (0.012) | 0.544 (0.010) | 0.589 (0.016) | 0.604 (0.005) | — | 257.0 (15.5) | 240.0 (7.1) | 250.0 (2.8) | 132.5 (9.6) | 192.5 (2.8) | 255.6 (6.9) |
| 6. *E. trachycaulus* | 0.629 (0.021) | 0.583 (0.010) | 0.625 (0.006) | 0.613 (0.017) | 0.600 (0.022) | — | 227.8 (8.7) | 235.1 (9.3) | 260.8 (9.7) | 274.7 (7.9) | 237.5 (12.3) |
| 7. *E. glaucus* | 0.638 (0.015) | 0.617 (0.014) | 0.645 (0.008) | 0.653 (0.017) | 0.611 (0.011) | 0.643 (0.012) | — | 215.5 (2.8) | 251.8 (8.5) | 258.0 (2.4) | 232.3 (8.4) |
| 8. *E. wawawaiensis* | 0.633 (0.017) | 0.600 (0.009) | 0.626 (0.011) | 0.642 (0.009) | 0.609 (0.016) | 0.643 (0.016) | 0.660 (0.013) | — | 265.0 (4.9) | 266.7 (6.0) | 220.1 (11.2) |
| 9. *E. caninus* | 0.577 (0.014) | 0.544 (0.009) | 0.576 (0.006) | 0.572 (0.008) | 0.794 (0.014) | 0.606 (0.015) | 0.605 (0.010) | 0.598 (0.014) | — | 206.4 (12.1) | 270.1 (5.5) |
| 10. *E. sibiricus* | 0.563 (0.013) | 0.535 (0.009) | 0.548 (0.008) | 0.578 (0.008) | 0.693 (0.006) | 0.574 (0.014) | 0.586 (0.008) | 0.585 (0.014) | 0.681 (0.021) | — | 268.8 (9.7) |
| 11. *E. lanceolatus* | 0.63 (0.018) | 0.590 (0.015) | 0.630 (0.016) | 0.637 (0.022) | 0.616 (0.010) | 0.653 (0.012) | 0.648 (0.013) | 0.677 (0.022) | 0.606 (0.007) | 0.600 (0.016) | — |

**Note:** Above diagonal: number of amplified fragment length polymorphisms between plants (*P*) averaged among taxa (SD in parentheses); below diagonal: proportion of shared fragments between plants (*F*) averaged among taxa (SD in parentheses).

**Table 4.** Genetic differentiation among *Elymus* taxa.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *E. elymoides* | — | 18.6 | 19.4 | 18.1 | 17.8[a] | 16.1 | 13.2 | 16.1 | 24.1 | 28.4 | 14.1 |
| | | **0.565** | **0.581** | **0.559** | **0.468** | **0.492** | **0.423** | **0.500** | **0.610** | **0.684** | **0.429** |
| 2. *E. multisetus* | 32.9 | — | 27.0 | 26.4 | 35.6[a] | 27.9 | 21.6 | 26.2 | 34.8 | 38.5 | 25.2 |
| | | | **0.769** | **0.756** | **0.807** | **0.721** | **0.639** | **0.720** | **0.784** | **0.846** | **0.660** |
| 3. *E. canadensis* | 33.4 | 35.1 | — | 12.6 | 29.3[a] | 22.4 | 18.2 | 22.7 | 29.9 | 36.3 | 19.9 |
| | | | | **0.519** | **0.790** | **0.683** | **0.607** | **0.696** | **0.765** | **0.846** | **0.612** |
| 4. *E. hystrix* | 32.4 | 34.9 | 24.3 | — | 26.5[a] | 23.3 | 16.8 | 20.3 | 29.8 | 31.5 | 18.6 |
| | | | | | **0.757** | **0.685** | **0.579** | **0.666** | **0.756** | **0.820** | **0.590** |
| 5. *E. mutabilis* | 38.0 | 44.1 | 37.1 | 35.0 | — | 22.5[a] | 17.8[a] | 22.3[a] | 4.2[a] | 19.2[a] | 16.5[a] |
| | | | | | | **0.634** | **0.528** | **0.650** | **0.276** | **0.780** | **0.487** |
| 6. *E. trachycaulus* | 32.7 | 38.7 | 32.8 | 34.0 | 35.5 | — | 15.7 | 17.9 | 23.1 | 29.7 | 14.4 |
| | | | | | | | **0.522** | **0.589** | **0.662** | **0.763** | **0.486** |
| 7. *E. glaucus* | 31.2 | 33.8 | 30.0 | 29.0 | 33.7 | 30.1 | — | 14.3 | 21.3 | 23.6 | 13.3 |
| | | | | | | | | **0.507** | **0.616** | **0.640** | **0.445** |
| 8. *E. wawawaiensis* | 32.2 | 36.4 | 32.6 | 30.5 | 34.3 | 30.4 | 28.2 | — | 24.7 | 28.7 | 12.2 |
| | | | | | | | | | **0.686** | **0.767** | **0.454** |
| 9. *E. caninus* | 39.5 | 44.4 | 39.1 | 39.4 | 15.2 | 34.9 | 34.6 | 36.0 | — | 18.0 | 21.4 |
| | | | | | | | | | | **0.692** | **0.605** |
| 10. *E. sibiricus* | 41.2 | 45.5 | 42.9 | 38.4 | 24.6 | 38.9 | 36.9 | 37.4 | 26.0 | — | 24.5 |
| | | | | | | | | | | | **0.682** |
| 11. *E. lanceolatus* | 32.9 | 38.2 | 32.5 | 31.5 | 33.9 | 29.6 | 29.9 | 26.9 | 35.4 | 35.9 | — |

**Note:** Above diagonal: nucleotide divergence ($D_A \times 1000$) corrected for nucleotide diversity ($\pi_t$) within groups (Table 2) and genetic differentiation ($G_{st}$) (bold); below diagonal: estimates of the total nucleotide divergence ($D \times 1000$).
[a]Not corrected for nucleotide diversity within *E. mutabilis*.

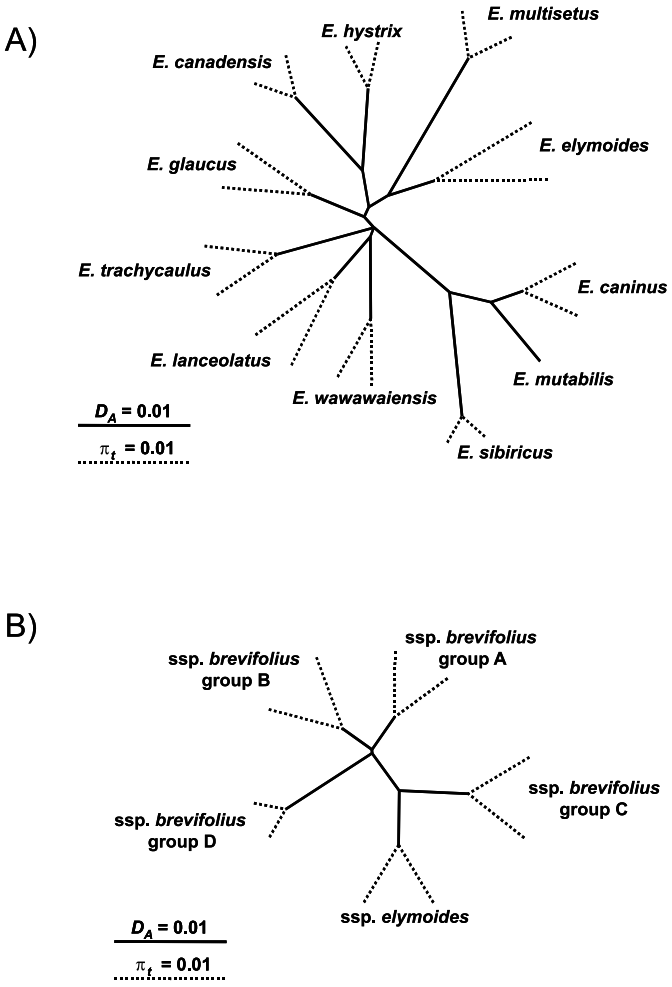
## Discussion

AFLP provided new and useful measures of genetic variation within and among *E. elymoides*, *E. multisetus*, and other allotetraploid *Elymus* species. Two key observations support the taxonomic ranks of *E. elymoides* and *E. multisetus*: (*i*) amplified fragment length polymorphism and nucleotide divergence among these taxa are similar to or greater than corresponding genetic differences among well-known and morphologically distinct *Elymus* species and (*ii*) *E. elymoides* and *E. multisetus* accessions can be reliably classified into genetically distinct monophyletic groups on the basis of glume and floret structures (Wilson 1963). *Elymus elymoides* and *E. multisetus* are self-compatible in nature. Thus, we are not surprised to find genetically distinct *E. elymoides* and *E. multisetus*plants growing at the same site or in the same regions (Fig. 3). Although most allotetraploid *Elymus* species can hybridize and form partially or fully fertile hybrids, gene flow within and among these species is probably controlled by self-fertilization (Jensen et al. 1990).

At least five natural groups within *E. elymoides* were discerned by morphology (Jones et al. 2002) and DNA fingerprinting. The monophyly of subsp. *elymoides* was supported by DNA fingerprinting; subsp. *brevifolius*, however, was paraphyletic and separated into four genetically distinct groups. In particular, subsp. *brevifolius* group C was more closely related to subsp. *elymoides* than it was to other subsp. *brevifolius* genotypes in groups A, B, and D. Interestingly, the *brevifolius* group C accessions originated from regions that are dominated by subsp. *elymoides* accessions (Fig. (3), at least in our germplasm assemblage. Thus, hybridization or introgression may account for the paraphyly of subsp. *brevifolius*. Alternatively, subsp. *elymoides* may be a recently derived lineage of a more diverse ancestral group, subsp. *brevifolius*. Two other taxonomic groups, *E. elymoides* subsp. *californicus* and *E. elymoides* subsp. *hordeoides*, were not examined. Thus, the monophyly of subsp. *elymoides*, or perhaps *E. elymoides* in general, may not hold up with the inclusion of additional variants. Likewise, the apparent paraphyly of subsp. *brevifolius* may be affected in some way by the inclusion of subsp. *californicus* and subsp. *hordeoides*. However, subsp. *hordeoides* is so elusive that authors of this study have come to doubt its existence. In any case, the general aspect of subsp. *hordeoides* is similar to that of subsp. *elymoides*, and subsp. *californicum* intergrades with subsp. *elymoides* where they are contiguous (Wilson 1963).

A significant correlation between amplified fragment length polymorphism and geographic distance was detected, especially within *E. elymoides*. Thus, geographic provenance was discerned by quantitative trait variation (Jones et al. 2002) and DNA fingerprinting (Fig. 3). However, geographic origin per se may not be a very reliable indicator of genetic identity or quantitative trait adaptations. For example, different accessions of *E. elymoides* subsp. *brevifolius* groups A and B were collected from the same site in Colorado (Fig. 3). Likewise, *E. elymoidies* subsp. *elymoides*, *E. elymoides* subsp. *brevifolius*, and *E. multisetus* accessions were collected from common areas on the Idaho Snake River Plain (Fig. 3). Conversely, genetically similar accessions of *E. elymoides* subsp. *brevifolius* group D (Fig. 2) are evidently widely dispersed across wide latitudes of the northern

**Fig. 2.** Unrooted neighbor-joining trees based on nucleotide divergence ($D$), the sum of average nucleotide diversity ($\pi_t$) within and corrected divergence ($D_A$) among (A) *Elymus* taxa or (B) subspecific groups of *E. elymoides*.




Great Plains region of western North America, sharply sectioned from other groups of bottlebrush squirreltail west of the Rocky Mountains (Fig. 3). Unfortunately, quantitative traits of subsp. *brevifolius* group D were not evaluated by Jones et al. (2002). Although subgroups of subsp. *brevifolius* were clearly distinguished by quantitative trait variation (Jones et al. 2002) and (or) amplified fragment length polymorphism, *E. elymoides* subsp. *brevifolius* is paraphyletic, and it may be difficult to develop dichotomous keys that will reliably classify plants into these five subspecific groups. Moreover, measures of amplified fragment length polymorphism and nucleotide divergence among these subspecific groups of *E. elymoides* are substantially less than corresponding measures of genetic divergence among *E. elymoides*, *E. multisetus*, and other *Elymus* taxa. A similar phenomenon was observed for South American accessions of *Bromus* sect. *Ceratochloa* (Massa et al. 2001). With the exception of big squirreltail (*E. multisetus*), we believe that these squirreltail taxa should be retained within *E. elymoides*. In addition to verifying two genetically distinct squirreltail species, these results help identify naturally important groups of bottlebrush squirreltail (*E. elymoides*).

**Table 5.** Summary of amplified fragment length polymorphism within five subspecific groups of *Elymus elymoides* detected using six *Eco*RI +3 – *Mse*I +3 selective primer pairs including average values for the number of fragments per plant ($M$), number of differences between plants ($P$), proportion of shared fragments between plants ($F$), total nucleotide diversity within groups ($\pi_t$), nucleotide diversity within accessions ($\pi$), and genetic differentiation among accessions within groups ($g_{st}$).

| | | | Among accessions | | | Within accessions | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Accessions (plants) | $M$ (SD) | $P$ (SD) | $F$ (SD) | $\pi_t \times 1000$ | $P$ (SD) | $F$ (SD) | $\pi \times 1000$ | $g_{st}$ |
| subsp. *brevifolius* group A | 14 (27) | 376.3 (9.6) | 120 (28.3) | 0.841 (0.038) | 11 | 20.3 (8.2) | 0.973 (0.011) | 1.8 | 0.847 |
| subsp. *brevifolius* group B | 3 (6) | 358.5 (4.6) | 132.5 (19.7) | 0.817 (0.024) | 13.7 | 16.3 (10.1) | 0.977 (0.014) | 1.5 | 0.891 |
| subsp. *brevifolius* group C | 3 (6) | 361.8 (13.0) | 124.5 (12.5 | 0.828 (0.018) | 12.7 | 30.3 (11.2) | 0.958 (0.015) | 2.8 | 0.780 |
| subsp. *brevifolius* group D | 4 (8) | 391.6 (4.0) | 64.1 (14.8) | 0.918 (0.018) | 5.8 | 13.4 (11.8) | 0.978 (0.025) | 1.5 | 0.741 |
| subsp. *elymoides* | 22 (42) | 356.0 (6.9) | 117.1 (20.5) | 0.836 (0.031) | 12.0 | 44.5 (24.1) | 0.931 (0.045) | 4.7 | 0.608 |

**Table 6.** Amplified fragment length polymorphism among five subspecific groups of *Elymus elymoides* detected using six *Eco*RI +3 – *Mse*I +3 selective amplification primer pairs.

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1. subsp. *brevifolius* group A | — | 179.3 (10.3) | 226.3 (18.0) | 206.4 (9.8) | 219.1 (6.1) |
| 2. subsp. *brevifolius* group B | 0.756 (0.014) | — | 232.3 (14.3) | 202.4 (10.4) | 217.5 (7.8) |
| 3. subsp. *brevifolius* group C | 0.693 (0.022) | 0.678 (0.016) | — | 249.8 (11.7) | 205.8 (14.2) |
| 4. subsp. *brevifolius* group D | 0.731 (0.013) | 0.730 (0.013) | 0.669 (0.012) | — | 237.1 (7.8) |
| 5. subsp. *elymoides* | 0.701 (0.010) | 0.696 (0.010) | 0.714 (0.017) | 0.683 (0.010) | — |

**Note:** Above diagonal: average number of amplified fragment length polymorphisms between plants (*P*) averaged among groups (SD in parentheses); below diagonal: average proportion of shared fragments between plants (*F*) averaged among groups (SD in parentheses).

**Table 7.** Genetic differentiation among subspecific groups of *Elymus elymoides*.

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1. subsp. *brevifolius* group A | — | 6.7 | 13.7 | 13.3 | 13.0 |
| | | **0.345** | **0.529** | **0.602** | **0.522** |
| 2. subsp. *brevifolius* group B | 19.4 | — | 14.1 | 12.9 | 12.5 |
| | | | **0.516** | **0.584** | **0.494** |
| 3. subsp. *brevifolius* group C | 25.9 | 27.3 | — | 19.6 | 11.1 |
| | | | | **0.681** | **0.474** |
| 4. subsp. *brevifolius* group D | 22.1 | 22.1 | 28.8 | — | 19.6 |
| | | | | | **0.726** |
| 5. subsp. *elymoides* | 24.9 | 25.3 | 23.4 | 27.0 | — |

**Note:** Above diagonal: nucleotide divergence ($D_A \times 1000$) corrected for nucleotide diversity ($\pi_t$) within groups (Table 5) and genetic differentiation ($G^{ST}$) (bold); below diagonal: estimates of the total nucleotide divergence ($D \times 1000$).

**Table 8.** Associations of geographic provenance, quantitative trait variation, and amplified fragment length polymorhism for the first germplasm assemblage described by Jones et al. (2002).

| | Geographic distance | Quantitative trait variation | DNA polymorphism *P* | Corrected DNA polymorphism $P_A$ |
|---|---|---|---|---|
| Geographic distance | — | 0.77 (0.002) | 0.61 (0.002) | 0.58 (0.003) |
| Quantitative trait variation | 0.75 (0.003) | — | 0.75 (0.002) | 0.70 (0.002) |
| DNA polymorphism *P* | 0.58 (0.003) | 0.65 (0.002) | — | 0.93 (0.002) |
| Corrected DNA polymorphism $P_A$ | 0.57 (0.002) | 0.67 (0.002) | 0.91 (0.002) | — |

**Note:** Above diagonal: matrix correlations (*r*) and corresponding significance values (*p*) (in parentheses) among 10 *Elymus elymoides* and three *E. multisetus* accessions considered together; below diagonal: *r* and *p* (in parentheses) strictly among the 10 *E. elymoides* accessions.

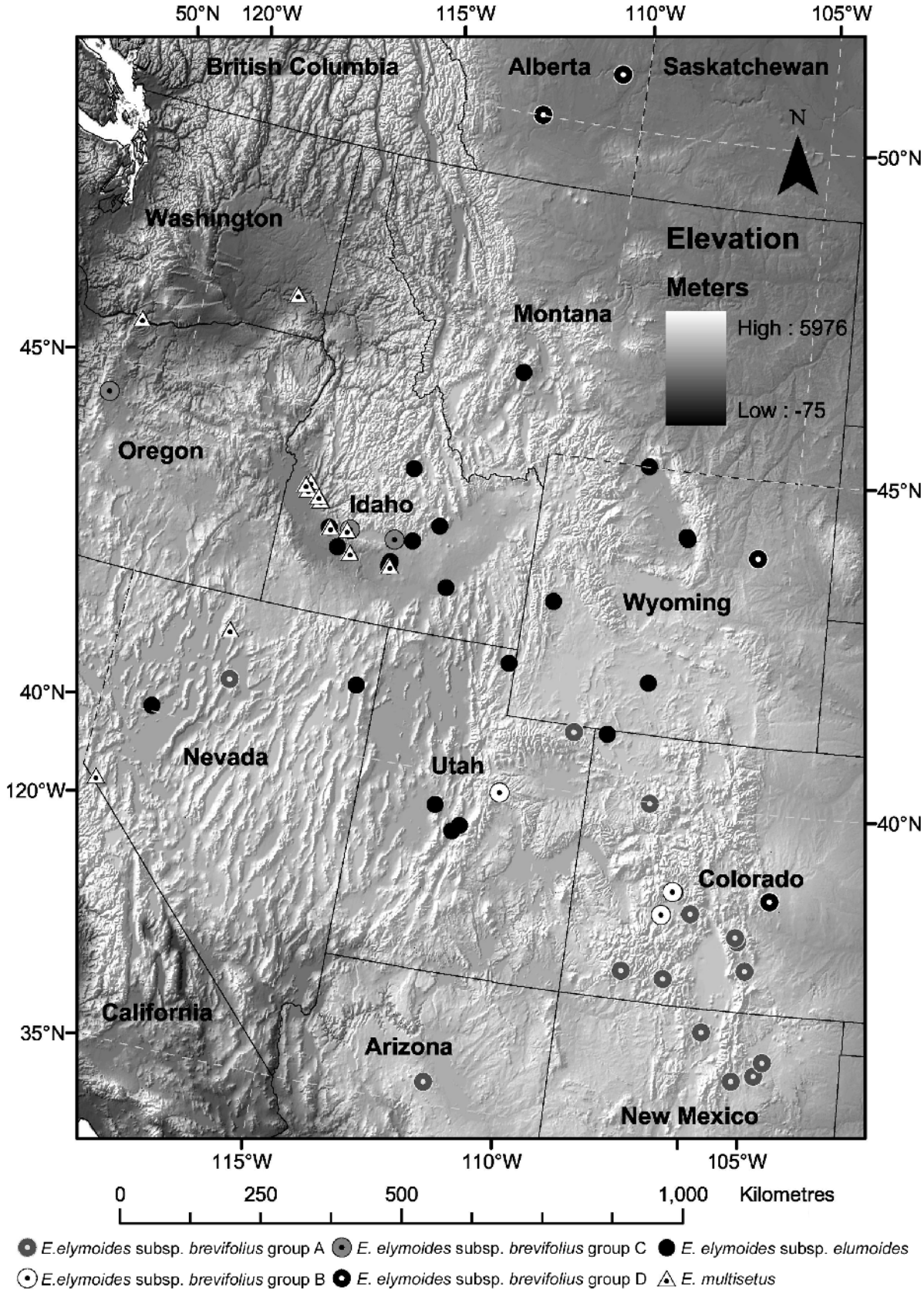
**Table 9.** Associations of geographic provenance, quantitative trait variation, and amplified fragment length polymorhism for the second germplasm assemblage described by Jones et al. (2002).

| | Geographic distance | Quantitative trait variation | DNA polymorphism *P* | Corrected DNA polymorphism $P_A$ |
|---|---|---|---|---|
| Geographic distance | — | 0.52 (0.002) | Not significant | Not significant |
| Quantitative trait variation | 0.58 (0.002) | — | 0.73 (0.002) | 0.75 (0.002) |
| DNA polymorphim *P* | 0.62 (0.002) | 0.65 (0.002) | — | 0.98 (0.002) |
| Corrected DNA polymorphism $P_A$ | 0.57 (0.002) | 0.66 (0.002) | 0.97 (0.002) | — |

**Note:** Above diagonal: matrix correlations (*r*) and corresponding significance values (*p*) (in parentheses) among 21 *Elymus elymoides* and six *E. multisetus* accessions considered together; below diagonal: *r* and *p* (in parentheses) strictly among the 21 *E. elymoides* accessions.

AFLP also provided new and informative measures of phylogenetic relationships and evolutionary divergence among three Eurasian and eight North American *Elymus* taxa. In the "Conspectus of the Triticeae", Löve (1984) recognized several sections in *Elymus*. Section *Dasystachyae* Löve includes *E. lanceolatus* as the type species along with other long-anther species (Löve 1984). Recently described *E. wawawaiensis* (Carlson and Barkworth 1997) was not

**Fig. 3.** Collecting sites of *Elymus elymoides* and *E. multisetus* squirreltail accessions distinguished by quantitative traits (Jones et al. 2002) and DNA fingerprinting.



classified into the sections recognized by Löve (1984); however, a close relationship between *E. wawawaiensis* and *E. lanceolatus* (Figs. 1 and 2) is not unexpected (Carlson and Barkworth 1997). *Elymus lanceolatus* and *E. wawawaiensis* are distinguished from other species examined here by having long anthers and self-incompatibility mechanisms. Section *Elymus* includes *E. sibiricus* as the type species, *E. glaucus*, and several other species. However, these latter two species did not form a group and *E. glaucus* did not have a sister species in this study (Figs. 1 and 2). On the other hand, *E. caninus* and *E. mutabilis* of sect. *Goulardia* (Husnot) Tzvelev were both closely related to the *Elymus* type species *E. sibiricus*. Section *Goulardia* includes *E. caninus* as the type species, *E. mutabilis*, *E. trachycaulus*, and several other species with one spiklet per node and a tough rachis (Löve 1984). However, *E. caninus* and *E. mutabilis* were more like *E. sibiricus*, and *E. trachycaulus* did not have an obvious sister among the species examined here (Figs. 1 and 2). Interestingly, an *E. sibiricus* accession from Sichuan, P.R.C., displayed more random amplified polymorphic DNA and microsatellite DNA similarity to Eurasian *E. caninus* accessions than did an *E. mutabilis* accession from Finland (Sun et al. 1997). Conversely, our *E. mutabilis* accession from Kazakhstan displayed more amplified fragment length polymorphism similarity to Eurasian *E. caninus* accessions than did our *E. sibiricus* accessions from P.R.C. and Russia (Figs. 1 and 2). In any case, DNA evidence does not seem to support distinction of *E. caninus* and *E. mutabilis* (sect. *Goulardia*) from the *Elymus* type species *E. sibiricus* (sect. *Elymus*). *Elymus hystrix* and *E. canadensis* are type species of sect. *Hystrix* (Moench) Á. Löve and sect. *Macrolepis* (Nevski) Jaaska, respectively, (Löve 1984). However, multiple accessions for both *E. hystrix* and *E. canadensis* group together in one well-defined lineage (Figs. 1 and 2). Thus, distinction of sect. *Hystrix* and sect. *Macrolepis* may not be useful. Section *Sitanion* essentially includes *E. elymoides* as the type species and *E. multisetus* exclusively (Löve 1984). The grouping of *E. elymoides* and *E. multisetus* in sect. *Sitanion* (Löve 1984) was supported by DNA fingerprinting (Figs. 1 and 2).

Estimates of nucleotide variation based on amplified fragment length polymorphism within and among *E. elymoides*, *E. multisetus*, and other *Elymus* taxa can be compared with those of other species. Corresponding estimates of nucleotide variation among purple needlegrass (*Nassella pulchra*) populations range from 1.1 to 3.8 differences per 1000 nucleotides (Larson et al. 2001). These values are considerably lower than $\pi_t$ in *E. elymoides*, *E. multisetus*, and other *Elymus* taxa. Yet morphological variation among these purple needlegrass populations is substantial (Knapp and Rice 1998). The content and structure of coding and noncoding DNA display substantial variation among different grass species. Thus, rates of nucleotide variation may not correspond to phenotypic diversity in divergent species. Estimates of nucleotide variation in bluebunch wheatgrass (*Pseudoroegneria spicata*) of the U.S. Palouse region approach 38 differences per 1000 nucleotides (Larson et al. 2000). These estimates are substantially higher than those of any of the *Elymus* species examined in this study, including self-incompatible *E. lanceolatus* and *E. wawawaiensis*. *Elymus* and *Pseudoroegneria* genera share the **St** genome, which is evidently very similar in size to the **H** genome of *Hordeum* and *Elymus* (Vogel et al. 1999). Genetic similarity between *Elymus* and *Pseudoroegneria* is also evident by the frequent difficulty in distinguishing *E. wawawaiensis* and *P. spicata*. Thus, differences in nucleotide variation between *Pseudoroegneria* and *Elymus* species cannot be easily attributed to mode of reproduction or genome structure. Interestingly, total nucleotide variation among predominantly *Elymus* taxa is comparable with nucleotide diversity within *P. spicata* (Larson et al. 2000). *Pseudoroegneria spicata* is a widely distributed, cross-pollinating grass with no other congeners recognized in North America. Thus, levels of DNA variation maintained within one widely distributed cross-pollinating species, *P. spicata*, may be comparable with DNA variation partitioned among numerous self-pollinating *Elymus* taxa. Although estimates of nucleotide variation based on amplified fragment length polymorphism may come into question, these standard parameters of genetic diversity and phylogenetic relationships provide a useful reference. Corrected nucleotide divergence ($D_A$) is very much dependent on the sampling of genotypes representing diversity ($\pi_t$) within the taxa or groups being compared. Therefore, the apportionment of $D_A$ and $\pi_t$ within *Elymus* species (Fig. 2) should be viewed skeptically. However, estimates of total nucleotide divergence ($D$) among *Elymus* taxa or groups should be largely independent of sampling within species. Compared with simple measures of amplified fragment length polymorphism (Fig. 1), we believe that estimates of nucleotide divergence should provide a more accurate assessment of phylogenetic relationships among divergent taxa or subspecific groups (Fig. 2).

## Acknowledgements

This study was funded by joint contributions of the U.S. Department of Agriculture, Agriculture Research Service, and the U.S. Department of Interior, Bureau of Land Management, Great Basin Restoration Initiative. We thank Dr. Lynn Clark, Dr. Elizabeth Zimmer, and other reviewers for their suggestions.

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