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# Genetic variability of *Bouteloua gracilis* populations differing in forage production at the southernmost part of the North American Graminetum

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**Abstract**

*Bouteloua gracilis* (blue grama grass) native populations have been shown to be highly variable, however the genetic basis of this variability has not been well established. Determining the extent of genetic variability within and among plant populations have important repercussions for the management and conservation of species, and in particular for those subjected to intensive use such as forage plants. Using RAPD, this study was undertaken to investigate the genetic variability of four *B. gracilis* native populations developed in three grasslands and one shrubland at the southernmost part of the North American Graminetum in México. Significant differences in grass aboveground production were found among the study sites, while considerable genetic variation within each of the four blue grama populations evaluated was detected. The molecular analysis, based on 55 individuals, revealed a total of 108 scorable repeatable bands, with 99 of them being polymorphic (overall polymorphism= 91.7%). Within every population each individual was genetically distinct and no population-specific bands (fixed marker differences) were identified. Pair-wise $\Phi_{ST}$ comparisons indicated that the four blue grama populations examined were significantly different in their genetic constitution ($P < 0.001$). AMOVA revealed that most of the genetic variation detected in *Bouteloua gracilis* was explained by intra- (88.53%), rather than by inter-population (11.47%) differences. UPGMA based on the $\Phi_{ST}$ values indicated that the blue grama population collected from the shrubland displayed the RAPD profiles that most differed among the study sites. Possible causes of these results could reside on intensive grazing reducing, and proper management conserving, the forage production and genetic diversity of blue grama native populations. Our results are consistent with previous studies analyzing population genetic variation in outcrossing grasses and, in particular, with ecological and cytological evidence for a high genetic variability in native populations of *B. gracilis*. The implications of our findings and prospective studies to be undertaken using molecular tools in the study of blue grama biology and ecology are discussed.

## Introduction

Native grassland communities located at the southernmost part of the North American Graminetum are dominated by *B. gracilis* and *B. scorpioides* (Aguado-Santacruz 1987). Historical use, grazing intensity, soil factors and climate (Aguado-Santacruz and García-Moya 1998; Aguado-Santacruz et al. 2002) are all factors determining vegetation change at this region with water and nitrogen as the main limiting factors of the shortgrass prairie productivity (Detling 1979). Although less studied, biotic factors, such as soil microorganisms, are also known to exert determinant effects on natural communities (John and Coleman 1983; Johnson et al. 1991; Newsham and Watkinson 1998).

When native blue grama populations at the southernmost part of the North American Graminetum are subjected to intense grazing or plowing and then permitted to recover, reestablishment of the vegetation to the state *prior* to the disturbance can proceed slowly (if any) or rapidly (Aguado-Santacruz and Fierro 1988; Aguado-Santacruz et al. 1989, Aguado-Santacruz 1994), a finding closely related to the fast- and slow-spread blue grama populations of Samuel (1985) and to the high internal variability in spread rate of blue grama populations reported by Samuel and Hart (1995).

At a local scale, this differential response in blue grama populations to disturbance can be reflecting differences in competitive abilities, phenotypic plasticity, genotypic background and/or soil factors (biotic or abiotic). Definition of which factors are the more outstanding determinants of blue grama grasslands response is, however, difficult because of the multifactorial and complex nature of the interrelationships operating in grazing communities.

As a dominant or co-dominant species over most of the native Mexican semiarid grassland, the role that *B. gracilis* plays in functioning and structure of these communities is fundamental. In this context, the genotypic composition of dominant species is anticipated to exert an important impact in determining vegetation response to environmental factors, biotic or abiotic, because behavior of functionally dominant species are related to processes operating at the ecosystem level (Carney 1989).

Several studies have revealed the non-random distribution of genetic variation in populations and highlighted the importance of increasing our current knowledge on the structure of native plant populations and their promoter agents (Loveless and Hamrick 1984). Kölliker et al. (1998) have stressed the importance of increasing our knowledge about how resource management influences structure and diversity of plant populations, while other authors have emphasized the importance of historical factors when trying to explain current patterns of genetic differentiation in plant populations (Newton et al. 1999).

Although growth of blue grama populations have been shown to be highly variable (Riegel 1940; Mueller 1941; McMillian 1956; Green and Goetz 1973; McGinnies et al. 1988; Samuel and Hart 1995) at local and regional scales, little is known concerning variability at the molecular level.

In this work we have studied the grass aboveground production and, using RAPD, the extent of genetic variation within and between four native populations of blue grama located at the southernmost part of the North American Graminetum, while examining the possible origins of this variability. In the light of these results, we advance new directions to be undertaken in the study of blue grama biology and ecology, which securely will have important repercussions on the management of grasslands at the southernmost part of the North American Graminetum.

## Materials and methods

### *Study area*

This study was carried out at the southernmost part of the North American Graminetum in "Los Llanos de Ojuelos" physiographic subprovince of central México, which includes five states: Zacatecas, San Luis Potosí, Guanajuato, Jalisco and Aguascalientes (Figure 1). Four localities located within this region, 'La Mesa', 'La Presa', 'Vaquerías' and 'Sto. Domingo', were considered in this research. The first three sites are open grasslands, with dominance of *Bouteloua gracilis* at 'La Mesa' and 'La Presa', and *B. scorpioides* at 'Vaquerías' site. Vegetation at 'Sto. Domingo' is represented by an *Acacia schaffneri*-shrubland with *B. scorpioides* dominating the herbaceous stratum. Site elevations range from 2,200 to 2,380 m. The study sites differ in historical and current grazing regimes and soil types (Table 1). 'La Mesa' is the only site, which has been managed properly, at least since 1925, while a relatively higher

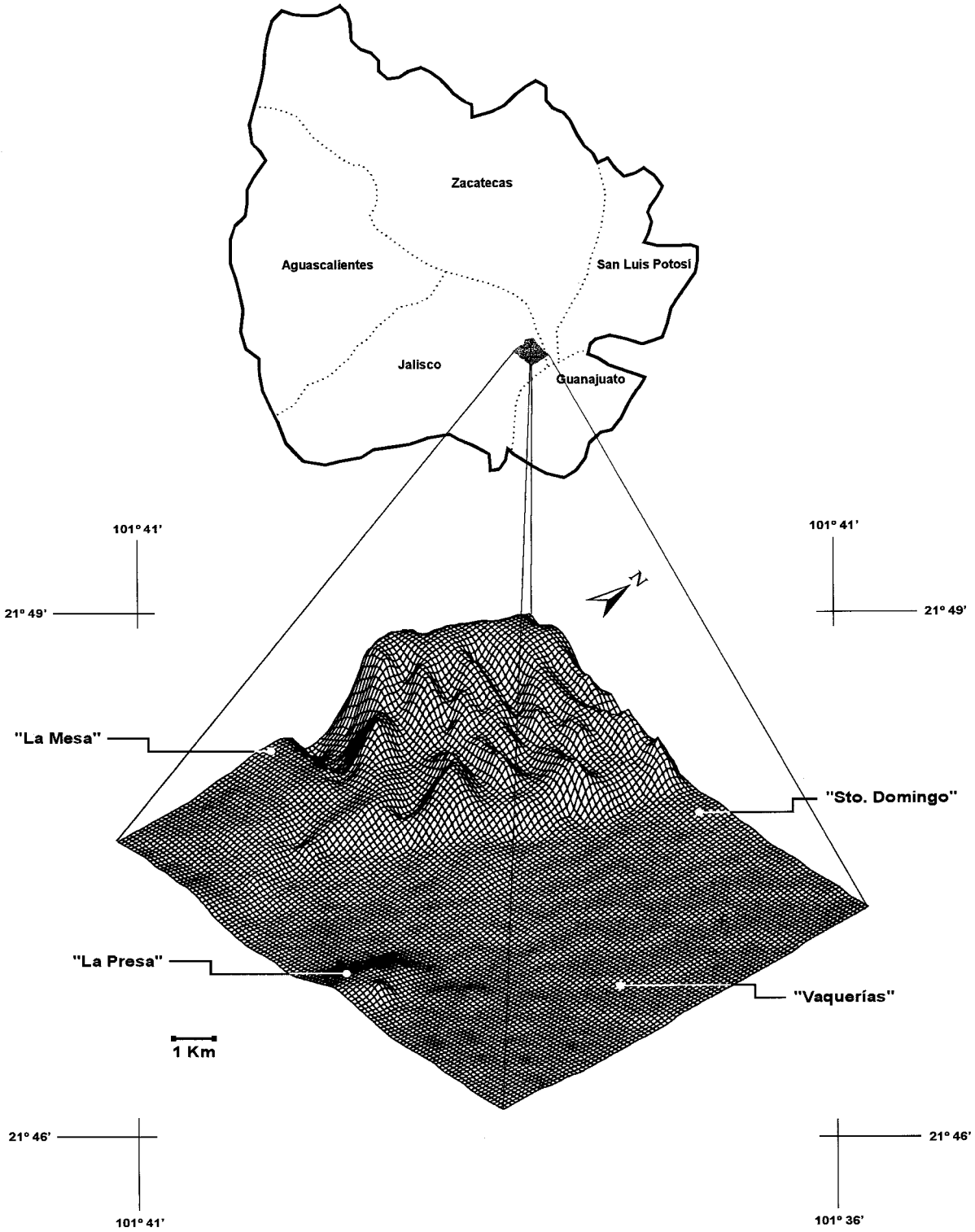


*Figure 1.* Localization of the study sites within "Los Llanos de Ojuelos" physiographic subprovince of central México.

*Table 1.* Characteristics of the study sites.

| | Vaquerías | La Mesa | La Presa | Sto. Domingo |
|---|---|---|---|---|
| Vegetation | *Bouteloua scorpioides* grassland | *Bouteloua gracilis* grassland | *Bouteloua gracilis* grassland | *Acacia schaffneri* shrubland |
| Elevation (m) | 2,200 | 2,380 | 2,280 | 2,200 |
| Soil type * | Haplic xerosol | Haplic phaeozem | Haplic xerosol | Haplic xerosol |
| Historical grazing regime | Heavy | Moderate (at least since 1925) | Heavy | Heavy |
| Current level of grazing | Moderate | Moderate | Heavy | Moderate |

*According to FAO/UNESCO classification.

overgrazing has been maintained at 'La Presa' site for a long time (Aguado-Santacruz et al. 2002).

*Field data*

Grass cover and aboveground production were determined in 1988 (annual precipitation= 444.1 mm, annual maximum temperature=22.7, annual minimum temperature=5.7) at the end of the rainy (growing) season at 'Vaquerías', 'La Mesa' and 'La Presa' sites. The grass cover was recorded on twelve twenty-meter-long permanent lines, established at each site, using the line intercept method (Canfield 1941). This method is based on the measurements of all plants intercepted by the vertical plane of a transect line placed at or near ground level. Measurements of grasses were taken on the line at the ground surface (basal diameter).

Grass aboveground production, both on individual and area basis, was determined within one-hectare exclosures established in 1979 or 1980 at each study site. Previously, the foliage accumulated in the quadrats since livestock removal was clipped and discarded one year before sampling. For determination of the grass biomass on an area basis, twenty-five 0.5 $m^2$ quadrats per site were sampled, separating the material corresponding to each species. The grass aboveground production per plant was estimated by randomly collecting the aerial biomass of 25 adult individuals showing well-developed inflorescences for each species. An individual grass plant was defined as all tillers connected by a single crown and clearly separated from its neighbors. The plant material was dried in a 70 °C oven for 72 h and the dry biomass recorded.

*Collection and preparation of plant material for molecular analysis*

Vegetative material was randomly collected from 'Vaquerías', 'La Mesa', 'La Presa' and 'Sto. Domingo' sites. Although forage production information was not originally collected for 'Sto. Domingo', we decided to included this site in the molecular analyses to compare blue grama genotypes being developed at open grassland to an ecologically contrasting community (shrubland). At each site, tillers were removed from fifteen independent plants (separated one another by at least 7 m), transferred to pots containing peat moss and grown in a greenhouse for propagation.

For molecular analyses, leaves from plants grown in the greenhouse were collected for DNA extraction according to a modification of the method developed by Lee and Taylor (1990). Because erratic amplification was noticed using the templates directly from this DNA extraction procedure, RNAse and a second cleaning step with chloroform:isoamyl alcohol 24:1 (v/v) were utilized in order to obtain a better quality DNA. Briefly, 1 μl RNAase (10 mg/ml) was added to 100 μl of water-resuspended DNA and incubated for 1 h at 37 °C. An equal volume of chloroform:isoamyl alcohol 24:1 (v/v) was added, the mixture gently shaked and centrifuged at 10,000 rpm, 10 min. The superior aqueous phase was recovered, precipitated with 2/3 vol. isopropanol at − 20ºC and then centrifuged at 11,000 rpm 10 min at 4 ºC. The resultant pellet was washed with 200 μl 70% ethanol and then dried at ambient temperature. The DNA was finally resuspended in 50 μl TE buffer (0.01 M Tris-HCl, 0.01 M EDTA, pH 8) and the concentration spectroscopically determined. This addition to the original DNA extraction procedure resulted in consistently repeatable amplifications.

*RAPD analysis*

We tested 15 out of 20 primers from the OPC series of Operon Technologies, Inc. (Alameda, Cal.); OPC-1, OPC-7, OPC-8, OPC-9, OPC-13 primers were not evaluated. PCR reactions (total volume= 12.5 μl) were carried out in a Perkin Elmer DNA thermal cycler using 0.5 ml eppendorf tubes which contained: 10 to 20 ng of DNA, 200 μM of each dNTP, 3 mM of $MgCl_2$, 0.4 μM of primer and 0.625 units of Taq DNA polymerase (5 U/μl; Gibco BRL, Mississauga, Canada). The PCR mixtures were covered with 15 μl of sterile mineral oil. The DNA amplifications were performed under the following conditions: a denaturation cycle (94 °C for 5 min) was followed by 40 cycles of amplification (94 °C for 1 min, 36 °C for 1 min, 72 °C for 2 min) and the final extension step (72 °C for 5 min). All PCR amplification products were separated by electrophoresis in 1.4% (w/v) agarose gels in 1X Tris–acetate. Gels were stained with ethidium bromide and photographed on a digital documentation system. RAPD profiles were replicated by at least two PCR amplifications for each individual plant and only clearly reproducible bands were scored for statistical analysis.

*Statistical analysis*

A completely randomized experimental design was used for analyzing differences, on a global level and by single species, in plant cover (%), forage production per unit area (kg dry wt·ha$^{-1}$) and by individual plant (g dry wt·plant$^{-1}$) among sites. Measures of population genetic diversity were calculated according to Nei's gene diversity (Nei 1973) and Shannon´s Information Index (Shannon and Weaver 1949) using the POPGENE program v 1.31 (Yeh et al. 1999) and differences among sites were also evaluated under a one-way analysis of variance with loci as replicates. Significant differences were accepted at the 0.05 level of probability. When significant *F*-values were found, Duncan's multiple range test was utilized to separate significant ($P < 0.05$) mean differences (Steel and Torrie 1960).

For molecular analysis, only markers that were reproducible and could be scored unequivocally in all genotypes were included for study. Polymorphic RAPD markers were transformed into an absence-presence binary matrix, which was utilized for partitioning the total genetic variation to between- and within-population variations using AMOVA (Excoffier et al. 1992) to analyze a distance matrix calculated according to Nei and Lee (1979) through AMOVA-PREP program (Miller 2000) and considering 1000 bootstrap replicates for determination of the significance level of $\Phi_{ST}$. A graphic representation of the similarity among sites by unweighted pair-group method of arithmetic averages (UPGMA) cluster analysis on the pair-wise $\Phi_{ST}$ values was elaborated.

*Table 2.* Grass cover (%) at three grassland sites from the southernmost part of the North American Graminetum.

| | Sites | | |
|---|---|---|---|
| Species | La Mesa | Vaquerías | La Presa |
| *Aristida divaricata* | | 0.59 a[1/] | 1.57 b |
| *Bothriochloa barbinodis* | | 0.38 | |
| *Bouteloua gracilis* | 4.97 a | 0.49 b | 7.37 c |
| *B. hirsuta* | | 2.44 | |
| *B. scorpioides* | 0.92 a | 2.55 b | 3.60 c |
| *Lycurus phleoides* | 0.23 a | 0.30 a | 1.36 b |
| *Microchloa kunthii* | 1.02 a | 0.33 b | 1.34 a |
| *Muhlenbergia rigida* | 2.51 a | 0.13 b | |
| *Urochloa meziana* | | | 0.08 |
| *Sporobolus pyramidatus* | 3.20 | | |
| Other grasses | 0.04 | 0.13 | 0.03 |
| Total | 12.89 a | 7.34 b | 15.35 c |

[1/]Means followed by different letters are significantly different using Duncan's multiple range test ($P < 0.05$).

## Results

*Grass aboveground production*

Previously collected information about grass aboveground production at the study area showed great differences in the quantified ecological variables among sites. Floristic composition, as revealed by plant cover, was significantly different among sites ($P < 0.05$) both on a global basis as for most of the grass species (Table 2). 'Vaquerías' site showed the lowest total cover (7.34%; $P < 0.05$) but also supported the higher number of grass species. *Bouteloua gracilis* cover was lower at this site and higher at 'La Presa' site ($P < 0.05$). *B. scorpioides*, a widely spread grass over the southernmost part of the North American Graminetum (Aguado-Santacruz 1987), was the dominant species at 'Vaquerías' site, while at 'La Mesa' and 'La Presa' sites, *B. gracilis* displayed the highest cover among all grass species evaluated

*Table 3.* Aboveground production for dominant grasses at three grassland sites from the southernmost part of the North American Gramineetum.

| *Species* | Sites | | | | | |
|---|---|---|---|---|---|---|
| | La Mesa | | Vaquerías | | La Presa | |
| | Ha | Plant | Ha | Plant | Ha | Plant |
| *Aristida divaricata* | | | 67.9 a[1/] | 2.49 a | 213.6 b | 2.98 a |
| *Bothriochloa barbinodis* | | | 69.2 | 3.31 | | |
| *Bouteloua gracilis* | 465.3 a | 7.74 a | 4.5 b | 3.30 b | 267.8 c | 4.00 b |
| *B. hirsute* | | | 292.0 | 3.27 | | |
| *B. scorpioides* | 69.4 a | 4.34 a | 282.2 b | 2.80 b | 345.0 c | 3.13 b |
| *Lycurus phleoides* | 5.4 a | 3.61 a | 60.2 b | 4.45 b | 301.8 c | 5.68 c |
| *Microchloa kunthii* | 72.7 a | 0.56 ab | 8.1 b | 0.25 b | 159.0 c | 0.76 a |
| *Muhlenbergia rigida* | 145.8 a | 27.48 a | 146.0 a | 62.17 b | | |
| *Sporobolus pyramidatus* | 184.6 | 0.79 | | | | |
| *Urochloa meziana* | | | | | | 1.48 |
| Other grasses | 0.8 | | 16.8 | | 17.2 | |
| Total | 944.0 a | | 946.9 a | | 1304.4 b | |

[1/]Means followed by different letters are significantly different for either variable, Ha (kg dry wt·ha$^{-1}$) or Plant (g dry wt·plant$^{-1}$), among sites using Duncan's multiple range test ($P < 0.05$).

(Table 2). Some other grass species were site-specific such as *Sporobolus pyramidatus* at 'La Mesa' site and *Urochloa meziana* at 'La Presa' site.

Total grass aboveground production per unit area was similar between 'La Mesa' and 'Vaquerías' sites, but significantly higher ($P < 0.05$) at 'La Presa' site (Table 3). The highest values for aboveground production of *B. gracilis* in terms of area as well as on an individual basis were found at 'La Mesa' site (Table 3; $P < 0.05$).

### *Molecular analysis*

After the initial acclimation period in the greenhouse, five plants died and, therefore, the final analysis was carried out with 55 plants distributed as follow: 'Vaquerías'= 13, 'La Presa'=15, 'La Mesa'= 13, and 'Sto. Domingo'=14.

RAPD analysis based on these 55 individuals, revealed a total of 108 scorable repeatable bands, with 99 of them being polymorphic (overall polymorphism= 91.7%; Table 4); nine invariant markers, shared by all of the four populations evaluated were excluded from further analysis. Only 10 primers produced polymorphic bands across all populations, namely, OPC-2, OPC-3, OPC-6, OPC-10, OPC-12, OPC-14, OPC-15, OPC-16, OPC-18 and OPC-20 (Table 4). Primers OPC-4, OPC-5 and OPC-19 were not polymorphic, while OPC-11 and OPC-17 showed no or little amplification. Within every population each individual was genetically distinct and no population-specific bands (fixed marker differences) were detected. Representative RAPD profiles for 52 individuals using primers OPC-14 and OPC-18 are shown in Figure 2.

AMOVA revealed that most of the genetic variation detected in *Bouteloua gracilis* was apportioned within (88.53%), rather than among (11.47%), populations (Table 5). Pair-wise comparisons of $\Phi_{ST}$ values (Table 6) were all significant ($P < 0.001$) demonstrating that the four blue grama populations evaluated differed statistically from each other in their genetic constitution.

AMOVA sum of squares calculated for the different blue grama populations revealed that the highest RAPD variability (Huff 1997) was present at 'La Mesa' (a site with a long history of moderate grazing), while the lowest one occurred at 'La Presa' (Table 7), a site characterized by prolonged intensive grazing. Shannon's and Nei's diversity indexes partially confirmed these results (Table 7); 'La Presa' site tended to be the least diverse population, showing significant differences to 'Vaquerías' and 'La Mesa' sites, but not to 'Sto. Domingo' site.

An graphic representation generated by UPGMA on the pair-wise $\Phi_{ST}$ values is shown in Figure 3. The highest genetic similarity was observed between 'Vaquerías' and 'La Presa', while 'Sto. Domingo' popu-

*Table 4.* Primers and their scorable amplified fragments for populations of *Bouteloua gracilis* from the southernmost part of the North American Graminetum.

| Primer | | *Number of scorable fragments** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Vaquerías | | La Presa | | La Mesa | | Sto. Domingo | | Global | |
| Code | Sequence (5´→3´) | Mono | Poly | Mono | Poly | Mono | Poly | Mono | Poly | Mono | Poly |
| OPC-2 | GTGAGGCGTC | 4 | 7 | 3 | 8 | 3 | 8 | 3 | 8 | 3 | 8 |
| OPC-3 | GGGGGTCTTT | 0 | 10 | 4 | 6 | 2 | 8 | 2 | 8 | 0 | 10 |
| OPC-6 | GAACGGACTC | 1 | 7 | 3 | 5 | 2 | 6 | 3 | 5 | 0 | 8 |
| OPC-10 | TGTCTGGGTG | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 | 1 | 7 |
| OPC-12 | TGTCATCCCC | 1 | 16 | 4 | 13 | 6 | 11 | 5 | 12 | 0 | 17 |
| OPC-14 | TGCGTGCTTG | 4 | 4 | 3 | 5 | 3 | 5 | 4 | 4 | 3 | 5 |
| OPC-15 | GACGGATCAG | 2 | 10 | 2 | 10 | 1 | 11 | 7 | 5 | 0 | 12 |
| OPC-16 | CACACTCCAG | 1 | 12 | 3 | 10 | 0 | 13 | 5 | 8 | 0 | 13 |
| OPC-18 | TGAGTGGGTG | 4 | 9 | 8 | 5 | 4 | 9 | 4 | 9 | 2 | 11 |
| OPC-20 | ACTTCGCCAC | 1 | 7 | 0 | 8 | 1 | 7 | 1 | 7 | 0 | 8 |
| *Total* | | 19 | 89 | 31 | 77 | 23 | 85 | 35 | 73 | 9 | 99 |

*Mono, monomorphic; Poly, polymorphic.

lation revealed the RAPD profiles that most differed among all of the evaluated sites. 'La Mesa' population showed more affinities with 'La Presa' and 'Vaquerías than with 'Sto. Domingo' population.

An alternative UPGMA dendrogram based on Nei's genetic distance (Nei 1972; not shown) yielded a similar graphic representation of the blue grama populations relationships, but due to a tighter relation between 'La Presa' and 'La Mesa' was found with this measure the position of 'La Presa' and 'Vaquerías' sites within the first cluster inverted. These results are in concordance with Kölliker et al. (1998) who found a significantly high correlation (r= 0.87) between interpopulation genetic distance, expressed as $\Phi_{ST}$, and Nei's genetic distance.

## Discussion

Significant differences in ecological variables among the four blue grama populations evaluated were evident. At 'Vaquerías', blue grama contribution to overall grass biomass was only 0.47%, while at 'La Mesa' and 'La Presa' sites it represented 49.3% and 20.5%, respectively. Although these variables are strongly affected by environmental factors (Aguado-Santacruz and García-Moya 1998; Aguado-Santacruz et al. 2002) the differences in productivity and floristic composition among these sites have been recognized since a long time (Aguado-Santacruz 1987), and they could arise from historical land use, competitive interactions, soil factors (biotic and abiotic) and possibly from population genetic composition.

Wide genetic variation was registered in blue grama populations. Each of the 55 blue grama individuals analyzed possessed a unique combination of the 99 RAPD markers and therefore each individual was considered genetically distinctive. The uniqueness of the marker profiles revealed a high degree of genetic variability within the evaluated populations. These results are entirely in keeping with what we might expect from an outcrossing species. *B. gracilis* is a highly cross-fertilized (Snyder and Harlan 1953) but facultative apomictic grass (Gustafsson 1946), that can spread vegetatively by tillers, short root stalks (Pool 1948; Allred 1950), and under special conditions through stolons (Stubbendieck et al. 1973). Thus, our results reflect the predominance on a spatial scale of the outcrossing mating system over the alternative ways of reproduction displayed by this species in nature.

When compared to levels reported in previous RAPD studies with outcrossing grasses, the global polymorphism detected in our blue grama populations (91.7%) results comparable to those of *Cymbopogon* spp (57-89.1%; Sangwan et al. 2001) and *Cynodon* spp (97%; Ho et al. 1997), but higher to those detected in *Chloris gayana* (60%, Pérez et al. 1999), *Festuca pratensis* (68.3%; Kölliker et al. 1998) and *Vetiveria zizanioides* (70.8%; Kresovich et al. 1994). This polymorphism in blue grama seemed higher to those of *Cymbopogon* spp and *Cynodon* spp because of the heterogeneous nature of the material utilized in these two latter RAPD studies.

An increasing number of RAPD studies (Hogbin and Peakall 1999; Nybom and Bartish 2000) are

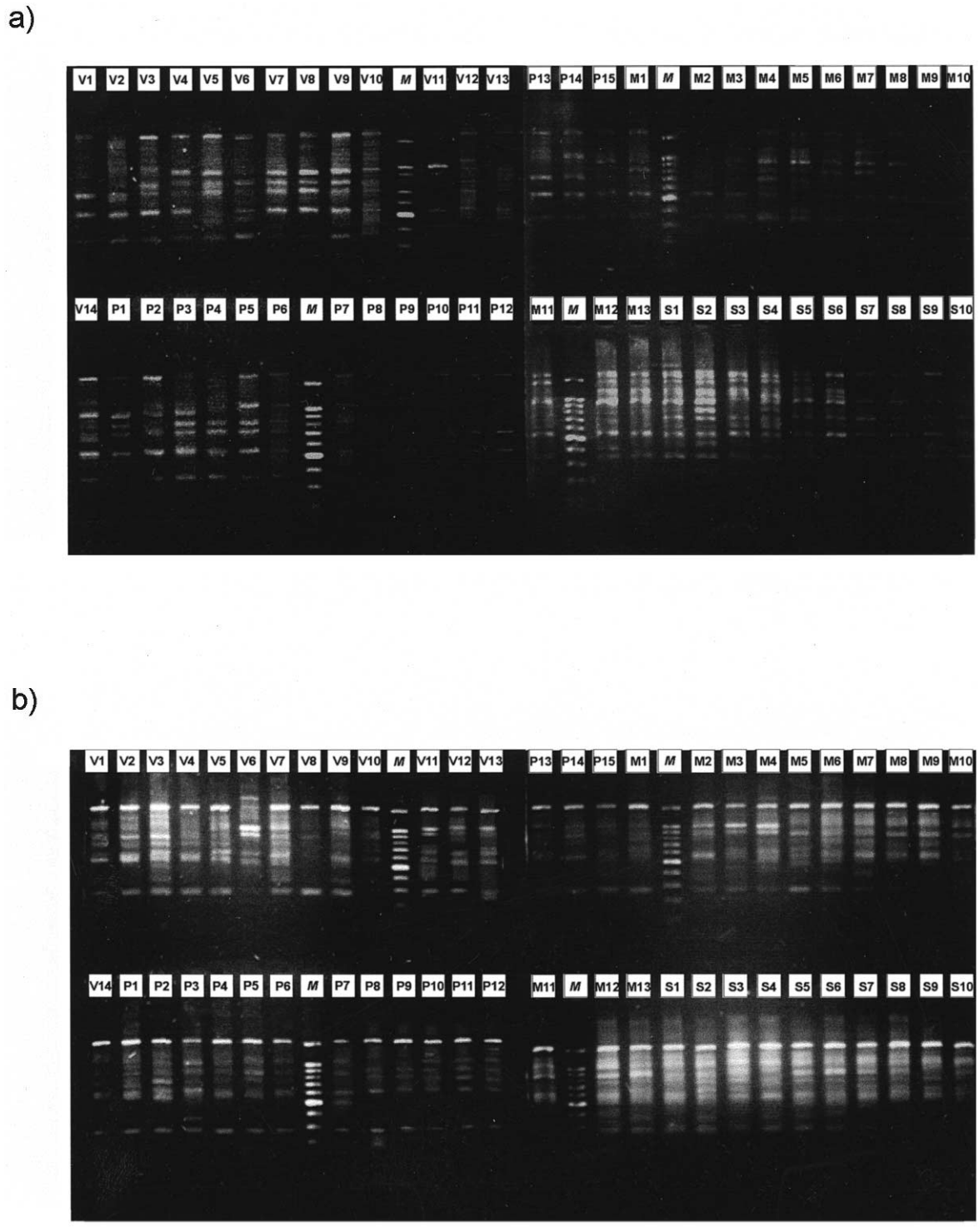


*Figure 2.* RAPD profiles for 'Vaquerías' (V; 14 individuals), 'La Presa' (P; 15 individuals), 'La Mesa' (M; 13 individuals) and 'Sto. Domingo' (S; 10 individuals) populations of *Bouteloua gracilis* using primers OPC-14, (a), and OPC-18, (b). *M*.- 100 bp molecular marker.

*Table 5.* Analysis of molecular variance (AMOVA) for native populations of *Bouteloua gracilis* located at the Southernmost part of the North American Graminetum using 99 RAPD markers derived from 55 individuals.

| | d.f. | Sum of squares | Mean squares | Variance | % of total variance | P |
|---|---|---|---|---|---|---|
| Among populations | 3 | 121.371 | 40.457 | 1.885 | 11.47 | $< 0.001$ |
| Within populations | 51 | 742.592 | 14.561 | 14.560 | 88.53 | |
| Total | 54 | 863.963 | | | | |

*Table 6.* Pair-wise $\Phi_{ST}$ values calculated by AMOVA for native populations of *Bouteloua gracilis.* All $\Phi_{ST}$ values computed were significantly larger than a random $\Phi_{ST}$ value ($P < 0.001$).

| | Vaquerías | La Presa | La Mesa | Sto. Domingo |
|---|---|---|---|---|
| Vaquerías | – | | | |
| La Presa | 0.0649 | – | | |
| La Mesa | 0.0680 | 0.0715 | – | |
| Sto. Domingo | 0.1442 | 0.2153 | 0.0981 | – |

coming to confirm previous results with enzyme markers, concerning lower intrapopulation genetic variation in self-pollinating than in cross-pollinating species (Hamrick and Godt 1989; Hamrick 1990; Moran 1992). Similarly, in *B. gracilis* we found that interpopulation genetic variation (11.47%) was lower than intrapopulation variation (88.53%). The percentage of genetic variation accounted by intrapopulation differences in *B. gracilis* is comparable or slightly lower to those reported earlier for outcrossing grasses: *Schizachyrium scoparium* (95%; Huff et al. 1998), *Buchloë dactyloides* (72.9-80.5%; Huff et al. 1993), *Bromus inermis*/*Bromus riparius* (65.8-82.3%; Ferdinandez et al. 2001). Conversely, in *Hordeum spontaneum* (a predominantly inbred species) the genetic variation explained by intrapopulation differences was only 57% (Dawson et al. 1993).

Because recruitment of *B. gracilis* seedlings occurs infrequently in the shortgrass, and at a lesser extent in the southern mixed prairie, due to seedling morphological constraints (Hyder et al. 1971; Wilson and Briske 1979), lack of a persistent seed bank (Coffin and Lauenroth 1989; Molina 1990), intraspecific competition by adult neighbors (Aguilera and Lauenroth 1993) and high spatial and temporal variability in seed production (McGinnies et al. 1988; Coffin and Lauenroth 1992), an *a priori* assumption on the genetic structure of native blue grama populations is that clonal spread will prevail over seedling recruitment (Fair et al. 1999). From the uniqueness in RAPD profiles found in this study for all *B. gracilis* individuals analyzed and the high internal variation, we can infer that clonal fragmentation is not important at the distance between individuals considered in the plant sampling of this work (at least 7m) and highlights the importance of sexual mechanisms in *B. gracilis* population structure and persistence. Accordingly, Fair et al. (1999) reported that sampling of *B. gracilis* individuals in the field is likely to represent a variable gene pool rather than clonal fragments.

Once a blue grama genet is completely established, it will retain community resources for an extended time, because of the long lifetime of this grass ($>$ 38-400 years; Coffin and Lauenroth 1990; Fair et al. 1999). This great persistence, together with the fact that genets will not spread beyond certain spatial limits, certainly would secure to *B. gracilis* populations to retain a great genetic variability. Simulation models have shown that low but repeated seedling establishment can be sufficient to prevent significant loss of genotypic diversity in clonal plant populations (Watkinson and Powell 1993). Unfortunately, little is known about the dynamics of seedling recruitment and genetic flow between the populations of *B. gracilis,* as well as about the competition intensity at the study area.

Our data are entirely in concordance with previous work based on direct or indirect evidence concerning a great genetic variability in native blue grama populations (Snyder and Harlan 1953; McGinnies et al. 1988; Fair et al. 1999). From our own observations and from those of other researchers, *B. gracilis* is considered a very plastic grass that shows, for example, multiple mechanisms of propagation, sexual and asexual. This could not be surprising because its genome has been molded under thousands of years of very stringent, but at the same time extremely stochastic, selection forces such as severe grazing, fires, droughts, frosts and other environmental factors characterizing the North American grassland. In this context, the retention of high genetic variability in blue grama populations to face this unpredictable environment is vital for the species surviving.

Besides mating system, gene flow (seed and pollen) and selection pressure are also important deter-

*Table 7.* Genetic variability in native populations of *Bouteloua gracilis.*

| Variable | Vaquerías | La Presa | La Mesa | Sto. Domingo |
|---|---|---|---|---|
| Sum of squares (AMOVA) | 177.2308 | 176.9333 | 198.0000 | 190.4286 |
| Nei´s gene diversity | 0.2821 a[1/] | 0.2257 b | 0.2871 a | 0.2616 ab |
| Shannon´s information index | 0.4278 a | 0.3469 b | 0.4322 a | 0.3945 ab |

[1/]Means followed by different letters are significantly different for Shannon's or Nei's diversity indexes among sites using Duncan's multiple range test ($P < 0.05$).

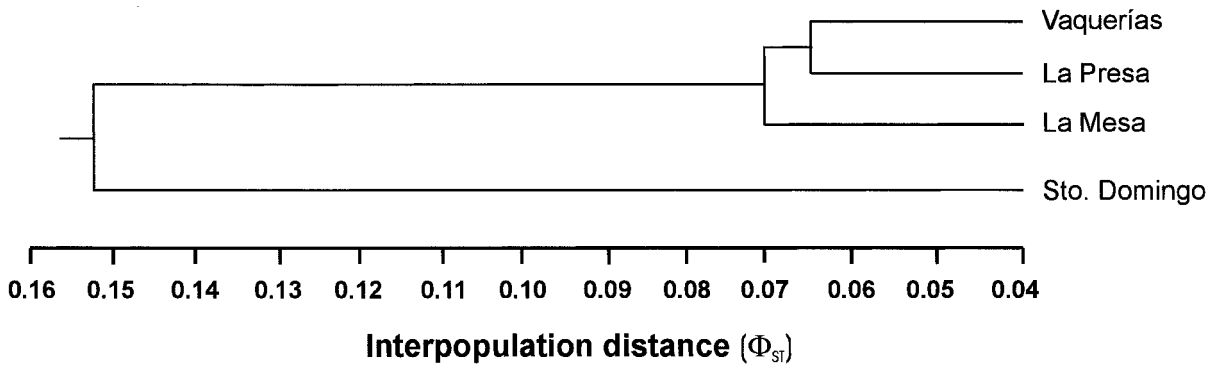


*Figure 3.* Dendrogram analysis of interpopulation distance ($\Phi_{ST}$) among the four populations of blue grama.

minants of genetic structure in native plant populations. From the distances prevailing among our study sites (Figure 1) and the relative positions of the sites within the dendrogram, we could advance the existence of higher gene flow between 'La Presa' and 'Vaquerías' sites, while the elevation of 'La Mesa' site could be an obstacle for major gene flow to this site.

On the other hand, known selection pressures at the study area might also contribute to these population differentiation patterns. Historical heavy grazing has been maintained in 'Vaquerías' and 'La Presa' sites (Aguado-Santacruz and García-Moya 1998), but intensity has been much more severe at 'La Presa' site, where forage removal by livestock can reach up to 95% (Aguado-Santacruz and Fierro 1998). Thus, it would not be surprising that a population with a long history of proper management (at least 76 years; Aguado-Santacruz 1987) and supporting the most productive and conserved blue grama population ('La Mesa' site) among all localities evaluated, showed the highest genetic diversity, while a population subjected to intensive and prolonged overgrazing displayed the lowest genetic diversity ('La Presa' site). Intensive grazing favors the persistence of grazing-tolerant genotypes while decreasing drastically the seed production of plants with the consequent reduction in recruitment. On the long term, overgrazing will limit, thus, the replenishment of the population genetic pool and the genetic diversity will be seriously affected. On a community basis, this has been the case at the southernmost part of the North American Graminetum. Prolonged overgrazing has reduced drastically plant community diversity at 'La Presa' site, as opposed to 'Vaquerías' and 'La Mesa' sites, where moderate grazing has increased this ecological variable (Aguado-Santacruz 1994).

Although stocking rate at 'Sto. Domingo' has been very variable in time, it is known that, on average, this site has been under heavy grazing. However, the separation of the genotypes found at this latter site from the other three blue grama populations might be mainly attributed to a relatively more heterogeneous environment prevailing in the shrubland selecting for different genotypes. When compared to open grasslands, shrublands have been found to be very heterogeneous communities at the southernmost part of the North American Graminetum (Aguado-Santacruz et al. 2002) and, therefore, selective forces in grassland should be expected to be different from those operating in shrubland. Shrubs and trees exert a profound influence on function and structure of plant communities (Aguado-Santacruz et al. 2002). However and opposite to our results, in *Schizachyrium scoparium*, Huff et al. (1998) found greater genetic similarity be-

tween populations of comparable fertility level in different biomes (grassland and forest) than between closely adjacent populations within the same biome, *i.e.*, fertility promoted a greater population genetic differentiation than the biome from which the individuals were collected. It is expected that under severe selection pressure population differentiation can occur relatively fast. According to Snaydon and Davies (1982) population divergence may occur over periods as short as six years, while Kölliker et al. (1998) found that fertilization and frequent defoliation led to a reduction in genetic variability within natural populations of *Festuca pratensis* in a study in which the fertilization and cutting treatments were applied at two different sites for 38 and 11 years. These interpretations should be taken, however, with caution because molecular markers not always show a significant correlation to selective forces (McKay and Latta 2002).

We consider our work as a first molecular approach to the study of genetic variability in *B. gracilis* populations located at the southernmost part of the North American Graminetum and its origins. We have tried to explain our results in the light of the available knowledge about the ecology of the sites. Nevertheless, further research specifically focused to directly correlate ecological information to molecular data should be carried out. In this context, the relation of adaptive traits to molecular markers is under keen debate (McKay and Latta 2002; Crnokrak and Merilä 2002; Hendry 2002) but *ad hoc* methodologies developed for correlating molecular markers to ecologically important information (Ritland, 2000) are expected to contribute to our current understanding of the evolutionary forces shaping F*st* and Q*st*.

Additionally, physiological and ecological work should be accomplished in order to evaluate the suitability of the 'La Mesa' genetic pool as a germplasm source to be used in breeding programs of *B. gracilis*. Correlation of physiological studies with genetic information will give insights about the relative contribution of the genetic vector to the final resultant measured in terms of productivity. The material of blue grama genetically characterized in this study is expected to support future programs for development of genotypes with improved agronomic traits. Ongoing physiological work conducted under greenhouse conditions is coming to confirm the ecological data presented here concerning more productive genotypes being developed at 'La Mesa' site (not published).

The integration of ecological and the steadily increasing molecular genetic approaches to study gene flow in population analysis is expected to contribute very much to our current understating of plant population differentiation processes in nature (Ouborg et al. 1999). Studies of reproductive biology in *B. gracilis,* complementary to molecular analysis, should be also necessary in order to increase our predictive capacity on successional trends in semiarid grasslands, because, for example, catastrophic events, which are common in these environments, are expected to exert a greater impact on genetically homogeneous populations of blue grama (Harper 1977). In this context, it is crucial to determine the genetic structure of native populations by determining, for instance, the actual extent of apomictic processes occurring in native populations of *B. gracilis* (Gustafsson 1946), a subject currently being addressed in a related species, *B. curtipendula* (Mosher and Smith 1997).

Molecular tools are expected to shed new light on central ecological issues in *Bouteloua gracilis* population biology such as the demography of genets and ramets (Fair et al. 1999) with important repercussions on our actual knowledge of the structure and dynamics of native grassland communities.

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