Genetic diversity of fringed brome (*Bromus ciliatus*) as determined by amplified fragment length polymorphism

Yong-Bi Fu, Bruce E. Coulman, Yasas S.N. Ferdinandez, Jacques Cayouette, and Paul M. Peterson

Abstract: Fringed brome (*Bromus ciliatus* L.) is found in native stands throughout a large area of North America. Little is known about the genetic diversity of this species. The amplified fragment length polymorphism (AFLP) technique was applied to assess the genetic diversity of 16 fringed brome populations sampled in Canada from the provinces of Alberta, British Columbia, Quebec, and Saskatchewan. Four AFLP primer pairs were employed to screen 82 samples with four to six samples per population and 83 polymorphic AFLP bands scored for each sample. The frequencies of the scored bands in all assayed samples ranged from 0.01 to 0.99 and averaged 0.53. Analysis of molecular variance revealed that 52.6% of the total AFLP variation resided among the 16 populations and 20.6% among the four provinces. The five Quebec populations appeared to be genetically the most diverse and distinct. The AFLP variability observed was significantly associated with the geographic origins of the fringed brome populations. These findings are useful for sampling fringed brome germplasm from natural populations for germplasm conservation and should facilitate the development of genetically diverse regional cultivars for habitat restoration and revegetation.

Key words: native grass, fringed brome, genetic variation, AFLP, habitat restoration.

Résumé : On retrouve le brome cilié (*Bromus ciliatus* L.) dans des peuplements indigènes, sur la majeure partie de l'Amérique du Nord. On sait peu de choses sur la diversité génétique de cette espèce. Les auteurs ont utilisé la technique du polymorphisme de la longueur des fragments d'amplification (AFLP) pour évaluer la diversité génétique de 16 populations du brome, échantillonnées au Canada, incluant les provinces d'Alberta, de la Colombie-Britannique, de Québec et de la Saskatchewan. Ils ont utilisé quatre paires d'amorces, pour cribler 82 échantillons constitués de quatre à six prélèvements par population, et il ont enregistré 83 bandes AFLP polymorphiques pour chaque échantillon. Les fréquences des bandes obtenues chez tous les échantillons testés vont de 0,01 à 0,99, avec une moyenne de 0,53. L'analyse de la variance moléculaire révèle que 52,6 % de la variation AFLP totale se retrouve entre les 16 populations, et 20,6 % entre les provinces. Les cinq populations du Québec semblent être les plus génétiquement diverses. La variabilité AFLP observée est significativement associée aux origines géographiques des populations du brome cilié. Ces constatations sont utiles pour échantillonner le matériel génétique provenant de populations naturelles, en vue de la conservation de ce matériel, et devraient rendre plus facile le développement de cultivars génétiques régionalement diversifiés, pour la restauration des habitats et de la végétation.

Mots clés : graminée indigène, brome cilié, variation génétique, AFLP, restauration des habitats.

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Introduction

Native plant species have gained renewed interest in research and plant breeding programs in recent years. Particular interest includes the use of these species for soil stabilization (Cooper 1957), mine site reclamation (Gaffney and Dickerson 1987), wildlife habitat restoration (Duebbert et al. 1981), and development of high-quality forage (Vogel and Pedersen 1993). One of the major limitations to the use of native plant species is the lack of commercial seed quantities. Efforts to develop cultivars with improved seed production have increased in the United States and Canada (Crowle 1970; Smoliak and Johnson 1980, 1983; Jones et al. 1991). Many improved germplasm lines have been released and made commercially available for rangeland restoration and large-scale revegetation (May et al. 1997; Englert et al. 2002). However, these improved plant materials may not maintain a sufficiently high level of genetic diversity necessary for adaptation in nonlocal environments (Knapp and Rice 1996; Roundy 1999; Larson et al. 2000). Such con-

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cern has its roots not only in adaptation but also in genetic diversity, as general knowledge about genetic diversity of many grass species native to North America is largely lacking (Huff et al. 1998; Larson et al. 2001*a*; Fu et al. 2004*b*). Little attention has been paid to assess the genetic diversity of native grass species, particularly using molecular techniques, for the development of diverse grass germplasm with improved seed production.

Fringed brome (*Bromus ciliatus* L.) is a native, diploid ($2n = 14$), predominantly outcrossing, tufted perennial species (McKone 1985; Pavlick 1995; Peterson et al. 2001). This species is widely distributed in North America from Alaska and the Northwest Territories to Newfoundland–Labrador, Canada, south to Maryland, North Carolina, Tennessee, Illinois, Nebraska, Colorado, and southern California (Wagnon 1952; Pavlick 1995; Peterson et al. 2001). It occurs in a variety of habitats such as wet meadows, streambanks, bogs, thickets, and occasionally talus slopes and roadsides. The diversity of habitats in which *B. ciliatus* grows has stimulated some interest in the development of ecological cultivars that are diverse and from a relatively unselected seed source and in the utilization of fringed brome seed sources for reseeding clearcuts and disturbed areas (May et al. 1997; Cayouette et al. 1997). Efforts have been made to collect the germplasm from the species range in Canada (Peterson et al. 2001) and to evaluate the seed yield, vegetative growth, and forage quality of this species with the hope of generating an additional source of abundant, high-quality seed specifically adapted to western Canada (Cayouette et al. 1997; May et al. 1998, 1999). However, little effort has been made to assess the genetic diversity of this species. Thus, challenges exist in improving seed production of this species while simultaneously maintaining genetic diversity for adaptation in nonlocal environments (Smith and Whalley 2002).

The amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) is a robust, highly effective method of DNA fingerprinting that can be used to assess molecular genetic variability. AFLP markers, although scored dominantly (i.e., without distinction between homozygotes and heterozygotes) and not always homologous (Koopman 2005), have been successfully applied to detect genetic variation in many grass species, including smooth bromegrass (*Bromus inermis* Leyss.) and meadow bromegrass (*Bromus riparius* Rehmann) (Ferdinandez and Coulman 2002), bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve) (Larson et al. 2000), bluegrasses (*Poa* spp.) (Larson et al. 2001*b*), crested wheatgrass (*Agropyron* spp.) (Mellish et al. 2002), blue grama (*Bouteloua gracilis* [Kunth] Lag. ex Griffiths) (Fu et al. 2004*a*), and little bluestem (*Schizachyrium scoparium* [Michx.] Nash) (Fu et al. 2004*b*). Some studies also assessed the correlation of AFLP variability with geographic origin of natural populations (Larson et al. 2001*b*; Larson et al. 2003; Fu et al. 2004*b*). In a companion study aimed at clarifying *B. ciliatus* and *Bromus richardsonii* Link as distinct species, Peterson et al. (2001) revealed large AFLP variability in fringed brome, but no diversity analysis was specifically made for fringed brome populations.

The objective of this study was to assess the patterns of genetic variability in fringed brome populations using AFLP markers with the hope to facilitate the development of diverse fringed brome germplasm for revegetating clearcuts and other disturbed areas in Canada.

Materials and methods

Plant materials

Seeds of fringed brome plants were collected at 16 locations across Canada (five locations in Alberta, three in British Columbia, five in Quebec, and three in Saskatchewan; see Table 1). Most of the collections were made between 1993 and 1999. On average, seeds were collected from 10 plants that were at least 5 m apart at each location. Collected seeds were germinated and four to six seedlings for each location were randomly selected. Such a small sample size may introduce some bias in the inference of within-population variability, but population and regional comparisons of genetic variability should be relatively robust. Young leaf tissue was individually collected, freeze-dried, and stored at –80 °C before DNA analysis.

DNA extraction and AFLP procedure

Two leaves of each sample were placed in a 2 mL microcentrifuge tube with two 2 mm glass beads. The tubes were placed on a horizontal shaker until the leaf tissue was ground to a fine powder. The DNA was extracted by using a DNeasy™ Plant Mini Kit (QIAGEN Inc, Mississauga, Ontario) according to the manufacturer's directions. Total genomic DNA was quantified by fluorimetry using Hoechst 33258 stain (Sigma Chemical Co., St. Louis, Missouri).

The AFLP analysis was performed as described by Vos et al. (1995) using the AFLP™ Analysis System 1 provided by Life Technologies (Burlington, Ontario). Initially, a restriction digestion of 250 ng of genomic DNA with *Eco*RI and *Mse*I restriction enzymes were carried out followed by ligation of adapters to the restriction fragments. This was followed by preamplification of the primary templates with AFLP primers with an additional single nucleotide at the 3′ end. A selective amplification of the preamplified fragments with labeled [γ-^{33}P]*Eco*RI primers having three selective nucleotides at the 3′ end and *Mse*I primers having three selective nucleotides at the 3′ end was performed. The selective amplification was performed in an MJ Research PTC-200 DNA engine thermocycler using the following amplification profile: one cycle with a denaturation step at 94 °C for 30 s, an annealing step at 65 °C for 30 s, and an extension step at 72 °C for 60 s. The next 12 cycles emulated a Touchdown polymerase chain reaction format decreasing the annealing temperature by 0.7 °C each cycle to 56 °C. Twenty-three more cycles were performed using the profile 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s. Finally, the amplification products were separated on 5% polyacrylamide gels for 2.30 h at 90 W. The gels were transferred to Whatman paper and dried on a gel dryer for 2 h at 80 °C. Gels were exposed to Kodak BIOMAX film at 80 °C for 1–7 d depending on the signal intensity. From the previous AFLP analysis of *Bromus* plants (Ferdinandez and Coulman 2002), four most informative *Eco*RI–*Mse*I primer combinations were selected and applied in this study (Table 2).

Table 1. Sampling site and variation pattern of AFLP for the 16 fringed brome populations studied.

Location (code)	Latitude	Longitude	Elevation (m)	NP	NPB	MBF	WPV
St-Denis, Quebec (DEN)	47°23′00″N	69°55′00″W	20	5	29	0.545	14.20
Lévis, Quebec (LEV)	46°40′00″N	71°11′00″W	100	4	6	0.500	3.33
ND-des-Bois, Mt Marbre, Quebec (MAR)	45°19′50″N	71°01′15″W	650	4	8	0.375	4.33
Pontiac-Station, Quebec (PON)	45°28′00″N	76°18′20″W	300	4	11	0.576	5.67
Rivière Ste-Anne-des-Monts, Quebec (RSA)	49°03′50″N	66°29′25″W	200	6	29	0.494	12.00
Brancepeth, Saskatchewan (BRA)	53°02′10″N	105°16′10″W	440	6	18	0.454	7.53
Okla, Saskatchewan (OKL)	51°59′10″N	103°06′05″W	617	6	28	0.512	12.93
Willowbrook, Saskatchewan (WIL)	51°11′30″N	102°48′10″W	540	4	26	0.510	14.67
Bezanson, Alberta (BEZ)	55°13′52″N	118°17′10″W	587	6	14	0.643	6.40
Hondo, Alberta (HON)	55°03′15″N	114°03′20″W	610	5	16	0.475	7.40
Nestow, Alberta (NES)	54°14′25″N	113°34′45″W	628	6	23	0.478	10.33
Athabaska River, Smith, Alberta (SMI)	55°04′25″N	114°05′45″W	564	5	27	0.615	12.40
Viking, Alberta (VIK)	53°02′40″N	113°30′12″W	701	4	15	0.633	8.33
Chetwind, British Columbia (CHE)	55°45′10″N	121°36′10″W	914	5	25	0.520	13.00
Dome Creek, British Columbia (DOM)	53°42′25″N	121°03′00″W	701	6	23	0.457	8.87
Shelley, British Columbia (SHE)	53°56′30″N	122°35′25″W	690	6	20	0.408	8.20

Note: NP, the number of plants assayed; NPB, the number of polymorphic AFLP bands scored; MBF, mean band frequency; WPV, within-population variation calculated from the sum of squares of AMOVA. The voucher information on these populations is described in Peterson et al. (2001) with the same code (or geographical identifier) as used in this study.

Data analysis

For gels generated from each primer pair, the numbers of observable and monomorphic AFLP bands were counted. Polymorphic AFLP bands with sufficient intensity for all of the samples were selected and manually scored as present (1) or absent (0). The selection may introduce bias into some of the AFLP variability measurements, but these selected bands should provide a relative measure of polymorphism for group comparison. The selected polymorphic bands were analyzed for each primer pair for the level of polymorphism by counting the number of polymorphic bands and generating the summary statistics on the band frequencies. To visualize the variation pattern, the numbers of polymorphic bands were plotted against their frequencies of occurrence in all samples. For each population, the number of polymorphic bands, the mean band frequency, and within-population variation measured from the sum of squares from the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) were calculated and regressed over the population latitude, longitude, elevation, and sample size using the SAS regression program (PROC REG) (SAS Institute Inc. 2004).

To assess AFLP variations across various groups (population and region), an AMOVA was performed using Arlequin version 2.001 (Schneider et al. 2002). This analysis not only allows the partition of the total AFLP variation into within- and among- group variation components but also provides a measure of intergroup genetic distances as the proportion of the total AFLP variation residing between any two groups (called Phi statistic; Excoffier et al. 1992; Huff et al. 1998). Models involving various types of structuring (population and region) were applied. Significance of resulting variance components and intergroup genetic distances was tested with 10 000 random permutations. In this study, populations were grouped by province to form four regions for comparing regional genetic variability.

To assess the genetic associations of the fringed brome plants representing 16 populations and four regions, the bootstrapped unweighted pair group with arithmetic averages (UPGMA) dengrograms were generated based on the pairwise F_{st} values that were obtained using the method of Reynolds et al. (1983) invoked in PHYLIP version 3.64 (Felsenstein 2005) from band frequencies. The association of the distance matrix of the Phi statistic for 16 populations with the corresponding geographical distance matrix was also examined with the MXCOMP program of NTSYS-pc 2.01 (Rohlf 1997) with 10 000 random permutations. To evaluate the genetic associations of 82 individual plants, an individual pairwise similarity matrix was generated using simple matching coefficient (Sokal and Michener 1958) and converted to the Euclidean distance matrix for a principal coordinate analysis using the NTSYS-pc program. The first three resulting principal coordinate scores were plotted to assess the clustering of individual fringed bromes.

Results

Four AFLP primer pairs amplified a total of 327 observable bands, with the number of observable bands per primer pair ranging from 47 to 104 (Table 2). There were 206 (63%) observable bands that were polymorphic, indicating the presence of large AFLP variation in this native grass species. Since many of the amplified polymorphic bands lacked clarity, only 83 of them were scored for further analyses. These scored bands were presumably sampled from the whole fringed brome genome, but their exact genome coverage is unknown. The frequencies of the scored bands in all assayed samples ranged from 0.01 to 0.99 and averaged 0.53. A large proportion (61%) of the scored bands had frequencies of either less than 0.16 or greater than 0.84. For each primer pair, statistics (mean, minimum, and maximum) of the band frequencies are given in Table 2. The lowest mean frequency was found with the primer pair E+ACG/M+CTG, and the highest mean frequency was observed with the primer pair E+AAG/M+CAC.

Genetic variations for 16 fringed brome populations were quantified in this study by the number of scored polymorphic

Table 2. AFLP in 82 fringed brome plants as revealed by four AFLP primer pairs.

Primer pair	No. of AFLP bands			Frequency of scored bands		
	Total	Mono	Scored	Mean	Min.	Max.
E+ACG/M+CTG	47	14	25	0.485	0.012	0.988
E+AAG/M+CAC	104	33	14	0.615	0.024	0.976
E+AGG/M+CGC	87	25	14	0.512	0.012	0.988
E+AAC/M+CAG	89	24	30	0.508	0.012	0.988
All	327	121	83	0.530	0.012	0.988

Note: Four *Eco*RI primers (E plus three selective nucleotides, 5′-GACTGCGTACCAATTC+ACG, AAG, AGG, and AAC) and four *Mse*I primers (M plus three selective nucleotides, 5′-GATGAGTCCTGAGTAA+CTG, CAC, CGC, and CAG) are shown for the primer pair combinations applied in this study. Total, total number of AFLP bands observed; mono, number of monomorphic AFLP bands detected; scored, number of polymorphic AFLP bands scored.

bands, the mean band frequency, and the within-population variation measured from the AMOVA sum of squares (Table 1). Within a population, the number of scored polymorphic bands ranged from 6 to 29 with an average of 19.9, the mean band frequency ranged from 0.38 to 0.64 with an average of 0.51, and the within-population variation ranged from 3.33 to 14.67 with an average of 9.35. The Quebec populations had the most and the least polymorphic bands (29 for St-Denis and Rivière de Ste-Anne-des-Monts, six for Lévis, and eight for ND-des-Bois). The Bezanson population in Alberta had the highest mean band frequency, and the ND-des-Bois population from Quebec had the lowest mean band frequency. The population with the most within-population variation was Willowbrook (14.7), Saskatchewan, followed by St-Denis (14.2), Quebec. The population with the least within-population variation was Lévis (3.3) followed by ND-des-Bois (4.3), Quebec. Linear regression analyses revealed nonsignificant associations of population latitude, longitude, elevation, and sample size with the population estimates of the three genetic parameters mentioned (results not shown).

Partitioning of the total AFLP variation into within- and among- population components by AMOVA showed that 47.4% of the total variation was present within populations and 52.6% resided among 16 populations (Table 3). These variation components were significantly different from zero ($P < 0.0001$) based on the permutation test. The largest between-population difference measured by interpopulation distance was observed between two Quebec populations (Lévis and ND-des-Bois; 0.83), and the least between-population difference measured was between the Okla population in Saskatchewan and the Athabaska River population in Alberta (0.09). These differences can also be visualized in the inferred genetic relationships of the 16 populations given in a dendrogram (Fig. 1A). Two populations from Quebec (Lévis and ND-des-Bois) represented the first two most distinct clusters with one member each. The third cluster consisted of the other three populations from Quebec, and the fourth cluster included 11 populations from the other three provinces. This pattern of clustering was similar to those made based on Euclidean distance (shown in Fig. 17 of Peterson et al. 2001). The AFLP variations of pairwise populations were significantly associated ($r = 0.68$, $P < 0.0001$) with geographical origin of the populations. Figure 2 illustrates the associations between genetic distances measured by the Phi statistic and geographic distances in kilometres of fringed brome populations. Further partitioning of the among-population variation into among-region and among-population, within-region components revealed that 20.5% of the total variance resided among four geographic regions (Alberta, British Columbia, Quebec, and Saskatchewan). Further assessment of the variation among the four regions showed that the fringed plants from Quebec were genetically the most distant (or distinct) from the remaining plants assayed followed by those from British Columbia (Fig. 1B).

To understand the genetic structure observed in fringed brome, the genetic associations of individual plants were assessed. The plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 83 AFLP bands revealed more or less three major groups (Fig. 3). The first group on the upper left of the figure consisted of 58 fringed brome plants from Alberta, British Columbia, and Saskatchewan. The second group on the upper right of the figure consisted of 15 plants from the three Quebec populations with higher latitudes (Rivière Ste-Anne-des-Monts, St-Denis, and Lévis). The third group on the lower part of the figure included one plant collected from Willowbrook, Saskatchewan, and eight plants from the two Quebec populations with lower latitudes (ND-des-Bois and Pontiac-Station). Clearly, the Quebec fringed bromes were not only diverse but also distinct from those fringed bromes collected from western Canada.

Discussion

This study represents the first attempt using AFLP markers to characterize the genetic variability and structure of Canadian fringed brome populations with the goal to enhance the development of fringed brome germplasm for habitat restoration. The study revealed several interesting patterns. First, 52.6% of the AFLP variation was present among the 16 populations examined, and 20.6% resided among the four provinces. Second, the five Quebec populations were both the most diverse and distinct. Third, the between-population AFLP variability was significantly associated with the geographic origins of the fringed brome populations. These findings are useful for sampling fringed brome germplasm from natural populations for germplasm conservation and should facilitate the improvement of fringed brome germplasm for habitat restoration.

Table 3. AMOVA based on AFLP data among 82 fringed brome plants with respect to region and population.

Source of variation	df	SS	Variance components	% variation
Among regions	3	190.87	2.06[a]	20.52
Among populations within regions	12	253.00	3.22[a]	32.10
Within populations	66	314.17	4.76[a]	47.38

Note: Populations were grouped by province to form a region.
[a]Significant at $P < 0.0001$, as calculated from 10 000 random permutations.

Fig. 1. UPGMA dendrograms illustrating the genetic relationships of fringed brome plants representing (A) 16 populations and (B) four geographic regions (BC, British Columbia; QC, Quebec; AB, Alberta; SK, Saskatchewan). Population labels are given in Table 1. Pairwise F_{st} genetic distances were calculated following Reynolds et al. (1983). Bootstrap values higher than 50 (out of 100 replicates) are indicated inside the node.

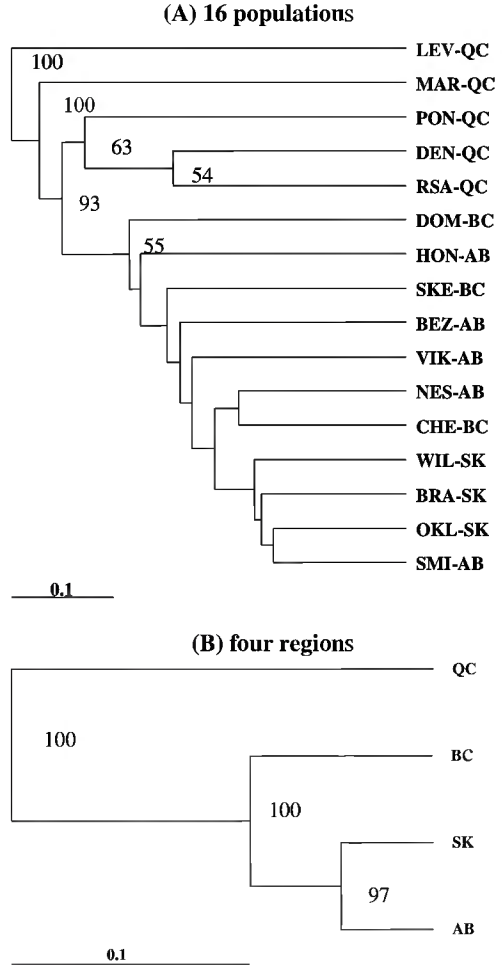


Fig. 2. Associations between genetic distances measured by the Phi statistic and geographic distances in kilometres of fringed brome populations. A linear regression is also presented.

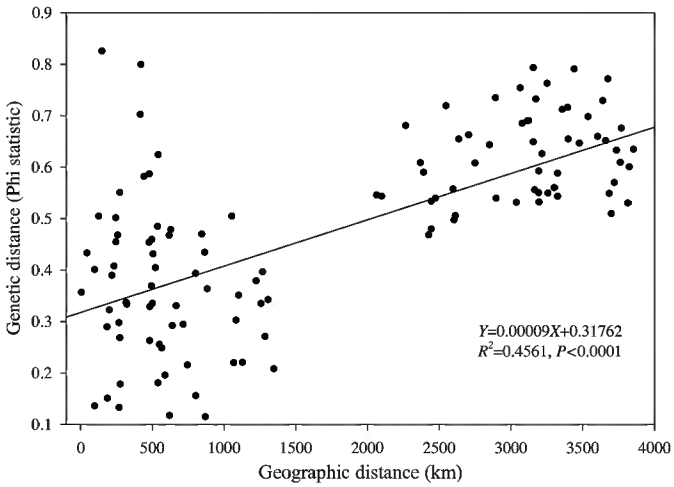
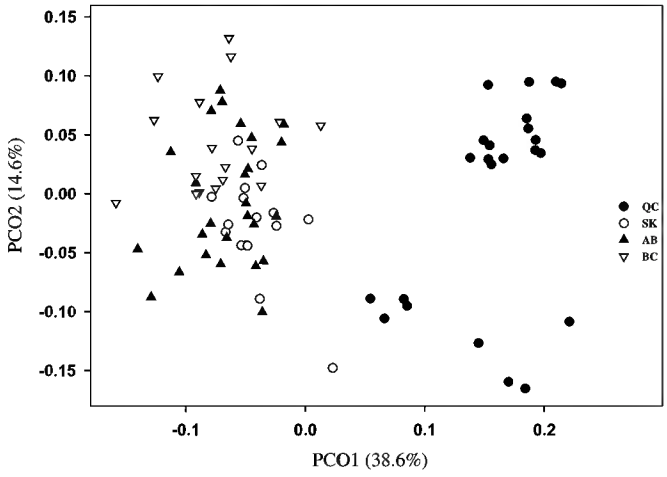


Fig. 3. Plot of the first two principal component scores based on the Euclidean distances converted from the simple matching coefficient matrix of 83 AFLP bands for 82 fringed brome plants. These two components accounted for 38.6% and 14.6% of the total AFLP variance, respectively. Individual plants were separately labeled for their geographic regions (QC, Quebec; SK, Saskatchewan; AB, Alberta; BC, British Columbia).



Fringed brome is known to be an outcrossing grass species also with self-compatibility (McKone 1985), although the level of self-fertilization in this species is not known. Thus, it is not fully surprising to observe the low within-population variation (47.4%) shown in these populations (Hamrick and Godt 1989), but a question remains on how much such a low estimate was accounted for by self-pollination. Clearly, a comprehensive study on the mating system of this species is needed for understanding this pattern of variation (Barrett et al. 2004). Also, it is possible that this low estimate may reflect the bias from the use of small samples for these populations. Larger sample size (>30 plants

per population) is needed for a more informative inference of within-population variation.

The extensive differentiations observed over the populations and regions may reflect limited gene flow via pollen and seed among the populations and (or) strong differential selection pressures existing among the sampled sites. Further studies are needed to provide information on gene flow between natural fringed brome populations and its effect on genetic diversity (Hutchison and Templeton 1999). Also, the high among-population or low within-population variations observed may not be directly associated with adaptive variations that exist in these populations, as AFLP markers presumably were selectively neutral (Vos et al. 1995).

The within-population AFLP variability was not associated with the population latitude, longitude, and elevation, probably owing to the use of small sample sizes for these populations. The significant association of the between-population AFLP variation with geographic origin of the populations surveyed may reflect that local adaptations existed in these populations, particularly in those of northern Quebec. A similar phylogeographic pattern was observed in *Nassella pulchra* (Hitchc.) Barkworth (Larson et al. 2001*b*), *Elymus* spp. (Larson et al. 2003), and little bluestem (Fu et al. 2004*b*). However, the generality of this association remains to be determined, as the populations assessed in this study were located in the northern fringe of the species' range. Also, further assessment in a breeding nursery on adaptive genetic variations in reproductive growth and seed yield is still needed for these fringed brome populations. Such adaptive assessment, currently underway, could not only help in the understanding of the nature of adaptation in fringed brome but also would facilitate the selection of fringed brome germplasm for habitat restoration.

The patterns of AFLP variation observed in this study are comparable only with some reports for other native plants. For example, 57% AFLP variation was observed among 58 natural populations of *Echinacea* (Mechanda et al. 2004) and 48% among six populations of arctic bramble (*Rubus arcticus* L. subsp. *arcticus*) (Lindqvist-Kreuze et al. 2003). In contrast, 19% AFLP variation was found among three populations of *Festuca campestris* Rydb. (Fu et al. 2005) and 7% among six populations of little bluestem (Fu et al. 2004*b*). It is difficult to explain such discrepancy only from modes of reproduction (Larson et al. 2001*a*, b) and also is beyond the scope of this study. However, it is certain that the applications of the AFLP technique can provide useful measures of genetic variability in native plants, even with the issues of dominance and homology (Koopman 2005).

The results of this study have implications for sampling fringed brome germplasm from natural populations for germplasm conservation. The low variation detected within single natural populations suggests that a rather large number of plants from single sites should be sampled to capture substantial genetic variation, but the optimal number of plants to be sampled from one site remains to be determined empirically. The observation of the large between-population variation implies a low genetic similarity between nearby native stands, and thus, more distant populations should be sampled to capture more localized germplasm, which is important for the development of diverse, well-adapted fringed brome germplasm for habitat restoration. The finding of the large regional variation (20.6%) underscores the need for germplasm collection across the species' range, as the current sampling was applied only to the populations in the northern part of the range and fringed brome plants in more southern latitudes may also harbour substantial genetic diversity.

The findings presented here appear to suggest the importance of developing localized germplasm that could capture the unique adaptations to a particular geographic area. Thus, ecological varieties specific to eastern and western Canada should be developed for habitat restoration. Emphasis should be placed on the balance of local adaptation and population differentiation by including more germplasm from distant populations to sample more among-population variability. To capture more genetic diversity in an ecological variety, equal representation of nearby seed sources could be applied when information is limited on adaptation and diversity (Fu et al. 2004*a*). To maximize the genetic diversity in a developed germplasm, proportional contributions of different seed sources based on their adaptability and genetic variability should be considered. As demonstrated here, the application of a molecular technique cannot only assess the genetic variability within and among fringed brome populations but also facilitate the selection of a fringed brome seed source that was either genetically diverse or distinct for the development of fringed brome germplasm for habitat restoration in Canada.

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