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Subtle topographical differences along a floodplain promote different plant strategies among *Paspalum dilatatum* subspecies and populations

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Abstract It was hypothesised that subtle topographical differences might cause the existence of ecotypes along a floodplain. The apomict grass *Paspalum dilatatum* subspecies *dilatatum* inhabits flood-prone lowlands as well as nearby uplands in the floodplains of Argentina, while the sexual *P. dilatatum* subspecies *flavescens* almost exclusively inhabits the uplands. The aim of the present study was to identify the different traits that allow these *P. dilatatum* populations to inhabit different habitats. Plants of *P. dilatatum* were reciprocally transplanted between uplands and lowlands. Morphophysiological traits related to flooding tolerance were measured during a flood. Subspecies *dilatatum* from the uplands and subspecies *flavescens* showed a high physiological performance in the uplands but a considerable decrease in stomatal conductance, net photosynthesis rates and tiller number in the flooded lowlands. In contrast, the subspecies *dilatatum* from the lowlands showed relatively lower and stable stomatal conductance, photosynthesis rates and leaf water potential at both sites. Subspecies *dilatatum* from the lowlands outperformed upland populations at the lowland site with respect to tillering. Leaves of subspecies *dilatatum* from the lowlands that had grown at the lowland habitat had a lower blade/sheath proportion than leaves of plants transplanted to the uplands. This behavior did not occur in both upland populations. Results suggest that *dilatatum* Lowland plants have the typical strategy of stress-tolerant genotypes and that the upland populations are adapted to habitats where competitive species are selected. In conclusion, habitats with subtle differences in topographic level can favour both ecotypic differentiations within an apomict subspecies but also the maintenance of morphophysiological similitudes between coexisting upland populations belonging to different subspecies.

Key words: ecotype, flooding, intraspecific variation, reciprocal transplant, water relations.

INTRODUCTION

Differences in natural selection pressures due to spatial heterogeneity in soil characteristics can cause the existence of locally specialized ecotypes (Snaydon & Davies 1982; Nyberg Berglund *et al.* 2003). Together with many other soil conditions, floods and waterlogging are able to produce variation among locally adapted populations (Keeley 1979; Davy *et al.* 1990; Ashraf 2003). However, temporal variation in selection factors favours plastic phenotypes (Bradshaw 1965; Kawecki & Ebert 2004). As a result, floodplains may favour the appearance of locally adapted ecotypes with different plasticity because they are subjected to contrasting effects among sequential flooding, drawdown and drought conditions. In addition, the temporally heterogeneous environmental characteristics of floodplains make difficult the prediction about the pattern of population differentiation (Keeley 1979). In this way, natural selection in floodplains could produce the existence of a mixture of genotypes adapted to habitats that do or do not flood or, by contrast, the selection of intermediate-like phenotypes (Keeley 1979).

The distributional ranges of floodplain species are likely to reflect their sensitivity to flooding (Oesterheld & McNaughton 1991; van Eck *et al.* 2006). In the fine-grained heterogeneous flooding Pampa grasslands of Argentina (South America), communities are arranged as a matrix of flood-prone lowland grasslands interrupted by few, never-flooded uplands triggered from eolic origin, which have a very small area within the landscapes (Aragón & Oesterheld 2008). The perennial grass *Paspalum dilatatum* Poir. is a highly palatable C_4 grass that is widely distributed in the flooding Pampa grasslands (Burkart *et al.* 1990; Loreti & Oesterheld 1996). However, *P. dilatatum* has two

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subspecies that display different spatial distributional patterns in this floodplain. The subspecies *dilatatum* dominates in lowland communities where winter or spring flooding occurs (Burkart *et al.* 1990). This subspecies is also very common in the upland communities where flooding does not occur (Burkart *et al.* 1990). Otherwise, *P. dilatatum* subsp. *flavescens* Roseng., B.R. Arrill. & Izag. almost exclusively inhabits the uplands (Mollard *et al.* 2008). These different distributional patterns of *P. dilatatum* subspecies associated with particular habitat conditions suggest the existence of locally adapted populations.

As well as distributional dissimilarities, *Paspalum dilatatum* subspecies also have important reproductive differences that can determine their adaptive potential. The subspecies *flavescens* is an allotetraploid cytotype with sexual reproduction and predominantly autogamous (García *et al.* 2007) while *P. dilatatum* subsp. *dilatatum* is an apomictic allopentaploid taxa (Souza-Chies & Cavalli-Molina 1995; Espinoza & Quarín 2000). The restricted gene flow among populations of subspecies *dilatatum* might favour the spread of potentially adapted genotypes without the recombinational problem that sexuality involves. In contrast, subspecies *flavescens* has more potential for micro-evolution due to its sexual character. Despite this, subspecies *flavescens*, as previously mentioned, has a more limited distribution compared with subspecies *dilatatum*. Since apomictic taxa have larger distributional ranges and tend to range to higher altitudes and latitudes than their sexual relatives (Hörandl 2006), the above-described differentiation in the distribution of *P. dilatatum* subspecies represents a non-previously recognized case of geographical parthenogenesis (*sensu* Hörandl 2006).

The flooding tolerance of plants depends on the development of a suite of physiological and morphological responses in order to survive partial submersion and anaerobic soil conditions (Armstrong 1979; Kozlowski & Pallardy 1984; Blom *et al.* 1994; Insausti *et al.* 2001; Voesenek *et al.* 2006). At the physiological level, flood-tolerant grasses are able to maintain their leaf water potential, stomatal conductance and photosynthesis during flooding without showing reductions in growth (Kozlowski & Pallardy 1984; Naidoo & Naidoo 1992; Blanch *et al.* 1999; Insausti *et al.* 2001; Mollard *et al.* 2008). By contrast, flood-sensitive grasses are not able to sustain such basic physiological processes during flooding, thus reducing their growth and tillering rates (Oesterheld & McNaughton 1991; Baruch 1994; Huang *et al.* 1994; Malik *et al.* 2001). At the morphological level, the most conspicuous responses suggesting flood tolerance in grasses are increments in the leaf length extension, the increase in leaf insertion angles and the development of aerenchyma in tissues (Naidoo & Naidoo 1992; Insausti *et al.* 2001; Mollard *et al.* 2008). These responses help plants to re-establish leaf contact with atmospheric oxygen to sustain the respiratory demands of submerged organs (Colmer 2003). Thereby, all aforementioned plant variables (i.e. stomatal conductance, photosynthesis rate, leaf water potential, leaf lengthening and tiller number per plant) can be useful features for studying the adaptation of plants to flooding.

The aim of the present paper is to test for population differentiation with respect to flood tolerance and related morphophysiological attributes in *Paspalum dilatatum* subspecies. Questions are:

1 Can be the distributional differences between *P. dilatatum* subspecies be explained by morphophysiological attributes related to flooding tolerance?
2 Does *P. dilatatum* have locally adapted populations?
3 Are the phenotypes displayed in the lowlands by the different *P. dilatatum* populations characteristic of flood-adapted species or merely the disadvantageous consequences exerted for a stressful habitat?

Addressing these questions will help us to identify some of the different strategies that allow populations to inhabit mild habitats, as well as nearby temporary stressful, habitats, like those that are found in floodplain grasslands.

METHODS

Plant material and study site

Similarly sized plants of *Paspalum dilatatum* subsp. *dilatatum* (i.e. tiller number) were selected in two different plant communities situated in an upland and a lowland site along a topographic gradient in the flooding Pampa grasslands. Additionally, similar plants of *P. dilatatum* subsp. *flavescens* were only selected in the aforementioned upland site due to the remarkable scarcity of plants of this subspecies in the lowlands. In this manner, we used three different populations for the present study: two populations of the subsp. *dilatatum*, one from the upland site and the other from the lowland site and, one population of the subsp. *flavescens* taken from the upland site. To avoid potential vegetative clones, plants inside each community were collected at sufficiently large distances (>10 m). The subsp. *flavescens* and subsp. *dilatatum* plants from the uplands (hereafter '*dilatatum* Upland') belong to a community characterized by *Melica brasiliana* Ard., *Borreria dasycephala* (Cham. & Schltdl.) Bacigalupo & E. L. Cabral, and *Echium plantagineum* L. Plants of subsp. *dilatatum* from the lowlands ('*dilatatum* Lowland') belong to a community characterized by *Piptochaetium montevidense* (Spreng.) Parodi, *Ambrosia tenuifolia* Spreng., *Eclipta bellidioides* (Spreng.) Sch. Bip. ex S.F. Blake and *Mentha pulegium* L., one of the most widespread plant communities of these grasslands (Burkart *et al.* 1990). Both communities are related to two different habitat types that have contrasting water regimes due to their relative topographical positions and soil characteristics: the uplands are associated with sandy soils of moderate drainage, are never-flooded, and are positioned 50–100 cm higher than the lowlands, which have clayey soils with poor drainage (Soriano 1991). Communi-

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ties are highly interwoven with irregular limits between them. Lowlands predominate in the landscape, covering approximately 70% of the area (Aragón & Oesterheld 2008). The small patches of the Uplands represent only 4.7% of the area, with a mean patch size of 2.72 ha (Aragón & Oesterheld 2008).

Reciprocal transplant experiment

Plants from each of the three *Paspalum dilatatum* populations (16 plants per population) were carefully extracted from each of the sites and were transplanted within and between sites inside fenced exclosures to exclude large herbivores (15 × 15 m). Inside each exclosure, a grid with six columns and four rows (24 places) was marked, leaving a walkway space of 0.5 m between plants. An experimental unit (each plant) was randomly assigned to each position. So, we used eight plants per population in each site. At each site, the soil volumetric water content was monitored with ECHO Probes EC-10 sensors (Decagon Devices, Pullman, USA) connected to Campbell 21-X dataloggers (Campbell Scientific, Logan, USA). The growing season of *P. dilatatum* ranges from spring to the end of summer, so the reciprocal transplant was carried out at the beginning of winter during the plant's latency period to minimize stress due to transplantation. Morphological and physiological parameters were assessed after 3 months of growth (6 months after transplanting). Thereby, all measurements were carried out on tillers and leaves formed under the influence of the environmental conditions of each site. On the date of the morphophysiological measurement, the soil oxygenation at the flooded lowlands and the non-flooded uplands was evaluated by measuring the oxygen diffusion rate (ODR) at a soil depth of 5 cm with platinum microelectrodes (Letey & Stolzy 1964).

Physiological and morphological measurements

The stomatal conductance and transpiration rate were measured in the youngest fully expanded leaf blade in a similar position of each plant (eight replications per population and site) using a Li-1600 M steady state porometer (Li-Cor Inc., Lincoln, NE, USA). Leaf water potential was recorded immediately on the same leaves with a Schölander-type pressure chamber (Bio-Control, Buenos Aires, Argentina). The net CO_2 exchange was measured on the same type of leaves with a Li-6200 portable system (Li-Cor Inc., Lincoln, NE, USA). Measurements were taken at noon on a clear day ($PPFD = 1850 \pm 70\ \mu mol\ m^{-2}\ s^{-1}$). At the same time, air temperature and relative humidity were measured (Temperature & RH Probe HUMICAP H, Vaisala, Finland). Both parameters were used to calculate the air vapour pressure deficit (VPDair), as a way to characterize the atmospheric evaporative demand, which was approximately 2.2 kPa.

For each plant, tiller number was recorded, and the blade and sheath lengths measured separately in the youngest fully expanded leaves of three tillers per plant.

Statistical analyses

Physiological and morphological data were analyzed through two-way ANOVAs with population and site as main factors. When significant interactions were detected, a subsequent Tukey test was applied to reach conclusions on the treatments effects. The relationship between leaf sheath and leaf blade lengths was determined by conducting regression analysis for each combination of population and site. This allows us to detect potential differences for leaf allometric relationships among plants growing at the upland or lowland sites and to reach conclusions on leaf size-scaling effects due to habitat quality. When a significant relationship between variables was detected, additional *F*-tests for differences in the regression parameters between sites were carried out. If differences between parameters were not detected, data were pooled to obtain a single linear regression (Schwinning 1996; Striker *et al.* 2007). All datasets were checked to ensure the normality and homogeneity of variances. Results are presented as untransformed means of eight replicates ± standard error. All tests were carried out using the GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Soil volumetric water content time courses

During the experimental period, soil in the lowlands was flooded several times in winter and spring, whereas no floods occurred in the uplands (Fig. 1). When physiological and morphological data were collected in late spring, the lowlands had been flooded for nearly 25 days, while the upland soil volumetric water content was slightly below field capacity (Fig. 1). On that day, the ODR values were $57 \pm 5 \times 10^{-8}\ g\ cm^{-2}\ min^{-1}$ in the well-oxygenated soil of the upland site, and $2.6 \pm 1.5 \times 10^{-8}\ g\ cm^{-2}\ min^{-1}$ in the soil of the lowlands, which indicates the hypoxia stress at the flooded site.

Physiological responses

The physiological performance of *Paspalum dilatatum* populations differed across grassland habitat types (see population × site in Table 1, site refers to the location where plants were transplanted). The two *P. dilatatum* populations from the uplands performed better at their home sites than in the flooded lowlands (Fig. 2). *Dilatatum* Upland and subsp. *flavescens* had 60% and 70% lower stomatal conductance ($P < 0.01$ and $P < 0.0001$) at the lowland site than at the upland, their original habitat. In addition, the net photosynthesis rates of plants of both populations were 30% lower in the lowland site in comparison to their counterparts growing in the upland site ($P = 0.023$ for *dilatatum* Upland and $P < 0.001$ for subsp. *flavescens*). The leaf water potential was similar at both sites for *dilatatum* Upland plants but was higher in subsp. *flavescens* growing on the lowlands ($P < 0.01$). The physiological performance of *dilatatum* Lowland plants was remark-

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ably different from the responses of the two upland populations: *dilatatum* Lowland plants showed similar stomatal conductance ($P = 0.29$), photosynthesis ($P = 0.34$) and leaf water potential ($P = 0.17$) in both sites.

Morphological responses

The populations of *Paspalum dilatatum* showed different responses in tiller number, leaf blade, leaf sheath and leaf length between sites (see population × site in Table 1). In particular, morphological differences were detected in the two populations native from the upland when comparing plants transplanted to different sites (Figs 3,4; Table 2). Tiller number per plant of *dilatatum* Upland and subsp. *flavescens* were

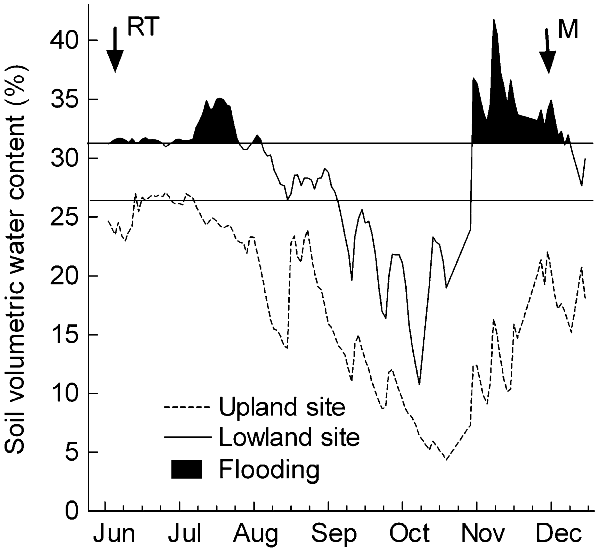

Fig. 1. Soil volumetric water content (%) time courses in both upland and lowland sites from the date of reciprocal transplanting until the date of measurements. M, measurement date; RT, reciprocal transplanting date. Horizontal solid lines at 32% and 27% of soil volumetric water content indicate field capacity for the soil of the lowland and the upland sites, respectively.

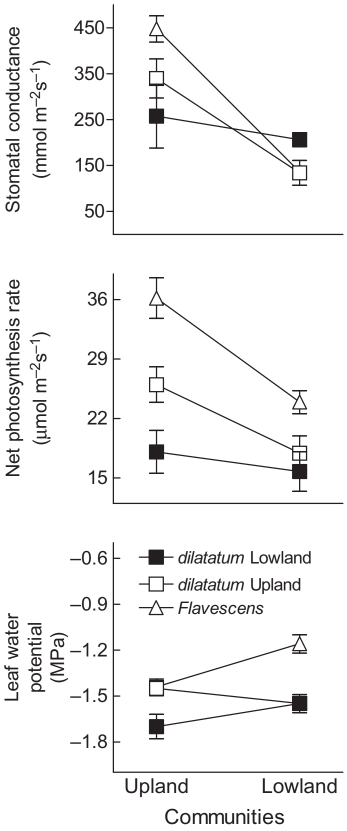

Fig. 2. Physiological measurements on leaves of *Paspalum dilatatum* populations transplanted to the upland and lowland sites: (a) stomatal conductance (b) net photosynthesis rate and (c) leaf water potential. During the measurement, the lowland site had been flooded for nearly 25 days, while in the uplands the soil water content was close to field capacity. Values are mean ± SE of eight replicates.

Table 1. Results of two way ANOVA for analyzed plant physiological and morphological variables of *Paspalum dilatatum* populations transplanted to the upland and flooded lowland sites

	Main effects		Interaction	Response at the lowland site		
Variable	Population	Site	Pop × site	*dilatatum* Lowland	*dilatatum* Upland	*flavescens*
Stomatal conductance	1.56	37.89***	6.05**	0	↓	↓↓
Net photosynthesis rate	18.96***	18.39***	3.68*	0	↓	↓
Leaf water potential	16.54***	5.58**	5.74**	↓	0	↑
Tiller number	3.56*	8.43**	5.73**	0	↓	↓↓
Leaf length	2.99	37.5***	5.90**	0	↓	↓
Leaf blade length	3.24*	36.41***	4.84*	0	↓	↓
Leaf sheath length	1.71	18.49***	5.82**	0	↓	↓

F-values and significance levels are presented (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Arrows indicate the net effect registered on plants growing at the flooded lowland site (in comparison with the upland) for each variable and population.

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41% and 63% lower, respectively, in the lowlands in comparison to their counterparts growing at their home site ($P = 0.023$ and $P < 0.01$, respectively; Fig. 3). In addition, both populations native to the upland registered on average lower leaf blade, leaf sheath and consequently, leaf lengths at the lowland site in comparison to their counterparts growing at the upland site ($P < 0.001$ in both cases; Fig. 4; Table 2). A strong positive relationship between leaf blade and leaf sheath lengths was detected in the two populations native to the uplands (Fig. 4). This relationship did not differ between sites (*F*-tests: $P = 0.23$ for upland *dilatatum* and $P = 0.71$ for subsp. *flavescens*). This allowed us to pool the data and obtain a single linear regression for each population (Fig. 4). These results indicate that simply size-scaling effects for these leaf traits accounted for the detected average differences between sites. This means that *dilatatum* Upland and subsp. *flavescens* developed longer leaves at the upland site and shorter leaves at the lowland site, but with a similar proportion of leaf blades and leaf sheath lengths (Fig. 4; Table 2).

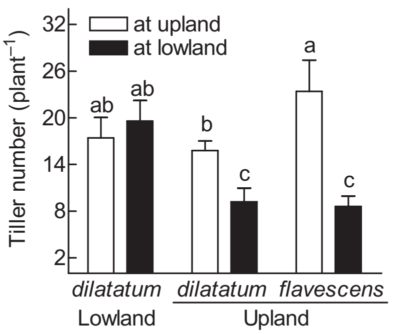

Fig. 3. Tiller number per plant of *Paspalum dilatatum* populations growing at the upland (open bars) and lowland (closed bars) sites. Different letters among bars indicate significant differences ($P < 0.05$) among population × site combinations based on the Tukey test. When tillers were counted, the lowland site had been flooded for nearly 25 days, while in the uplands the soil water content was close to field capacity. Values are mean ± SE of eight replicates.

The morphological traits of *dilatatum* Lowland plants were relatively stable across grassland habitats (Figs 3,4; Table 2). In the *dilatatum* Lowland population, tiller number was similar in plants growing at both sites ($P = 0.30$) and twofold higher than in plants of both upland native populations growing at the flooded lowland site (Fig. 3). Additionally, the average lengths of leaves, leaf blades and leaf sheaths did not vary between the two habitats ($P = 0.29$ and $P = 0.86$, respectively; Fig. 4; Table 2). This population also registered a positive relationship between leaf blade and leaf sheath lengths. However, in contrast to that previously pointed out for the populations native to the upland, regression parameters differed between sites (slope test: $P = 0.85$, intercept test: $P < 0.0001$; Fig. 4). In the *dilatatum* Lowland population, the leaves at the lowland site had a higher sheath proportion than leaves in the upland site (Fig. 4) although in

Table 2. Total leaf length (sheath + blade) of *Paspalum dilatatum* populations transplanted to the upland and lowland sites

	Sites	
Total leaf lengths (cm)	Upland	Lowland
dilatatum Lowland	34.7 ± 3.2 bc	30.1 ± 1.5 bc
dilatatum Upland	41.6 ± 2.0 ab	26.5 ± 2.2 c
Flavescens	50.1 ± 3.2 a	28.2 ± 3.4 c

Values are means ± SE of eight replicates. Different letters indicate significant differences ($P < 0.05$) among population × site combinations based on the Tukey test. When leaf length was measured, the lowland site had been flooded for nearly 25 days, while in the uplands; the soil water content was close to field capacity.

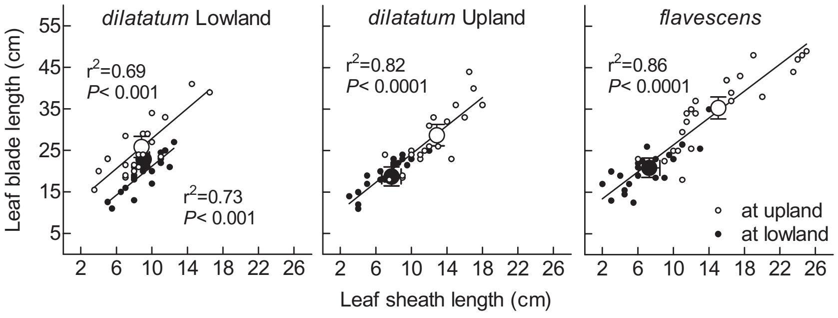

Fig. 4. Allometric relationships between leaf blade and leaf sheath lengths of *Paspalum dilatatum* populations growing at the upland site (open circles, $n = 20–24$) and at the lowland site (closed circles, $n = 20–24$). When differences between parameters of equations were not detected, data were pooled to obtain a single linear regression. Fitted equations are $y = 1.73x + 3.86$ (*dilatatum* Lowland at the lowland site), $y = 1.81x + 9.81$ (*dilatatum* Lowland at the upland site), $y = 1.71x + 8.10$ (*dilatatum* Upland) and $y = 1.65x + 10.19$ (Subsp. *flavescens*). Bigger circles indicate the average leaf blade length and leaf sheath length ± SE of eight plants.

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both cases, leaf sheath was scaled in a similar proportion to the leaf blade length (similar slopes between sites). This means that, irrespective of the similar leaf blade length between sites, the individual leaves of *dilatatum* Lowland formed in the lowlands had larger leaf sheaths than the individual leaves formed in the uplands (Fig. 4).

DISCUSSION

This reciprocal transplant experiment reveals that subspecies *flavescens* and *dilatatum* Upland performed similarly in both the upland and lowland habitats in terms of carbon fixation capacity, stomatal conductance, tiller number and leaf traits (Table 1). The morphophysiological responses of both populations native to the upland point in the same direction: lower physiological performance and a restricted growth in the flooded habitat. These features were very different from those depicted by *dilatatum* Lowland. In effect, only the performance found in *dilatatum* Lowland plants suggested the existence of specific adaptations to flooding tolerance since there was no decrease shown in most of the morphophysiological features measured. Results also indicate the existence of different strategies between upland and lowland populations of *Paspalum dilatatum* to cope with disturbances such as flooding. In fact, the intrinsic lower values of physiological parameters and leaf size recorded in the *dilatatum* Lowland plants in both habitats suggest a typical strategy of stress-tolerant genotypes (*sensu* Grime 1977) adapted to a flood-prone habitat and characterized by stable functional levels (Chapin 1991; Grime 2001). In contrast, both *dilatatum* Upland and subsp. *flavescens* showed a considerable reduction in net photosynthesis rates and tiller number when grown in the flood-prone lowland habitat. This indicates that in both populations native to the upland, the habitat with milder conditions in which competitive species are predominantly selected (Grime 1977), showed lower tolerance to the more stressful conditions of the lowland site. Our results were in line with those of Loreti and Oesterheld (1996) in which the more productive clones of *P. dilatatum* were also the less resistant to flooding and drought. So, the intrinsic differences between genotypes within a population could be shown at a populational level. Therefore, the different morphophysiological features and strategies described here could help to understand in part the distributional differences between *P. dilatatum* subspecies.

Floods generally exert negative effects on stomatal conductance both in upland and lowland grassland species (Oesterheld & McNaughton 1991; Ashraf 2003; Striker *et al.* 2005). Subsp. *flavescens* and *dilatatum* Upland plants had lower stomatal conductance in the flooded lowland than in the uplands. Researchers have shown that stomatal closure under flooding is a mechanism to regulate the water balance of plants and prevent leaf dehydration in conditions were hypoxia can affect the absorption of water by roots (Bradford & Hsiao 1982; Jackson & Hall 1987; Baruch 1994; Pezeshki 1994; Ashraf 2003). The high leaf water potential of subsp. *flavescens* showed in the flooded lowland suggests that stomatal conductance was effective in regulating the water status of this subspecies. In addition, both populations native from the uplands had a lower net leaf photosynthesis in the flooded lowlands, responses that could be related to their partial stomatal closure, and therefore an increased resistance to CO_2 diffusion into leaves (Björkman & Demming-Adams 1995). In contrast, *dilatatum* Lowland maintained its stomatal conductance and photosynthesis at similar levels at both sites; the flooded lowland and the drained upland (Fig. 2), indicating the high physiological adjustment of this population to flooding conditions, which is the most significant stress factor in the lowland habitat. Consequently, evidence indicates the better adaptation of each population to their home site in physiological terms.

Increases in leaf lengths under flooding conditions is a common response of flooding-tolerant plants as it helps their leaves to emerge from water to recover contact with the air (Laan *et al.* 1990; Grimoldi *et al.* 1999; Voesenek *et al.* 2006). On the other hand, flood-sensitive plants do not increase and even diminished leaf length and plant heights in flooding or waterlogged conditions (Oesterheld & McNaughton 1991; Dixon 1996; Malik *et al.* 2001). In the current experiment, both *Paspalum dilatatum* populations native to the upland site had smaller leaves in the flooded lowland habitat, than in the upland site (Fig. 4). The different sites did not modify the relationship between leaf blade and sheath lengths in the upland populations, indicating that the smaller leaves mainly reflected a smaller size resulting from passive reductions in growth due to resource limitations or to suboptimal conditions exerted by the flooding stress, both associated with the lower habitat quality of the flooded lowlands (van Kleunen & Fischer 2005). In contrast, the average leaf size of *dilatatum* Lowland was similar at both upland and lowland sites (Fig. 4; Table 2). However, this apparent lack of response was associated with a higher sheath proportion registered on individual leaves formed in the lowlands than in those formed in the uplands (Fig. 4). This unexpected allometric feature could be associated with the fact that *P. dilatatum* populations increase leaf sheaths aerenchyma in flooding conditions (Mollard *et al.* 2008). This improves oxygenation of submerged tissues by facilitating the flow of oxygen from shoots to roots (Laan *et al.* 1990; Jackson & Armstrong 1999). Due to

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its novelty, the finding that a lowland ecotype invests more in leaf sheath lengths in detriment to blade lengths in a flood-prone habitat merits further experimental research to find its adaptive meaning.

The number of tillers per plant can be seen as an integrative description of plant performance throughout the period of vegetative growth in each site. In spite of the low differences in tiller number among populations in the uplands, the lowland habitat had a clearly negative effect on tillering on both populations native to the upland. In fact, *dilatatum* Lowland outperformed upland populations at the lowland site with respect to tiller number by twofold. Tillering potential is a relevant variable because it determines vegetative reproduction and thus, the plant's adaptation to stress factors (e.g. frequent grazing; Nelson 2000). On the other hand, it also determines the number of inflorescences that can be produced (Huang *et al.* 1994) and thus the potential of reproductive regeneration. Both reproduction features clearly have an important impact on the persistence of plant populations and could explain the uncommonness of subspecies *flavescens* in the lowlands.

Selection can act on morphophysiological traits, which in turn have implications for evolutionary outcomes, potentially enabling populations to adapt to flooding in a relatively short time span (Huber *et al.* 2009). So, after enough generations, selection for traits that enhance success under the flooding conditions can drive the local adaptation of ecotypes. However, in the absence of sexual reproduction, such as in the case of subsp. *dilatatum*, the divergence of novel genotypes becomes a difficult event, only attained by mutation accumulation. So, for range expansions in spatially and temporally heterogeneous environments, a certain level of initial clonal diversity should be involved in colonization events (Hörandl 2006). In addition, uniparental reproduction makes apomictic lineages potentially disperse more quickly than their self-incompatible sexual relatives. Consequently, the appearance in the flooding Pampa grasslands of different subsp. *dilatatum* ecotypes adapted to different habitats could be explained by multiple colonizations of asexual apomictic lineages.

In conclusion, morphophysiological features of *dilatatum* Upland and subspecies *flavescens* were quite similar in plants growing in both the upland and the flooded lowland habitats despite the differences in genetic background. Furthermore, at both sites they had a different behaviour from that of *dilatatum* Lowland. So, the novelty of the present work is that very subtle differences in topographic level in a fine-grained heterogeneity floodplain can drive the evolution or habitat colonization of locally adapted populations, thus favouring both ecotypic differentiations within an asexual subspecies (i.e. subsp. *dilatatum*) but also the maintenance of morphophysiological similitudes between coexisting upland populations belonging to different subspecies that have dissimilar genetic backgrounds and type of reproduction (asexual in subsp. *dilatatum*, sexual in subsp. *flavescens*).

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REFERENCES

Aragón R. & Oesterheld M. (2008) Linking vegetation heterogeneity and functional attributes of temperate grasslands through remote sensing. *Appl. Veg. Sci.* **11,** 117–30.

Armstrong W. (1979) Aeration in higher plants. In: *Advances in Botanical Research*, Vol. 7 (ed. H. W. Woolhouse) pp. 225–332. Academic Press, London.

Ashraf M. (2003) Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (*Panicum antidotale* Retz.) under salinity or waterlogging. *Plant Sci.* **165,** 69–75.

Baruch Z. (1994) Responses to drought and flooding in tropical forage grasses. II. Leaf water potential, photosynthetic rate and dehydrogenase activity. *Plant Soil* **164,** 97–105.

Björkman O. & Demming-Adams B. (1995) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: *Ecophysiology of Photosynthesis* (eds E. D. Schulze & M. M. Caldwell) pp. 17–47. Springer Verlag, Berlin.

Blanch S. J., Ganf G. & Walker K. F. (1999) Growth and resource allocation in response to flooding in the emergent sedge *Bolboschoenus medianus*. *Aquat. Bot.* **63,** 145–60.

Blom C. W. P. M., Voesenek L. A. C. J., Banga M. *et al.* (1994) Physiological ecology of riverside species: adaptive responses of plants to submergence. *Ann. Bot.* **74,** 253–63.

Bradford K. J. & Hsiao T. C. (1982) Stomatal behaviour and water relations of waterlogged tomato plants. *Plant Physiol.* **70,** 1508–13.

Bradshaw A. D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **13,** 115–55.

Burkart S. E., León R. J. C. & Movia C. P. (1990) Inventario fitosociológico de un pastizal de la depresión del Salado (Prov. de Bs. As.) en un área representativa de sus principales ambientes. *Darwiniana* **30,** 27–69.

Chapin I. F. S. III (1991) Effects of multiple environmental stresses on nutrient availability and use. In: *Response of Plants to Multiple Stresses* (eds H. Mooney, W. Winner & E. Pell) pp. 67–88. Academic Press, San Diego.

Colmer T. D. (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ.* **26,** 17–36.

Davy A. J., Noble S. M. & Oliver R. P. (1990) Genetic variation and adaptation to flooding in plants. *Aquat. Bot.* **38,** 91–108.

doi:10.1111/j.1442-9993.2009.02026.x

Dixon J. M. (1996) Waterlogging and survival in *Sesleria albicans*. *New Phytol.* **133,** 415–22.

van Eck W. H. J. M., Lenssen J. P. M., van de Steeg H. M., Blom C. W. P. M. & De Kroon H. (2006) Seasonal dependent effects of flooding on plant species survival and zonation: a comparative study of 10 terrestrial grassland species. *Hydrobiologia* **565,** 59–69.

Espinoza F. & Quarín C. L. (2000) 2n + n Hybridization of Apomictic *Paspalum dilatatum* with diploid Paspalum species. *Int. J. Plant Sci.* **161,** 221–5.

García M. V., Balatti P. A. & Arturi M. J. (2007) Genetic variability in natural populations of *Paspalum dilatatum* Poir. analyzed by means of morphological traits and molecular markers. *Genet. Resour. Crop [ALLERGY]*. **54,** 935–46.

Grime J. P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* **111,** 1169–94.

Grime J. P. (2001) *Plant Strategies, Vegetation Processes and Ecosystem Processes*. Wiley, New York.

Grimoldi A. A., Insausti P., Roitman G. G. & Soriano A. (1999) Responses to flooding intensity in *Leontodon taraxacoides*. *New Phytol.* **141,** 119–28.

Hörandl E. (2006) The complex causality of geographical parthenogenesis. *New Phytol.* **171,** 525–38.

Huang B., Johnson J. W., Nesmith S. & Bridges D. C. (1994) Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply. *J. Exp. Bot.* **45,** 193–202.

Huber H., Jacobs E. & Visser E. J. W. (2009) Variation in flooding-induced morphological traits in natural populations of white clover (*Trifolium repens*) and their effects on plant performance during soil flooding. *Ann. Bot.* **103,** 377–86.

Insausti P., Grimoldi A. A., Chaneton E. J. & Vasellati V. (2001) Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. *New Phytol.* **152,** 291–9.

Jackson M. B. & Armstrong W. (1999) Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* **1,** 274–87.

Jackson M. B. & Hall K. C. (1987) Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant Cell Environ.* **10,** 121–30.

Kawecki T. J. & Ebert D. (2004) Conceptual issues in local adaptation. *Ecol. Lett.* **7,** 1225–41.

Keeley J. E. (1979) Population differentiation along a flood frequency gradient-physiological adaptations to flooding in *Nyssa sylvatica*. *Ecol. Monogr* **49,** 89–108.

van Kleunen M. & Fischer M. (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol.* **166,** 49–60.

Kozlowski T. T. & Pallardy S. G. (1984) Effects of flooding on water, carbohydrate and mineral relations. In: *Flooding and Plant Growth* (ed. T. T. Kozlowski) pp. 165–93. Academic Press Inc, Orlando, FL.

Laan P., Tosserams M., Blom C. W. P. M. & Veen B. W. (1990) Internal oxygen transport in *Rumex* species and its significance for respiration under hypoxic conditions. *Plant Soil* **122,** 39–46.

Letey J. & Stolzy L. H. (1964) Measurement of oxygen diffusion rates with a platinum microelectrode. I. Theory and equipment. *Hilgardia* **35,** 54–5.

Loreti J. & Oesterheld M. (1996) Intraspecific variation in the resistance to flooding and drought in populations of *Paspalum dilatatum* from different topographic positions. *Oecologia* **108,** 279–84.

Malik A. I., Colmer T. D., Lambers H. & Schortemeyer M. (2001) Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Funct. Plant Biol.* **28,** 1121–31.

Mollard F. P. O., Striker G. G., Ploschuk E. L., Vega A. S. & Insausti P. (2008) Flooding tolerance of *Paspalum dilatatum* (Poaceae: Paniceae) from upland and lowland positions in a natural grassland. *Flora* **203,** 548–56.

Naidoo G. & Naidoo S. (1992) Waterlogging responses of *Sporobolus virginicus* (L.) Kunth. *Oecologia* **90,** 445–50.

Nelson C. J. (2000) Shoot morphological plasticity of grasses: leaf growth *vs*. tillering. In: *Grassland Ecophysiology and Grazing Ecology* (eds G. Lemaire, J. Hodgson, A. de Moraes, C. Nabinger & P. de Faccio Carvalho) pp. 101–26. CAB International, Wallingford.

Nyberg Berglund A.-B., Dahlgren S. & Westerbergh A. (2003) Evidence for parallel evolution and site-specific selection of serpentine tolerance in *Cerastium alpinum* during the colonization of Scandinavia. *New Phytol.* **161,** 199–209.

Oesterheld M. & McNaughton S. G. (1991) Interactive effect of flooding and grazing on the growth of Serengeti grasses. *Oecologia* **88,** 153–6.

Pezeshki S. R. (1994) Plant response to flooding. In: *Plant-Environment Interactions* (ed. R. E. Wilkinson) pp. 289–321. Marcel Dekker, New York.

Schwinning S. (1996) Decomposition analysis of competitive symmetry and size structure dynamics. *Ann. Bot.* **77,** 47–57.

Snaydon R. W. & Davies T. M. (1982) Rapid divergence of plant populations in response to recent changes in soil conditions (*Anthoxanthum odoratum*). *Evolution* **36,** 289–97.

Soriano A. (1991) Río de la Plata Grasslands. In: *Ecosystems of the World 8A. Natural Grasslands. Introduction and Western Hemisphere* (ed. R. T. Coupland) pp. 367–407. Elsevier, Amsterdam.

Souza-Chies T. T. & Cavalli-Molina S. (1995) Variability in seed production and germination in *Paspalum* – *Dilatata* group (Gramineae). *Rev. Bras. Biol.* **55,** 127–39.

Striker G. G., Insausti P., Grimoldi A. A., Ploschuk E. L. & Vasellati V. (2005) Physiological and anatomical basis of differential tolerance to soil flooding of *Lotus corniculatus* L. and *Lotus glaber* Mill. *Plant Soil* **276,** 301–11.

Striker G. G., Insausti P., Grimoldi A. A. & Vega A. S. (2007) Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. *Plant Cell Environ.* **30,** 580–9.

Voesenek L. A. C. J., Colmer T. D., Pierik R., Millenaar F. F. & Peeters A. J. M. (2006) How plants cope with complete submergence. *New Phytol.* **170,** 213–26.

doi:10.1111/j.1442-9993.2009.02026.x