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Lack of intraspecific variation in resistance to defoliation in a grass that evolved under light grazing pressure

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Abstract

Grass species frequently show marked intraspecific variation in morphology and tolerance to defoliation. Remarkably, most of this evidence comes from grasslands with long evolutionary history of grazing. Here, we explore the intraspecific variation in grazing tolerance and morphometric traits associated with grazing avoidance of *Paspalum dilatatum* (Poir.), a grass from the Flooding Pampa (Argentina), where grazing is a novel disturbance in evolutionary time. We performed a clipping experiment in a greenhouse with two populations from sites with contrasting short term grazing regime: continuous grazing and 20 year-old grazing exclosure. The populations did not differ in their tolerance to clipping, and showed minor differences in the way clipping affected plant height, a trait associated with grazing avoidance. Our results indicate that there are exceptions to the generalized findings of high levels of intraspecific variation in grazing resistance among populations of grasses. These exceptions may be associated to evolutionary history of grazing.

Introduction

The end of the Pleistocene (about 10000 yr ago) was marked by extinctions of megaherbivores in grassland ecosystems. These extinctions were greater in South America than in Africa and in many parts of North America (Ojasti 1983; Markgraf 1985; Owen-Smith 1987, 1989). Consequently, grasslands from these regions have been subjected to different grazing intensities during evolutionary time and have been classified as having long or short evolutionary history of grazing (Milchunas et al. 1988; Milchunas and Lauenroth 1993).

The evolutionary history of grazing -together with climate, fire history, and soil attributes- explains structural and functional differences in the responses to current grazing among grassland ecosystems (Milchunas et al. 1988). Experiments showed that grass species from grasslands with different evolutionary histories of grazing differ in their resistance to grazing, which is determined by their abilities to regrow after defoliation (tolerance) and to escape grazing (avoidance) (Rosenthal and Kotanen 1994). For instance, Simoes and Baruch (1991) showed that grasses from South American savannas, which have been lightly grazed during evolutionary time, were less tolerant than African grasses. A similar result was obtained by Caldwell et al. (1981) who compared two species of *Agropyron* Gaertn. with contrasting evolutionary histories of grazing. In addition, Wilsey et al. (1997) found that grasses from the Great Basin of North America and the Flooding Pampa of Argentina, which have evolved under light grazing pressures, were more affected by defoliation in terms of biomass allocation than grasses from long-term heavily grazed Serengeti ecosystems. In contrast, Klink (1994) did not find differential tolerance to defoliation between Brazilian and African grasses.

Divergent evolutionary histories of grazing also may have acted upon the intraspecific variation in resistance to grazing. However, most studies investigating intraspecific variation in grazing resistance of grassland species have focussed on species from ecosystems with a long evolutionary history of grazing. These studies showed conspicuous intraspecific variation in grazing tolerance and avoidance among clones or populations from sites subjected to different grazing regimes within the same landscape for time periods ranging from 10 to 50 to 100 years (Detling and Painter 1983; Carman 1984; McNaughton 1984; Carman and Briske 1985; Jaramillo and Detling 1988; Oesterheld and McNaughton 1988; Polley and Detling 1988, 1990; Painter et al. 1993). In contrast, only two papers analysed intraspecific variation in grazing resistance of species with short evolutionary history of grazing, and they found minor or no variation. One reported ecotypic differences in plant height (a trait related to grazing avoidance) but not in tolerance to defoliation between populations of *Festuca idahoensis* Elmer. from grasslands in Oregon (USA) that evolved under low grazing pressure (Jaindl et al. 1994). The other one found no genetic-based differences in growth and morphology between populations of *Piptochaetium napostaense* (Speg.) Hack from the Southern Pampas of Argentina (Tomás et al. 2000), which have lacked large herbivores for millennia until Europeans introduced domestic livestock in the 16th century (Sala et al. 1986). Clearly, more evidence is needed in order to elucidate if evolutionary history of grazing had a role in determining intraspecific diversity in grass resistance to herbivory.

Here, we determined the intraspecific variation in grazing resistance of *Paspalum dilatatum* (Poir.), an apomictic grass co-dominant in the Flooding Pampa of Argentina. Extrapolating to this grassland what we know about grazing ecotypes from other ecosystems suggests that populations of *P. dilatatum* from sites with different short-term grazing histories will greatly diverge in the components of grazing resistance: tolerance and avoidance (Rosenthal and Kotanen 1994). However, since grazing of the Flooding Pampa by large herbivores is novel in evolutionary time, we considered it particularly interesting to test the generality of this prediction when applied to species from a system with a short evolutionary history of grazing. Thus, we studied the tolerance to clipping, and the morphometric traits associated with grazing avoidance in populations of *P. dilatatum* from sites with contrasting short-term grazing histories.

Materials and methods

We performed a clipping experiment with two populations of *P. dilatatum* from sites with contrasting grazing regimes: a 20-yr grazing exclosure and an area subjected to continuous grazing by domestic cattle for ca. 100 yrs, with a mean herbivore density of about 0.5 animals per hectare. Each collection site encompassed approximately 1 ha of land. Both sites were located on a same stand that belongs to the community B_3, defined by *Piptochaetium montevidense* (Spreng.) Parodi, *Ambrosia tenuifolia* Spreng., *Eclipta bellidiodes* (Spreng.) Sch. Bip. ex S.F. Blake, *Mentha pulegium* L. and *Briza subaristata* Lam. (Burkart et al. 1990). This community is subjected to winter and spring flooding events almost every year (Paruelo and Sala 1990). The collection sites were situated in a livestock ranch in the central Flooding Pampa of Argentina (36 °S 58 °30′W).

At each site, we randomly collected 20 individual plants that were at least 20 m apart. Due to the tussock growth form of the species, we assumed that each plant belonged to a different clone. We cultivated the clones on garden soil in pots and propagated them vegetatively in a greenhouse during seven months, until the start of the experiment. Throughout the propagation process, each original plant was divided until there were approximately 15 individual plants/clone. We consider that any influence of the original environment was eliminated during this process for two reasons. First, seven months of cultivation under greenhouse conditions is sufficient time for a fast-growing herbaceous C_4 plant like *P. dilatatum* to accumulate significant amounts of biomass and to senesce old tissue. These two combined processes greatly diluted the contribution of the original tissue to the final experimental plants. Second, the vegetative propagation determined that most tillers of the final plants were originated under greenhouse conditions (we started the propagation with about five tillers per clone and ended with 75).

The experiment was a 2 × 2 factorial with two levels of *population* (population from the grazing exclosure and population from the continuously grazed area) and two levels of *clipping* (clipped plants and unclipped plants). Each combination of population and clipping treatment had 20 true replicates (clones) for the population from the exclosure, and 19 for the population from the grazed area. Each clone × treatment combination was represented by 2 individual plants, which were considered subsamples (and, thus,

Table 1. ANOVA probability values for the effects of clipping on traits of two populations of *Paspalum dilatatum* from sites with different recent grazing history (error degrees of freedom = 37). Note Bonferroni corrected significance level $\alpha' = 0.05/12 = 0.0042$.

Trait	Population	Clone (within population)	Clipping	Population × Clipping
Proportion of aboveground biomass removed	0.2505	0.57	—	—
Final biomass	0.0001	0.31	0.0001	0.56
Total biomass	0.0001	0.33	0.0005	0.94
Final aboveground biomass	0.0853	0.82	0.0001	0.19
Total aboveground biomass	0.0234	0.45	0.06	0.98
Leaf blade biomass	0.01	0.15	0.0001	0.10
Leaf sheath biomass	0.30	0.83	0.0001	0.30
Crown biomass	0.01	0.03	0.0001	0.20
Root biomass	0.0001	0.10	0.05	0.40
Standing dead	0.01	0.34	0.0001	0.30
Leaf area	0.05	0.51	0.0001	0.40
Blade:sheath	0.01	0.64	0.001	0.8
Plant height	0.0001	0.70	0.001	0.005

averaged for statistical analysis) to avoid pseudo replication (Hurlbert 1984). Summarizing, there were a total of 78 experimental units (20 + 19 clones × 2 clipping treatments) and 156 plants (78 experimental units × 2 subsamples within them). At the start of the experiment, one additional individual plant per clone was harvested to determine the initial biomass of each population.

The experimental plants, composed of approximately 5 tillers, were grown on washed sand in 14 cm tall × 12 cm diameter plastic pots in the same greenhouse where the original plants were grown and propagated. The experiment began 60 days after the last propagation event. At this time, initial biomass was determined. Clipping treatments were assigned at random within clones and clipping × population combinations were segregated in space to avoid aboveground competition among them (they were periodically rotated). The clipping treatment intended to mimic heavy defoliation conditions observed in the field (Sala et al. 1986). It consisted of one defoliation event at the start of the experiment and resulted in the removal of 60% of the aboveground biomass (all blade biomass and part of the sheath biomass) for both populations. The soil of all pots was maintained at field capacity by watering all plants daily to avoid the possibility of any plant experiencing drought. In addition, 50 ml of Hoagland's solution (Hoagland and Arnon 1950) were provided weekly. At the end of the experiment, on the 35th day, the height and the number of tillers of each plant were recorded. Then, each plant was harvested and its leaf area measured with a Li-3000 leaf area meter (LICOR, Inc.). The biomass of each plant was separated into leaf blades, leaf sheaths, roots, crowns and standing dead material. Since the plants were cultivated on sand, roots and crowns were not ashed because they were easily separated from the substrate by washing them by hand with running water on a sieve. Finally, the weight of each compartment was recorded after oven-drying at 70 °C for 72 hours.

Data were analysed by two-way type III ANOVA, since the design was unbalanced because of the lower number of clones from the grazed area. The ANOVA model was a nested-factorial, because the clones could not be crossed among populations (Montgomery 1997). All data were checked for normality and homoscedasticity, and Tukey's tests for *a posteriori* means comparisons were performed when required. The effects of the main factors and the interactions were considered significant at $\alpha' < 0.0042$ after applying the Bonferroni correction for multiple testing: $\alpha' = \alpha/k$, where k is the number of tests performed (i.e., $\alpha' = 0.05/12$; see Legendre and Legendre (1998)).

Results

The two populations of *P. dilatatum* showed similar tolerance to defoliation, as indicated by the lack of significant clipping × population interaction in a variety of growth measures (Table 1). Defoliation reduced final biomass of both populations by 26% (Figure 1a). This was the result of negative effects of defoliation on the final size of all plant compartments,

Table 2. Effects of clipping on traits of two populations of *Paspalum dilatatum* from sites with different recent grazing history. The values are means and standard errors (in brackets).

Trait	Population			
	Grazing exclosure		Grazed area	
	Unclipped	Clipped	Unclipped	Clipped
Proportion of aboveground biomass removed (%)	0	57 (1.6)	0	59 (1.6)
Leaf blade biomass (g/plant)	2.2 (0.1)	1.4 (0.05)	2.4 (0.1)	1.5 (0.05)
Leaf sheath biomass (g/plant)	4.0 (0.15)	2.0 (0.1)	4.3 (0.2)	2.0 (0.1)
Crown biomass (g/plant)	4.5 (0.2)	3.3 (0.2)	4.8 (0.2)	3.9 (0.2)
Root biomass (g/plant)	5.5 (0.3)	5.2 (0.3)	7.2 (0.3)	6.4 (0.3)
Standing dead (g/plant)	1.7 (0.1)	1.0 (0.05)	1.9 (0.1)	1.2 (0.1)
Leaf area (cm^2/plant)	501 (18)	418 (20)	559 (20)	447 (12)

particularly, leaf sheath and leaf blade biomass (52 and 37% reduction respectively) (Table 2). Total plant biomass (i.e. final biomass plus biomass removed by clipping) was only 10% lower in defoliated plants than in control plants (Figure 1a). Defoliation had no effect on total aboveground biomass, (final aboveground biomass plus biomass removed by clipping) (Table 1, Figure 1b). The different values of the biomass of the two populations (Figure 1) cannot be attributed to different growth rates because the differences in biomass at the end of the experiment (ca. 2 g dry weight plant^{-1}; Figure 1a) equalled the differences of initial biomass (9 g dry weight plant^{-1} vs 11 g dry weight plant^{-1} for the populations from the grazing exclosure and the grazed area respectively; $p < 0.05$). The different values of initial biomass between populations were due only to differences in belowground biomass ($p < 0.05$), since the initial aboveground biomass values of the two populations were equal ($p = 0.5$). This explains the larger difference between populations in total biomass than in aboveground biomass (Figure 1a vs. 1b).

The populations showed minor or no differences in morphometric traits. Plant height, a morphological trait associated with grazing avoidance, was reduced by clipping by 20% in the population from the grazed area and was unaffected in plants from the grazing exclosure. Surprisingly, the population from the grazed area was slightly taller than the population from the exclosure when plants were not clipped (Figure 2a, Table 1). Blade:sheath ratio, an allometric variable that usually differs between populations with contrasting grazing history, was similar between the *P. dilatatum* populations and was marginally increased by clipping (10%) equally across populations (interaction not significant, Table 1 and Figure 2b). Finally, other variables such as the number of tillers, specific leaf area and leaf area ratio did not differ between populations ($p > 0.1$; data not shown).

Discussion

Unlike grasses with long evolutionary history of grazing, *P. dilatatum* did not display conspicuous intraspecific variation in traits that determine grazing resistance. The populations analysed did not differ in their tolerance to defoliation and diverged only slightly in plant height, a morphometric trait related to the ability to avoid grazing.

The lack of differences in defoliation tolerance across populations from species that inhabit sites with contrasting grazing regimes in grasslands with short evolutionary history of grazing is not restricted to *P. dilatatum*. Absence of intraspecific differences in grazing resistance was also found in *Piptochaetium napostaense* from southern areas of the Argentinean Pampas (Tomás et al. 2000). This pattern is not unique to the Argentinean pampas. Jaindl et al. (1994) also reported a lack of intraspecific variation in grazing tolerance among *F. idahoensis* populations from grassland sites subjected to different grazing intensities in Oregon (USA), which also have experienced light grazing intensities during evolutionary time. In contrast, all grasses surveyed from ecosystems subjected to heavier grazing intensities by large herbivores during evolutionary time displayed noticeable differences in tolerance to defoliation between populations subjected to different grazing regimes during some decades (Detling and Painter 1983; Carman 1984; McNaughton 1984; Carman and Briske 1985; Jaramillo and Detling 1988; Oesterheld and McNaughton 1988; Polley and Detling 1988, 1990; Painter et al. 1993).

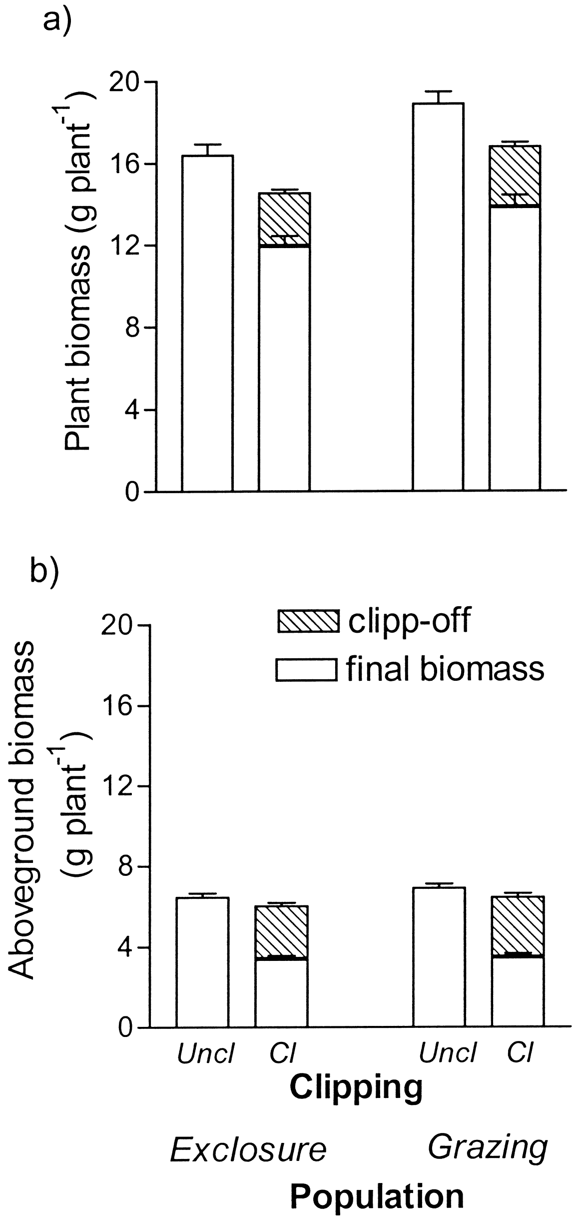

Figure 1. Clipping effects on **a)** total plant biomass (final biomass plus clipp off), and **b)** total aboveground biomass (final aerial biomass plus clipp off) of two populations of *Paspalum dilatatum* from a 20-yr old grazing exclosure (*Exclosure*) and from a continuously grazed area (*Grazing*). *Uncl* = unclipped plants, *Cl* = clipped plants. The error bars correspond to standard errors.

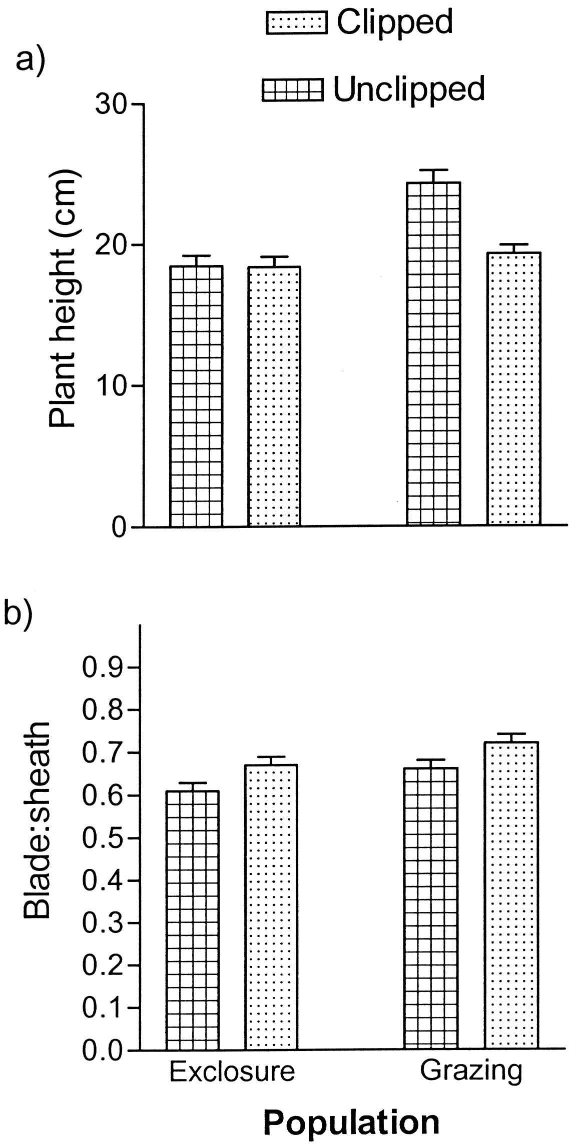

Figure 2. Clipping effects on **a)** plant height, and **b)** blade:sheath ratio of two populations of *Paspalum dilatatum* from a 20-yr old grazing exclosure (*Exclosure*) and from a continuously grazed area (*Grazing*). *Unclipped* = unclipped plants, *Clipped* = clipped plants. The error bars correspond to standard errors.

Although the *P. dilatatum* populations analysed here did not differ in growth-related variables, they did differ in plant height, a morphometric trait associated with grazing avoidance. The difference, however, was smaller and opposite in direction to that found among populations of grasses from ecosystems with long evolutionary history of grazing: The two *P. dilatatum* populations differed in plant height by less than 5 cm and the population from the grazed site was taller than the population from the grazing exclosure. In contrast, the grazing ecotypes of the grass *Agropyron smithii* Rydb. from the long-term heavily grazed North American mixed grass prairie were noticeably shorter in stature than the non-grazing ecotypes of the same species (Detling and Painter 1983). The lack of differences between the *P. dilatatum* populations in allometric traits, such as blade:sheath ratio, also contrasted with evidence on species from grasslands with long evolutionary history of grazing, which presented conspicuous differences in these traits (Detling and Painter 1983; Jaramillo and Detling 1988).

Why does high grazing pressure lead to the evolution of populations with differences in grazing resistance and the lack of grazing lead to a more uniform intraspecific composition? The answer to the first part of this question may bear on a particular aspect of grazing: it is heterogeneous in space and time due to animal migration and their preference for particular food sources in different times of the year. This leads to the coexistence of vegetation patches subjected to different grazing regimes and, hence, disruptive selection pressures within a same landscape, which results in population differentiation (McNaughton 1983, 1984). In contrast, the more uniform intraspecific composition in ungrazed mesic ecosystems can be expected to be the result of a more homogeneous selection pressure exerted by uniform closed canopy conditions. This selection pressure operates in opposite direction to that exerted by grazing. For example, grazing favors prostrate growth forms, whereas the lack of grazing favors tall growth forms with high competitive ability for light (McNaughton 1984; Milchunas et al. 1988). Clearly, the opposite direction of these selection pressures is associated with costs: grazing resistance is expected to be costly in terms of competition for light and vice-versa (Milchunas et al. 1988).

It may be argued that the lack of population differentiation in our study could be partly associated with the duration of the grazing regimes in the field, determined here by the age of the exclosure (20 yr). There are two facts against this argument. First, there is evidence from studies in the mixed grass prairie from North America that differentiation of populations in response to grazing may occur in less than 15 years (Detling and Painter 1983; Jaramillo and Detling 1988). This rapid differentiation has been attributed to strong selection pressure combined with high heritability and simple genetics governing grass morphometric traits such as plant height and internode length (McNaughton 1984). In addition, there is also evidence on rapid evolution in grasses in response to fertilization (Snaydon 1970) or fire (Scheiner and Teeri 1987). Second, although we lack specific information on the life span of *P. dilatatum* individuals in the Flooding Pampa, we know that the exclusion of grazing provokes a sudden and drastic change in canopy structure and species composition: forbs and prostrate grasses are replaced by taller bunch grasses in less than four years (Sala et al. 1986; Sala 1988). This indicates that any form of *P. dilatatum* not able to survive under the environmental conditions generated by the exclusion of grazing should also have been eliminated from the exclosure. Demographic information on grasses is scarce in the literature. An almost unique data set on the C-4 grass *Bouteloua gracilis* in the North American mixed prairie showed that the mean life span was 3.7 years and that only 4.5% of the genets of a population sampled during 40 years lived longer than 30 years (Fair et al. 1999). Hence, there is no strong reason to believe that a 20-year period may not be long enough for extreme grazing situations to lead to population differentiation in a grass species like *P. dilatatum.*

The absence of *P. dilatatum* populations specialized in coping with contrasting grazing regimes agrees with the effects of grazing on plant community structure in the Flooding Pampa: In this grassland, livestock grazing alters community structure mainly through changes in species composition (Sala et al. 1986; Sala 1988; Chaneton et al. 1988). On the contrary, grazing does not cause drastic floristic changes in ecosystems with long evolutionary history of grazing like the Serengeti grasslands because they contain grass species and ecotypes adapted to the different grazing situations (McNaughton 1983, 1984).

In addition to occupying sites with different grazing regimes, *P. dilatatum* is also found as co-dominant in communities with contrasting flooding regimes along topographic gradients in the Flooding Pampa (Burkart et al. 1990). A previous study with populations from different topographic positions revealed the existence of intraspecific variation in the resistance to flooding in *P. dilatatum* (Loreti and Oesterheld 1996). Why does *P. dilatatum* possess intraspecific variation with respect to flooding and not to grazing, since both are main disturbances in this grassland (Sala et al. 1986; Chaneton et al. 1988; Chaneton and Facelli 1991)? The answer may bear on the historical regimes of these factors in the Flooding Pampa. The heterogeneity regarding the water regime comprises long evolutionary time: it was generated during the last late Pleistocene glaciations, when the current relief of the region was shaped (Tricart 1973). In contrast, the current heterogeneity of the grazing regime was generated recently in an evolutionary time scale, when cattle were introduced by European settlers a few centuries ago (Sala et al. 1986; Sala 1988). Hence, the novelty of grazing as an important evolutionary force in the Flooding Pampa may account for the lack of ecotypes specialized for different grazing regimes.

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