Effects of heavy grazing pressure on the random amplified polymorphic DNA marker diversity of mountain rough fescue (*Festuca campestris* Rydb.) in south western Alberta

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[1]*College of Ecology and Environmental Science, Inner Mongolia Agricultural University, Huhhot, Inner Mongolia 010018, P.R. of China;* [2]*Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1. LRC contribution no. 38704064, received 10 November 2004, accepted 29 March 2005.*

Mengli, Z., Willms, W. D., Bing, H. and Laroche, A. 2005. **Effects of heavy grazing pressure on the random amplified polymorphic DNA marker diversity of mountain rough fescue (*Festuca campestris* Rydb.) in south western Alberta**. Can. J. Plant Sci. **85**: 623–629. The Fescue Grassland is found in the western portion of the Northern Great Plains in Canada. Grazing and cultivation threaten this grassland, and a better understanding of its character is needed to preserve its integrity. Mountain rough fescue is highly sensitive to grazing during the growing season, which results in smaller plants and the death of some. The death of plants suggests the potential loss of genetic diversity. Therefore, we compared the genetic diversity of mountain rough fescue plants from sites in south western Alberta (50°12′N, 113°54′W) that had either been heavily grazed by livestock or left ungrazed for 52 yr to determine if grazing pressure had affected their genetic composition. Thirty-four and 43 plants were sampled in the spring of 2001 from very heavily grazed and ungrazed subpopulations, respectively, and their DNA was analyzed using random amplified polymorphic DNA (RAPD). Of the 15 primers used, 12 generated an average of seven polymorphic loci each. Ten loci were present at a frequency of 0.10 or less in the heavily grazed subpopulation and six in the ungrazed subpopulation. RAPD marker diversity between the heavily grazed and ungrazed subpopulations of mountain rough fescue was mainly the result of frequency differences ($P < 0.05$) produced by 20% of the total markers that were examined, while the subpopulations accounted for only 4.37% of total heterozygosity. Therefore, grazing affected frequency of some markers but did not eliminate genes that may be linked with grazing sensitivity or tolerance. Lack of clear genetic segregation between the subpopulations might be caused by a high gene flow (*Nm* = 10.92). This mechanism requires further testing in order to prescribe a suitable management response for restoring overgrazed grasslands.

Key words: RAPD frequency, *F*-statistics, genetic identity, genetic distance, gene flow

Mengli, Z., Willms, W. D., Bing, H. et Laroche, A. 2005. **Incidence d'un fort taux de charge des pâturages sur la diversité des marqueurs d'ADN à régions polymorphes amplifiées à l'aide de séquences aléatoires chez la fétuque scabre (*Festuca campestris* Rydb.) dans le sud-ouest de l'Alberta**. Can. J. Plant Sci. **85**: 623–629. Les prairies à fétuque couvrent la partie occidentale du nord des grandes plaines canadiennes. La paissance et l'agriculture menacent ces prairies et il convient de mieux en comprendre la nature si on veut en préserver l'intégrité. Durant la période végétative, la fétuque scabre est très sensible à la paissance, ce qui entraîne le rabougrissement et parfois la mort des plants. La destruction des peuplements laisse supposer la disparition de la diversité génétique. Pour le savoir, les auteurs ont comparé la diversité génétique de la fétuque scabre dans des prairies surexploitées et à des endroits vierges après 52 années d'élevage, dans le sud-ouest de l'Alberta (50°12′N, 113°54′O), l'idée étant de déterminer si le taux de charge des pâturages modifie la composition génétique de l'espèce. Au printemps 2001, les auteurs ont respectivement prélevé 34 et 43 plants de populations secondaires situées sur des pâturages très exploités et des prairies intactes, puis en ont analysé l'ADN par amplification des régions polymorphes à l'aide de séquences aléatoires (RAPD). Sur les 15 amorces employées, 12 ont donné en moyenne sept locus polymorphes. Dix locus se caractérisaient par une fréquence de 0,10 ou moins pour la sous-population des pâturages surexploités contre six pour celle des endroits intouchés. La diversité des marqueurs RAPD dans les populations secondaires des pâturages surexploités et des prairies intactes de fétuque scabre résulte surtout des variations au niveau de la fréquence ($P < 0,05$) attribuables à 20 % des marqueurs examinés, les populations secondaires n'expliquant que 4,37 % de l'hétérozygotie. Par conséquent, la paissance affecte bien la fréquence de quelques marqueurs, mais n'élimine pas les gènes qui pourraient être associés à la sensibilité à la paissance ou à la tolérance de cette dernière. La faible ségrégation génétique entre les populations secondaires pourrait résulter d'un important flux génétique (*Nm* = 10,92). Pour le savoir, il faudrait entreprendre d'autres essais, après quoi on pourrait formuler des recommandations sur la manière de restaurer les prairies surexploitées.

Mots clés: RAPD, fréquence, statistique *F*, identité génétique, distance génétique, flux génétique

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Abbreviations: **DNA**, deoxyribonucleic acid; **PCR**, polymerase chain reaction; **RAPD**, random amplified polymorphic DNA

The Fescue Grassland is an important genetic reservoir found in the western portion of the Northern Great Plains in Canada. Climax communities of this grassland are dominated by mountain rough fescue (*Festuca campestris* Rydb.), a large-tufted species, which defines the character and function of the grassland. Historically, mountain rough fescue was likely the key forage species used by bison in winter. Presently, it is used for grazing livestock, but the species is extremely sensitive to summer defoliation, which will reduce its vigor and cause it to decline in the plant community. Mountain rough fescue was reduced from about 40% of basal area to about 1% after 32 yr of heavy grazing pressure, with most changes occurring within 6 yr (Willms et al. 1985). This was caused by fewer and smaller plants (Willms, unpublished data) and suggests that grazing-sensitive plants were eliminated. For example, the density in a heavily grazed paddock was 0.8 plants m^{-2}, while the density in a less-heavily grazed paddock was 1.4 plants m^{-2} (Willms, unpublished data). Furthermore, the plants in the former paddock had an average of 37 tillers, while those in the latter paddock had an average of 65 tillers. Thus, grazing may impose selection pressure for genotypes that are more grazing-tolerant and thereby reduce the genetic diversity of mountain rough fescue populations.

Grazing can affect the morphology of grass plants with traits that are maintained when grown in a common garden (Painter et al. 1989; Jaindl et al. 1994). Grazing pressure seemed to favor plants of western wheatgrass (*Agropyron smithii* Rydb.) and blue grama [*Bouteloua gracilis* (H.B.K.) Griffiths] that had fewer and shorter flowering tillers (Painter et al. 1989), while protected Idaho fescue (*Festuca idahoensis* Elymer) plants were taller and exhibited a more rapid rate of growth than grazed plants (Jaindl et al. 1994). These results were contrary to May et al. (2003) who reported, from a preliminary study, that heavy grazing pressure resulted in mountain rough fescue plants that had taller flowering tillers and were more winter hardy than ungrazed plants.

The structure, function, and species diversity of grassland ecosystems are inter-related (Tilman and Downing 1994) and can be altered by grazing. Improper utilization of rangeland with heavy grazing pressure can reduce the cover and diversity of native plant species. Although theoretical models predict that moderate grazing may enhance species diversity compared with ungrazed lands (Milchunas et al. 1988), information on the effect that heavy grazing pressure has on the genetic diversity of grass species is relatively sparse and presently unknown for mountain rough fescue. It is important to understand the potential effects that grazing has on the genetic diversity of mountain rough fescue in order to interpret its potential effect on the grassland ecosystem. This knowledge would also assist in determining the potential for selecting grazing-resistant genotypes that might be useful where summer grazing was practiced.

Random amplified polymorphic DNA (RAPD) markers are well suited for population genetic analysis. Each random primer typically yields several variable markers, and since many random primers are available, a large number of RAPDs can be identified for analysis. Random amplified polymorphic DNA markers have been shown to segregate in a biparental dominant Mendelian manner (Carlson et al. 1991; Roy et al. 1992; Heun and Helentjaris 1993). Their use as markers for the genetic characterization of populations has been well established for a variety of organisms (Chalmers et al. 1992; Huff et al. 1993; Caccone et al. 1997; Esquibet et al. 1998; Hogbin et al. 1998; Lou et al. 1998; Suazo et al. 1998). Using RAPD markers in this study, we compared the genetic diversity of two mountain rough fescue subpopulations that had been either heavily grazed or ungrazed for 52 yr to determine whether grazing pressure affected their genetic composition.

MATERIALS AND METHODS

Population Sampling

The study was situated in the Fescue Grasslands at the Agriculture and Agri-Food Canada Range Research Substation west of Stavely, AB (50°12′N, 113°54′W). The site (population) was part of a grazing capacity study begun in 1949 that included a 16.1-ha paddock (heavily grazed subpopulation; 200 × 805 m, oriented in an east to west direction) and two exclosures (ungrazed subpopulation). Both exclosures were outside the paddock but one (0.2 ha) was contiguous with the south side while the second was within 50 m of the north side (0.5 ha). The paddock was heavily grazed for up to 6 mo beginning in mid-May of each year since 1949. In any year, grazing was discontinued when there was insufficient forage to maintain animal weight. Average grazing pressure was estimated to be about 80% of annual net primary production (Willms 1988).

Heavy grazing pressure during the spring and summer suppressed the composition of rough fescue to about 2.5% within 6 yr after grazing began in 1949 (Willms et al. 1985). The same grazing pressure was maintained since then until the present. In comparison, the composition of rough fescue in the exclosures ranged from 23 to 60% between 1975 and 1981 (Willms et al. 1985) and is not expected to have changed significantly since then.

Leaf and root material were sampled in the spring of 2001 from 34 individual plants in the ungrazed subpopulation (17 in each of two exclosures) and from 43 plants in the heavily grazed subpopulation. Mountain rough fescue plants in the heavily grazed paddock were reduced in size and density and difficult to detect among other vegetation by mid-June. However, rough fescue begins growth earlier in spring than many other species, which enables its detection. The subpopulations were sampled at points spaced at least 10 m apart along transects oriented to cover the greatest area. The sampled plants were transplanted to root-trainers and grown in a greenhouse to increase biomass for analysis. Leaves were then harvested in preparation for analyses.

DNA Extraction and Amplification

The collected leaves were lyophilized before DNA extraction. From each sample about 0.1 g of leaves was placed in a 2-mL microcentrifuge tube with one 3-mm glass bead. The tubes were placed in a FastPrep FP120 (Savant Instruments, Inc, Farmingdale, NY, USA) instrument in which the leaf tissue was ground for 3 s (speed 5) to a fine powder. The

DNA was extracted by using FastDNA Kit® (Q-Biogene, Irvine, CA) according to the manufacturer's directions. Total genomic DNA concentrations were determined spectrophotometrically. For RAPD analysis, DNA samples were diluted to 5.0 ng μL^{-1}. Polymerase chain reaction (PCR) amplification of DNA was carried out in a 25 μL volume in microtitre plates using an MJ Research PTC-100® thermal cycler (Global Medical Instrumentation, Inc., Ramsey, MN). Polymerase chain reaction amplification of genomic DNA was carried out in a 25 μL volume containing 10 mM *Tris*-HCl pH 9.0, 50 mM KCl, 1.9 mM MgCl$_2$, 0.1% Triton X-100, 0.2 mM dNTP, 0.2 μM primer, 1 unit Taq DNA polymerase (Promega Corporation) and 25 ng DNA. The reactions were overlaid with mineral oil and amplification was performed as follows: 45 cycles at 92°C for 1 min, 35.5°C for 1 min, 72°C for 2 min, and 72°C for 10 min. Amplified fragments were separated in 1.4% agarose gel and electrophoresis was performed at 70 V for 5 h. The gel was visualized and photographed on a digital gel documentation system after staining with ethidium bromide.

The molecular sizes of RAPD fragments were estimated using *HindIII-EcoRI* digested Lambda DNA (MBI Fermentas Inc., Burlington, ON) as a standard marker. Samples that had amplification failure or had unscorable fragments were treated as missing data.

Fifty-eight primers were screened for effectiveness in identifying loci in a test using four plants from each of two geographically distant populations (Stavely and Kamloops, 50°47′N, 120°29′W). The purpose of this was to assess the greatest expected variability. As a result, 15 primers were selected that produced clearly defined markers.

Data Analysis

The RAPD markers were transformed into a binary matrix and analyzed using POPGENE 1.31 (Yeh et al. 1997) to determine gene frequency, the allele number, and the percentage of polymorphic loci. This program also calculated estimates of genetic distance and genetic diversity according to Nei (1973), estimates of gene flow following Slatkin and Barton (1989) and *F*-statistics according to Wright (1965).

RESULTS

Twelve of the 15 primers generated 84 polymorphic loci. The remaining three primers produced either complex patterns that were difficult to interpret or had insufficient information to be useful, and were eliminated from the analyses. Of 84 bands that were scored, 77 (91.7%) were reproducibly polymorphic. The number of markers generated by each primer varied from 6 to 11 (Table 1). The size of the scored fragments ranged from 0.3 to 3 kb. A simple measure of intrapopulation variability, based on the number of polymorphic markers scored in a single subpopulation divided by the total number of polymorphic markers, was 90.1 and 92.3% in the heavily grazed and ungrazed subpopulations, respectively. An example of the band profiles is shown in Fig. 1.

The number of polymorphic loci was 82 in the heavily grazed subpopulation and 84 in the ungrazed subpopulation (Table 2). Ten RAPD markers (OPA13-2110, OPC04-2000,

OPH04-1510, OPL10-1200, OPL10-1500, OPL17-810, OPM01-1030, OPM01-1250, OPM01-1510, and OPM19-1800) were present at a frequency of 0.1000 or less in the heavily grazed subpopulation and six (OPC04-2000, OPL10-1500, OPL17-810, OPM01-1030, OPM01-1250 and OPM19-1800) in the ungrazed subpopulation. No rare RAPD was restricted to a single subpopulation, but two RAPDs (OPH04-500 and OPM01-1300) showed no polymorphism (i.e., were always present) in the heavily grazed subpopulation. Of the 84 RAPDs, the frequencies of 17 (20%) were divergent as indicated by significant ($P < 0.05$) G^2-tests (log-likelihood ratio; Zar 1999) for heterogeneity (Table 2). Heterozygosity (H_e) of the heavily grazed and ungrazed subpopulations was 0.1591 ± 0.018 and 0.1692 ± 0.016, respectively, while Wright's fixation indexes (Wright 1965; 1-[H_o/H_e], ranges from 1 to –1 and is the fraction of the total genetic variation that is distributed among subpopulations) for the same subpopulations were –0.1230 and –0.1297, respectively. These data suggest that RAPD marker diversity between heavily grazed and ungrazed subpopulations of mountain rough fescue was affected mostly by their frequency differences (Table 2) and not by the fixation of rare alleles.

The *F*-statistics were: *Fit*, 0.0050; *Fis*, –0.0405; and *Fst*, 0.0437. A negative value of *Fis* indicates a small surplus of heterozygotes relative to the Hardy-Weinberg expectation (an index of 1 indicates complete inbreeding). The *Fst* can vary from 0 to 1, with *Fst* = 0 indicating the subpopulations are not differentiated (i.e., the subpopulations do not account for any heterozygosity that is observed in the entire population). This is in contrast to *Fst* = 1, which indicates complete divergence and the subpopulations account for all observed heterozygosity in the entire population. Therefore, in our study only 4.37% of total heterozygosity was found between the heavily grazed and ungrazed subpopulations, while 95.63% was within the population.

A high *Fit* reflects divergence from the expected due to the combined effects of self pollination and the subdivision of the population. A low *Fit*, which we report, indicates a small level of inbreeding. The number of migrants across the two subpopulations (*Nm*) was large (10.94) suggesting a high gene flow. Nei's Unbiased Measures of Genetic Identity and Distance were 0.9618 and 0.0390, respectively, suggesting that the two subpopulations had a high level of similarity.

DISCUSSION

Genetic diversity may be present in natural populations due to the effects of genetic drift or selectively neutral mutation in finite populations (Kimura 1986). Nonetheless, it is well recognized that various kinds of selection processes, which include grazing pressure, can also influence genetic diversity in natural populations (Gillespie 1994). In our study, we observed a clear indication that marker frequency was different (20% of markers examined) between the heavily grazed and ungrazed subpopulations (Table 2), but these subpopulations accounted for only 4.37% of total heterozygosity in the population. Therefore, grazing affected the frequency of some markers but did not eliminate genes that may be linked with grazing sensitivity.

Table 1. Number of amplification markers generated with 12 arbitrary oligonucleotide primers in a test of mountain rough fescue plants exposed to heavy and zero grazing pressure for 52 yr

| | Number of RAPD markers | | Number of polymorphic amplification markers | | Markers detecting polymorphism | |
| | | | Grazing pressure | | | |
Primer	Total primer^{-1}	Total	Heavy	Ungrazed	(%)	Sequences of primers
OPA09	6	6	6	6	100.0	5′-GGGTAACGCC-3′
OPA13	11	11	11	11	100.0	5′-CAGCACCCAC-3′
OPC04	9	9	9	9	100.0	5′-CCGCATCTAC-3′
OPC15	6	4	4	4	66.6	5′-GACGGATCAG-3′
OPH04	8	8	7	8	100.0	5′-GGAAGTCGCC-3′
OPJ13	7	4	4	4	57.2	5′-CCACACTACC-3′
OPL10	9	9	9	9	100.0	5′-TGGGAGATGG-3′
OPL17	6	5	5	5	83.4	5′-AGCCTGAGCC-3′
OPM01	6	5	4	5	83.4	5′-GTTGGTGGCT-3′
OPM02	9	9	9	9	100.0	5′-ACAACGCCTC-3′
OPM19	8	8	8	8	100.0	5′-CCTTCAGGCA-3′
OPM20	6	6	6	6	100.0	5′-AGGTCTTGGG-3′
Total	91	84	82	84		
Polymorphism (%)		92.3	90.1	92.3		
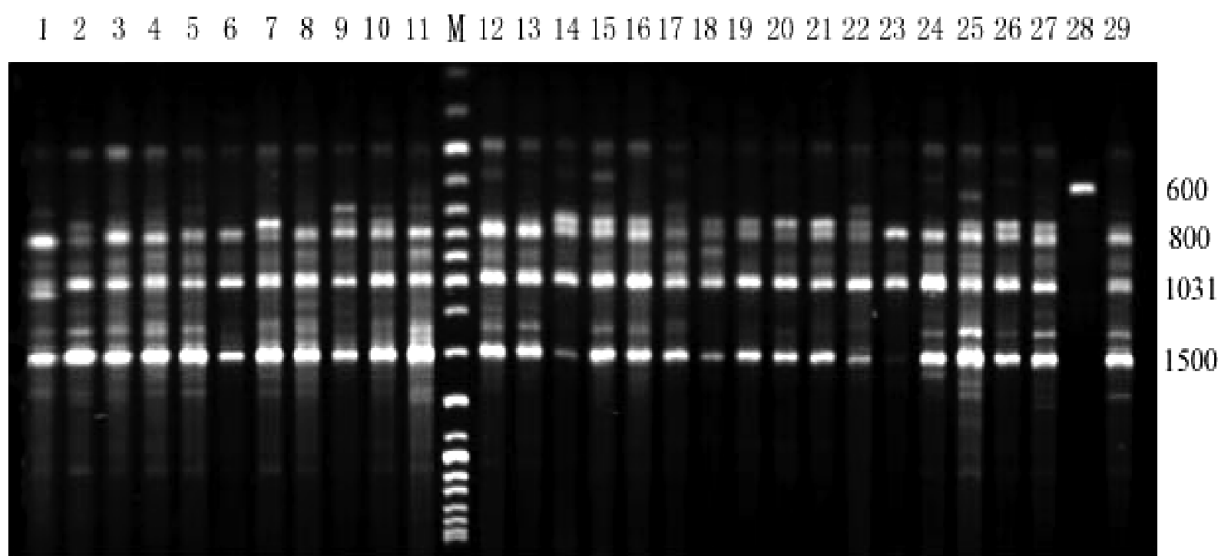
Fig. 1. RAPD profiles amplified with OPL10. Lanes 1 to 11 are amplified fragments of template DNAs from grazed plants. Lane M is the DNA molecular weight marker (*HindIII-EcoRI*® digested Lambda DNA). Lanes 12 to 29 are amplified fragments of template DNAs from ungrazed plants.

A reduction in heterozygosity caused by grazing might be expected if grazing-sensitive plants were killed and grazing sensitivity had a genetic control. The lack of a clear effect suggests the absence of a strong genetic control of vulnerability to grazing, the masking of its expression with the replacement of killed genotypes, or our inability to detect the linked genes using RAPD. Of the 17 markers that differed ($P < 0.05$) in frequency between the heavily grazed and ungrazed subpopulations, 11 were higher in frequency under grazing pressure and none were eliminated from that subpopulation (Table 2). This suggests that grazing pressure produced a genetic shift that appears to have resulted in a reduction of heterozygosity. If genes were eliminated due to grazing pressure, they were not detected with the RAPD primers that we selected.

The shift in the frequency of genes, but not their loss, indicates that heterozygosity would recover if grazing pressure were removed. This prediction is also supported by a strong gene flow ($Nm = 10.92$) across the two populations and a slight excess of heterozygotes ($Fis = –0.0405$) relative to the Hardy-Weinberg expectation.

The shift in gene frequency also suggests that vulnerable genotypes may be killed by an additive effect that is unrelated to grazing pressure. Such stresses might be caused by niches that were marginal for these genotypes due to edaphic or biotic conditions such as shallow soil that is more likely to desiccate during drought than deep soils.

Other studies have indicated that all plants are sensitive to defoliation (Willms 1991; Willms and Fraser 1992). In the study by Willms and Fraser (1992), a severe defoliation treat-

Table 2. Frequencies of polymorphic RAPD markers and the G^2 test for heterogeneity of their frequencies between subpopulations of rough fescue exposed to heavy or zero grazing pressure for 52 yr

RAPD marker	Grazing pressure		G^2	RAPD marker cont'd	Grazing pressure		G^2
	Heavy	Ungrazed			Heavy	Ungrazed	
OPA09-800	0.2770	0.3970	1.22	OPL10-500	0.2462	0.1652	0.76
OPA09-1050	0.2615	0.5076	4.92*	OPL10-600	0.1472	0.1472	0.00
OPS09-1100	0.6629	0.6985	0.11	OPL10-800	0.3784	0.3724	0.00
OPA09-1200	0.6985	0.8259	1.69	OPL10-850	0.8492	0.5076	10.65**
OPA09-1500	0.6011	0.4495	1.75	OPL10-900	0.2167	0.1124	1.50
OPA09-1800	0.8492	0.6985	2.51	OPL10-1100	0.2770	0.2615	0.02
OPA13-610	0.1743	0.2412	0.52	OPL10-1200	0.0955	0.1652	0.83
OPA13-700	0.6011	0.4778	1.16	OPL10-1250	0.8492	0.5394	8.97**
OPA13-800	0.2167	0.3037	1.75	OPL10-1500	0.0230	0.0465	0.32
OPA13-915	0.4359	0.3724	0.32	OPL17-650	0.1882	0.2215	0.13
OPA13-1031	0.5736	0.6985	1.27	OPL17-700	0.5736	0.3037	5.64*
OPA13-1050	0.3429	0.2615	0.59	OPL17-810	0.0707	0.0308	0.63
OPA13-1310	0.4778	0.5394	0.29	OPL17-2500	0.3884	0.5076	1.28
OPA13-1410	0.4359	0.5736	1.43	OPJ17-2700	0.1743	0.1124	0.59
OPA13-1500	0.6629	0.6985	0.11	OPM01-1030	0.0970	0.0487	0.19
OPA13-1920	0.6629	0.6108	0.22	OPM01-1250	0.0114	0.0955	3.11
OPA13-2110	0.0230	0.3487	16.05**	OPM01-1300	1.0000	0.6518	22.23**
OPC04-600	0.5233	0.5736	0.19	OPM01-1510	0.0230	0.1296	3.45
OPC04-700	0.1743	0.1296	0.29	OPM01-1600	0.1210	0.1472	0.11
OPC04-800	0.2929	0.4495	2.00	OPM02-320	0.4564	0.3724	0.55
OPC04-950	0.4778	0.2412	4.61*	OPM02-600	0.3784	0.2023	2.85
OPC04-1031	0.1340	0.2615	1.98	OPM02-680	0.4161	0.4778	0.29
OPC04-1150	0.3784	0.4226	1.15	OPM02-850	0.5477	0.3258	3.80
OPC04-1300	0.3784	0.3724	0.22	OPM02-1030	0.4161	0.4226	0.00
OPC04-1500	0.6629	0.3487	7.58**	OPM02-1200	0.3970	0.3724	0.05
OPC04-2000	0.0347	0.0465	0.07	OPM02-1400	0.6985	0.8259	1.69
OPC15-810	0.6629	0.1652	20.14**	OPM02-1600	0.2462	0.1472	1.17
OPC15-900	0.6011	0.4495	1.75	OPM02-2010	0.2167	0.2023	0.02
OPC15-1050	0.3604	0.1472	4.56*	OPM19-500	0.1081	0.2823	3.81
OPC15-1500	0.2313	0.1296	1.31	OPM19-720	0.3970	0.3970	0.00
OPH04-500	1.0000	0.8259	10.36**	OPM19-800	0.5233	0.8259	8.01**
OPH04-610	0.2167	0.5067	7.13**	OPM19-1020	0.4359	0.6108	2.32
OPH04-710	0.5233	0.4778	0.16	OPM19-1100	0.3092	0.6180	7.04**
OPH04-880	0.3970	0.7538	10.03**	OPM19-1400	0.5736	0.6985	1.27
OPH04-920	0.7389	0.5394	3.30	OPM19-1800	0.0585	0.0789	0.12
OPH04-1100	0.6629	0.5376	0.64	OPM19-2300	0.1743	0.1296	0.29
OPH04-1510	0.0955	0.2412	2.99	OPM20-350	0.1210	0.2412	1.89
OPH04-2500	0.1882	0.1931	0.48	OPM20-500	0.4778	0.5394	0.29
OPJ13-1030	0.4161	0.1835	4.91*	OPM20-600	0.5477	0.3487	3.04
OPJ13-1400	0.7389	0.4226	7.94**	OPM20-700	0.2023	0.1296	0.71
OPJ13-2100	0.2462	0.2823	0.13	OPM20-900	0.2313	0.1652	0.52
OPJ13-2820	0.2929	0.3487	0.27	OPM20-1050	0.3092	0.3970	0.64
Sample size (no. of plants)					33	44	
Polymorphic markers					82	84	

*, **G^2 significant at $P = 0.05$ and $P = 0.01$, respectively.

ment killed 37% of all plants and reduced the number of tillers of the survivors from an average of 347 to 25 tillers plant^{-1}. Furthermore, all plant mortality occurred by the second year, which indicates that surviving plants had become adapted, permitting them to persist under the defoliation treatment. Thus plasticity was at least partly responsible for plant survival and supports the conclusions by Trlica and Orodho (1989) and Tomás et al. (2000) on the morphological changes in Indian ricegrass [*Oryzopsis hymenoides* [(Roem & Schult.) Ricker] and Flechilla negra [*Piptochaetium napostaense* (Speg.) Hack], respectively. We concluded that genotypes of mountain rough fescue plants exhibit a variable sensitivity to grazing, but that additional extraneous factors may be required to kill the most sensitive plants.

The second possibility for a weak genetic expression is a strong gene flow ($Nm = 10.94$) that would swamp the effects of selection pressure, similar to the observation of Matlaga and Karoly (2004) for Idaho fescue (*Festuca idahoensis* Elmer). For example, seed or pollen from adjacent areas might be introduced, which could balance the genetic losses. Small numbers of mountain rough fescue seed were found in the seed pool during an extensive survey of the site of the present study (Willms and Quinton 1995). This was unexpected because grazing had reduced the ground cover of mountain rough fescue to less than 3% by 1956 (Willms et al. 1985) and seed production appeared restricted to a few plants protected by shrubs confined to the east end of the site. Furthermore, seed immigration would require a strong

south to north wind, while the prevailing winds are from west to east. In any case, recruitment of new plants was likely not an important factor because the site had been subjected annually to heavy grazing pressure that would have compromised the establishment of seedlings.

In a preliminary study on the effects of long-term grazing on mountain rough fescue, May et al. (2003) reported that grazed mountain rough fescue plants produced a greater number of tillers and taller flowering tillers, and were more winter hardy than ungrazed plants when grown in a common garden. However, plants in that study were selected late in spring and were identified by their inflorescences (found near shrubs). Therefore, the response of those plants may not have been due to grazing pressure but to other unknown factors, perhaps one or more environmental variables of the plant niche, that were expressed by flowering.

With over 30% of genetic diversity (H_o), the mountain rough fescue populations contain a large amount of genetic potential to respond to selection pressure produced by grazing. The relatively small effect that grazing may have on genetic diversity and the apparent lack of genetic drift caused by grazing indicates that any loss of genetic diversity would recover if grazing pressure were relieved since gene flow was occurring. Therefore, despite the highly sensitive response of mountain rough fescue to summer grazing, the inherent genetic diversity of the survivors suggests that the greatest effect of grazing pressure is on the loss of productivity. The studies of May et al. (2003) and the present study indicate that further research is required to elucidate the nature of genetic diversity between the subpopulations and their morphological and performance-based characteristics. Validation of our conclusions is also necessary to develop confidence that plant breeding for grazing-resistant mountain rough fescue is futile. The possibility exists that a gene or genes linked with grazing sensitivity was missed due to the random nature of RAPD. It would also be useful to confirm the observation of high gene flow and identify its cause. The Fescue Grassland is an important resource that is threatened by grazing and cultivation and a better understanding of its character is needed to preserve its integrity.

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