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Variation in $\delta^{13}C$ among species and sexes in the family Restionaceae along a fine-scale hydrological gradient

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ABSTRACT

Consistent, repeatable segregation of plant species along hydrological gradients is an established phenomenon that must in some way reflect a trade-off between plants' abilities to tolerate the opposing constraints of drought and waterlogging. In C_3 species tissue carbon isotope discrimination ($\delta^{13}C$) is known to vary sensitively in response to stomatal behaviour, reflecting stomatal limitation of photosynthesis during the period of active growth. However, this has not been studied at fine-spatial scale in natural communities. We tested how $\delta^{13}C$ varied between species and sexes of individuals in the family Restionaceae growing along a monitored hydrological gradient. 20 Restionaceae species were investigated using species-level phylogeny at two sites in the Cape Floristic Region, a biodiversity hotspot. A spatial overlap analysis showed the Restionaceae species segregated significantly ($p<0.001$) at both sites. Moreover, there were significant differences in $\delta^{13}C$ values among the Restionaceae species ($p<0.001$) and between male and female individuals of each species ($p<0.01$). However, after accounting for phylogeny, species $\delta^{13}C$ values did not show any significant correlation with the hydrological gradient. We suggest that some other variable (e.g. plant phenology) could be responsible for masking a simple response to water availability.

Key words: Cape floristic region, carbon isotope discrimination, hydrological gradient, plant gender, Restionaceae, soil water regime trade-off, water use efficiency

INTRODUCTION

Plants are in general very sensitive to small differences in soil moisture, even within a range of moisture tensions where water is still freely available to them (Davies & Gowing 1999). Classical experiments by Ellenberg (1953) with meadow grassland plants demonstrated that interspecific competition leads to segregation of species' distributions along soil moisture gradients and Silvertown *et al.* (1999) further showed that this structures meadow plant communities in the field.

Niche separation on fine-scale hydrological gradients may well be common in plant communities, although the physiological basis of this segregation is still unclear. In general, separation of species into distinct niches along any resource or environmental axes is caused by trade-offs that force species to specialize (MacArthur 1972). Possible causes of hydrological niche specialization that have been suggested include trade-offs between tolerance of aeration stress (caused by waterlogging) and drought stress (Davies & Gowing 1999; Silvertown *et al.* 1999) and tolerance of low soil nutrient availability (in wet conditions) and drought stress (e.g. Neill 1990; Castelli *et al.* 2000; Araya 2005).

Whatever the precise trade-offs may be that underlie the specialization of species into hydrological niches, these must involve the water economy of plants and hence must be related to stomatal behaviour. Stomatal function imposes a fundamental trade-off between water conservation and carbon assimilation (and hence growth) because stomata must open to allow CO_2 uptake, but must close to limit water loss. Thus water stress may cause stomatal limitation of photosynthesis during periods of active growth (e.g. Henson *et al.* 1989). The ratio of CO_2 assimilated to stomatal conductance determines the Intrinsic Water Use Efficiency (WUE) of a plant. Could this fundamental trade-off arising from the two conflicting functions of stomata be the ultimate explanation for niche separation along hydrological gradients? If it is, then intrinsic WUE ought to vary between species in a systematic manner along soil moisture gradients.

Precisely how intrinsic WUE is expected to vary along soil moisture gradients depends upon how other influences upon growth such as soil-available nitrogen or oxygen supply vary along the gradient too. If no other factor is limiting to growth, intrinsic WUE ought to decrease monotonically with increasing water supply. If soil-available nitrogen or oxygen (or both) become limiting at the wet end of the gradient, WUE ought to reach a maximum near the middle of the gradient. In theory, these patterns ought to be detectable through changes in ^{13}C isotope discrimination, a method often used for assessment of intrinsic WUE (Farquhar *et al.* 1982; Dawson *et al.* 2002). When stomatal resistance is high because of closure or increased photosynthetic demand, the influence of enzymatic discrimination is diminished and $\delta^{13}C$ leaf values are enriched. On the other hand, if resistance is reduced the relative influence of enzymatic discrimination increases, favouring ^{13}C depletion during fixation (Van de Water *et al.* 2002).

In experiments with crop plants, changes in stomatal resistance and behaviour eventually led to changes in plant water use efficiency (e.g. Farquhar and Richards 1984; Martin & Thorstenson 1988; Ebdon *et al.* 1998). However, this relationship is not as straightforward as has often been assumed in the past (Seibt *et al.* 2008). This is because, particularly in natural vegetation, Ci/Ca (the ratio of CO_2 concentrations in the leaf intercellular spaces to that in the atmosphere), which partly determines isotope discrimination and in turn WUE, could be influenced by external biotic and abiotic factors (Griffiths *et al.* 2000). Hence, WUE and $\delta^{13}C$ can vary independently of one another, making the use of $\delta^{13}C$ as a surrogate for WUE questionable (Griffiths *et al.* 2000, Seibt *et al.* 2008). This is particularly the case where $\delta^{13}C$ is used alone, without other independent estimates of gas exchange or environmental conditions.

In this paper, we test for a correlation between $\delta^{13}C$ and the location of species on a soil moisture gradient in the field, using two methods for controlling variation that could obscure the relationship. First, our 20 study species all belong to the same clade, the African Restionaceae, which are dioecious, graminoid C_3 perennials (Linder *et al.* 2005). We selected the Restionaceae for study because many species belonging to this family co-occur within fynbos habitats and species are found across a wide range of water regimes (Linder *et al.* 1998, Hardy *et al.* 2008). By use of a phylogeny for this clade that is resolved to species level (Hardy *et al.* 2008), we are able to use phylogenetically independent contrasts in correlations between $\delta^{13}C$ and other variables, thus eliminating the potential influence of phylogenetically-correlated hidden third variables. Second, we make intraspecific comparisons between males and females of Restionaceae species. Females are generally expected to have lower WUE than males in dioecious species (Dudley 2006).

METHODS

Site and species

Restionaceae species were sampled from two study sites, located at the heart of the Cape Floristic Region, South Africa a global biodiversity hotspot (Myers *et al.* 2000). The rationale of site selection was to test our hypothesis in sites with contrasting altitude, species composition and water regime. The first site was at Riverlands Nature Reserve (33° 29’ 14.2” S, 18° 35’ 44.1” E) at an altitude of 120 m a.s.l, while the second one was at New Years Peak, within Limietsberg Nature Reserve (33° 41’ 19.6” S, 19° 06’ 03.1” E) at an altitude of 1085 m. The annual rainfall at Riverlands was 375 mm, while for New Years Peak it was 1660 mm. Potential evapotranspiration rates were 3.5 mm and 2.8 mm per day for Riverlands and New Years Peak respectively.

In a plot of 11,442 m^2 at New Years Peak and 2500 m^2 size at Riverlands, 235 and 305 1 m^2 quadrats were surveyed for the presence/absence of Restionaceae species, and when possible, for the sex of each plant. For the study species, samples for $\delta^{13}C$ analysis were taken in replicates of ten along a transect placed through the plot.

Carbon isotope analysis

From flowering culms of sampled species between 6-14 months old, a ten cm long stem sample was taken ten cm above ground surface. The collected plant material was then oven dried at 60 °C for 48 hours before samples were analysed in the laboratory. For this analysis, a piece of the stem was scraped to remove any external dirt, and a small sliver of the clean stem, approximately 0.60 mg in weight, taken with a scalpel and put into a tin capsule. The tin capsule was then combusted in a Flash EA® 1112 series elemental analyzer (Thermo Finnigan, Italy). The CO_2 produced was fed into a Delta® Plus XP IRMS isotope ratio mass spectrometer (Thermo Electron, Germany) via a Conflo III® gas control unit (Thermo Finnigan, Germany). The carbon isotope ratio ($^{13}C/^{12}C$) output was then reported relative to the PDB standard. The resulting delta notation, $\delta^{13}C$, was determined using the following equation (Kloeppel *et al.* 1998):

$$\delta^{13}C(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} x1000$$

Hydrological monitoring

To enable an accurate understanding of soil water regime within the plots, a hydrologic model based on Gowing & Youngs (1997) was used. The model was built from inputs of water-table-depth behaviour in the field, topographic variation, soil characteristics and meteorological data.

The water-table depth was monitored from an array of dip wells, supported by automatic logging 'divers' (Eijkelkamp®, The Netherlands). The dip wells were read manually every two weeks, while automatic divers in a sub-sample of wells were set to read every four hours for 15 months' duration. We confirmed the suitability of water table depth as a proxy for assessing water availability in our sites, by regressing it against volumetric water content during the monitoring season.

To account for micro-topographical differences, topography was surveyed at all quadrat and dip well locations using a Leica Geosystems® TPS300 (Switzerland) total station device.

Using the hydrological monitoring from dipwells and divers, it was possible to interpolate the water-table depths for each quadrat location. The mean water-table depths of each quadrat from the spring season, was then weighted by the % presence of the respective species present, to get its hydrological metric (i.e. mean water-table depth).

Phylogeny

The Restionaceae comprise 350 species, 342 of which form a clade that is endemic to the Cape Floristic Region. The phylogeny of Restionaceae used in this study was built on the basis of *rbcl* markers and morphological traits (Hardy *et al.* 2008) and includes 94% of the Cape clade, resolved to species level.

Statistical analysis

Initially, the botanical data collected for each site were tested for spatial community structure using Stone and Roberts' C-score in the EcoSim software (Gotelli & Entsminger 1997). The C-score measures the average number of "checkerboard units" (i.e. species pairings that do not occur together in a quadrat) between all possible pairs of species. In a competitively structured community, the C-score should be significantly larger than expected by chance. 10,000 simulations with a random seed of 10 were run and compared against the actual field distribution.

The species-level δ^{13}C values were analysed using one-way and when appropriate two-way analysis of variance. The difference in δ^{13}C values between male and female plants was tested using a two-sample t-test. All the analysis was conducted using Statistica® Release 8.0 software.

The correlation between mean water table depth and δ^{13}C was measured by the method of phylogenetically independent contrasts, an approach often used for cross-species comparisons (Felsenstein 1985). Such analysis needs to be conducted to account for the potential similarity of closely related species (phylogenetic history); which otherwise prevents individual species from being statistically independent data points (Felsenstein, 1985). The comparative contrast was implemented using the CACTUS 1.13 (Comparative Analysis of Continuous Traits Using Statistics; Schwilk & Ackerly 2001) software.

RESULTS

Species

There was an average of 2.1 Restionaceae species per quadrat at each site. The botanical survey at Riverlands found 18 Restionaceae species, of which we studied the ten most prevalent species for logistical reasons. Of these ten species it was only possible to identify gender in individuals of five species. At New Years Peak a total of

12 species were found, of which we used 11 in our study (Table 1). Gender could be scored in eight of the species.

<Table 1>

Community structure

Statistical analysis of the field distribution of Restionaceae using Robert's C-score for co-occurrence (Gotelli & Entsminger 1997) revealed significant structuring, compared to what would be expected by chance (with observed values > simulated values at $p<0.001$).

Water regime

The water-table depths in individual quadrats, as modelled from biweekly monitoring, ranged from -0.20 to 0.65 m for New Years Peak site and between 0.30 and 1.46m for Riverlands. The mean spring water-table depths for the quadrats occupied by the studied Restionaceae species are given in Table 1.

δ^{13}C values

δ^{13} C values observed ranged from -26.9 ‰ (*Calopsis viminea*) to -28.77 ‰ (*Willdenowia arescens*) in Riverlands and from -25.2 ‰ (*Anthochortus crinalis*) to -27.2 ‰ (*Elegia coleura*) in New Years Peak. The standard errors within each species ranged from 0.20 - 0.51 (Riverlands) and 0.22 – 0.62 (New Years Peak). One way analysis of variance showed there were significant differences in δ^{13}C signatures among the species at both sites (Table 2).

<Table 2>

δ^{13}C values for females were generally more negative, -27.5 ‰ (New Years Peak) and -28.2 ‰ (Riverlands) than for their male counterparts -26.7 ‰ (New Years Peak) and -27.9 ‰ (Riverlands). Combined analysis of the difference between males and females of all species at the two sites, showed this difference was significant (two sample t-test $p<0.001$)

Two-way analysis of variance, for species whose gender was known, also showed significant differences between the species (both sites) as well as genders of the plants (only at New Years Peak). However, there was no significant interaction between species and gender at any of the sites (Table 3).

<Table 3 >

δ^{13}C versus water table depth

Taking account of phylogenetic relationships, through PICS analysis, mean water-table depth was plotted against δ^{13}C (Figure 1). The results show that there was no significant linear correlation between species' δ^{13}C and their mean water-table depth along the hydrologic gradient at either site (r^2=0.23 p=0.34, New Years Peak and r^2=0.06 p=0.60, Riverlands).

<Figure 1>

DISCUSSION

Soil moisture availability is a key environmental variable affecting plant distribution and coexistence along hydrological gradients (Silvertown 2004; Engelbrecht *et al.* 2007). In this study, we found that Restionaceae species showed niche segregation, as quite unrelated species also do for example in wet meadows in England (Silvertown *et al.,* 1999). Intrinsic water use efficiency is expected to vary with hydrological conditions and has implications for plant distribution and survival strategies. For example, where water supply is abundant, plants with a non-conservative water-use strategy seem to be the most successful (Chen *et al.* 2007). In the past, δ^{13}C has been used as a surrogate for WUE and so we tested whether there was a correlation between δ^{13}C and hydrological conditions measured in the field.

The Restionaceae δ^{13}C values observed in these sites, shown in Table 1 (from -25.2 ‰ to -28.8 ‰) are well within the range of what would be expected of C_3 plants (Griffiths *et al.* 2000; Linder 2005). The larger values of δ^{13}C at New Years Peak than at Riverlands (Table 1) could be attributed to differences in altitude, precipitation or temperature (Panek & Waring 1995; Van de Water 2002; Zheng & Shangguan 2007), since New Years Peak is 965 m higher than Riverlands and has rainfall of 1500 mm per year, four times that of Riverlands.

The more negative δ^{13}C signature that we found in female plants is in agreement with previous findings in other species (Dawson & Ehleringer 1993; Dudley 2006). The gender difference is usually interpreted as evidence of water use efficiency, which is improved through reduction of both water loss and carbon gain (Dawson and Geber 1999). In this context, females of dioecious species often show higher reproductive effort than males because females allocate more biomass to reproduction in the form of flowers and fruits (Correia & Diaz Barradas 2000) thereby reducing their WUE.

Stomatal limitation of photosynthesis mediated by water stress is currently accepted as one of the main limitations to plant productivity in dry-land ecosystems (Diaz-Espejo *et al.* 2007). The close relationship between WUE and transpiration efficiency, is also known to be reflected in carbon isotope discrimination (Farquhar *et al.* 1982). Thus, the significant differences in the δ^{13}C values observed (Tables 2 and 3) amongst species within a single family and having similar life form, suggests that they have different stomatal behaviour. Physiologically, this is a strong suggestion of environmental control (in this case by water) on plants' response (stomatal behaviour and hence carbon isotope discrimination). Ecologically, the implication goes even further: it could be regulating species distribution pattern by modifying their competitive interaction. For example, Mole *et al.* (1994) found that prairie grass *Agropyron smithii*, which has a more negative mean δ^{13}C, showed further range expansion than other coexisting species following drought. Even the level of diversity in leaf gas-exchange regulation has been associated with high species diversity in tropical rainforest (Bonal & Guehl 2001).

δ^{13}C values and water-table depth

Previous studies in the investigation of δ^{13}C and water availability, have been conducted either at coarse continental, altitudinal or large landscape vegetation units (e.g. Stewart *et al.* 1995; Kloeppel *et al.* 1998; Van de Water *et al.* 2002; Wang *et al.* 2005; Chen *et al.* 2007) or only between genotypes of a species level (e.g. Yoneyama & Ohtani 1983; Farquhar & Richards 1984; Martin & Thorstenson 1988). Only very few have looked at subtle topo-edaphic gradients within a landscape scale (Peñuelas *et al.* 1999; Bai *et al.* 2008). Furthermore, the most prevalent generalization from the above studies has been for plant δ^{13}C values to be positively correlated with water availability, which was often expressed as precipitation. In these studies, more negative values of δ^{13}C have been interpreted as indicators of greater enzymatic discrimination, implying higher stomatal conductance and hence lower water use efficiency (e.g. Farquhar & Richards 1984).

In woody fynbos plants, species in the same genera show similar drought vulnerability curves, regardless of whether or not they were wetland adapted or dryland adapted species. This suggests strong phylogenetic constraint on xylem function (Aston 2007). In this study even though we accounted for phylogeny of our species, we still found no significant correlation between the δ^{13}C and the subtle hydrologic gradient, even as the species segregated along the gradient (Figure 1).

Although this comes as a surprise, it reinforces recent views by Seibt *et al.* (2008), on the insufficiency of δ^{13}C values alone as reliable surrogates to WUE to explain plant response distributions in relation to water regime, particularly in natural field populations. This is because a number of other environmental interactions could influence δ^{13}C (Vitousek *et al.* 1990; Griffiths *et al.* 2000). For example, Bai *et al.* (2008) found the relationship between δ^{13}C and water regime was the opposite of what would be expected and they suggested that another environmental factor i.e. nitrogen availability could be a mediating factor between water availability and plant δ^{13}C response. In our case, we have accounted for the factors: species, gender, phylogeny, and water regime, but not the seasonality component i.e. phenology of the species. It is known that plant carbon isotope discrimination varies with the plant's stage of development and season (e.g. Mole *et al.* 1994; Zhao *et al.* 2004; Chen & Chen 2007). Smedley *et al.* (1991) showed there is a general trend for a decrease of carbon isotope discrimination as the growing season progresses i.e. a response to increase in evaporative demand and decrease in soil moisture. Hence early flowering species discriminate more than later flowering ones, because such species are active during the initial, less water-stressed months of the growing season and tend to use water less efficiently. As such phenology is a possible explanation for the differences we saw in our species, particularly as Restionaceae flower year-round, with different species flowering in different months (Linder 2002).

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Table 1. Species studied at each site, % presence, height, δ^{13}C and mean water table depth

Table 2. Analysis of variance results for δ^{13}C among species of Restionaceae at New Years Peak and Riverlands

Table 3. Two-way analysis of variance of δ^{13}C for Restionaceae by species and gender at New Years Peak and Riverlands

Figure 1. Phylogenetically independent contrasts of mean water table depth and δ^{13}C for species sampled at New Years Peak and Riverlands.

Table 1. Species studied at each site, % presence, height, δ^{13}C and mean water table depth

Site	Species	% Presence	Average height (m)	Mean δ^{13}C (‰)	Mean Water table (m)
Riverlands	*Calopsis viminea*	10.2	0.48	-26.9	0.54
	[+]*Cannomois acuminata*[‡]	12.1	0.78	-27.5	0.86
	Chondropetalum nudum	21.0	0.74	-28.7	0.63
	Elegia filacea	28.2	0.68	-28.0	0.43
	Hypodiscus willdenowia[‡]	13.1	0.45	-27.8	0.57
	Ischyrolepis capense	10.5	0.50	-28.2	0.58
	[+]*Ischyrolepis monanthos*	12.5	0.60	-27.5	0.83
	[+]*Staberoha distachyos*	24.9	0.65	-28.5	0.68
	[+]*Thamnochortus punctatus*	38.4	0.91	-28.2	0.83
	[+]*Willdenowia arescens*	17.0	0.68	-28.8	0.78
New Years Peak	*Anthochortus crinalis*	35.7	0.33	-25.2	0.07
	[+]*Elegia coleura*	13.2	0.48	-27.2	0.15
	Elegia filacea	23.8	0.27	-26.3	0.36
	Elegia neesii	50.6	0.49	-26.5	0.25
	[+]*Ischyrolepis curviramis*[§]	26.8	0.20	-26.8	0.34
	[+]*Restio pedicellatus*	10.6	0.44	-27.1	0.33
	[+]*Restio bolusii*	23.8	0.31	-25.9	0.30
	[+]*Hypodiscus arescens*	<10.0	0.51	-28.1	[na]
	[+]*Restio obscurus*	<10.0	0.55	-27.5	[na]
	[+]*Staberoha cernua*	<10.0	0.24	-26.3	[na]
	[+]*Ceratocarium fimbriatum*[§]	<10.0	0.69	-27.7	[na]

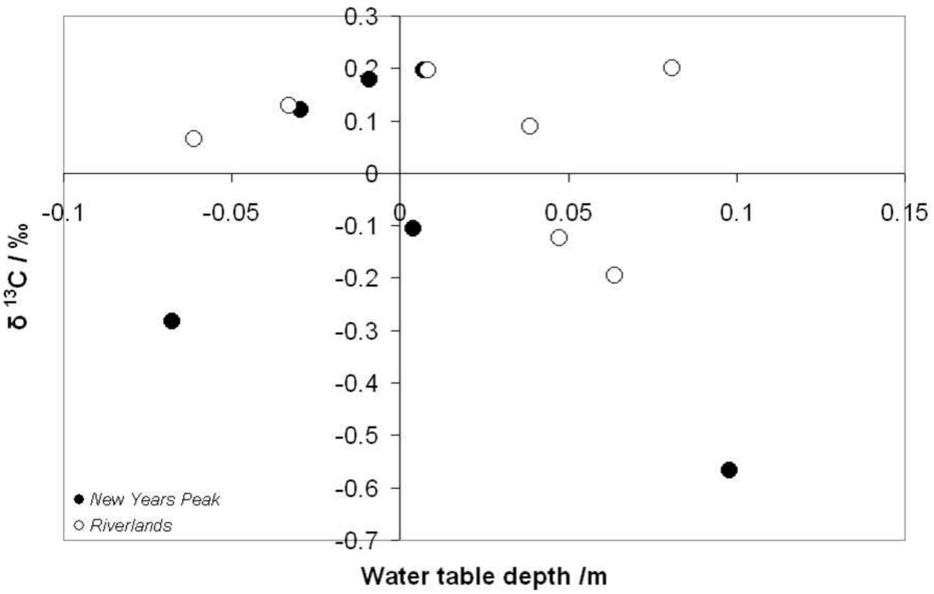
[+] species in which males and females were distinguished; [‡]species missing from phylogeny [§] there were only 5 replicates for the species (10 for the other species); [na] no sufficient hydrologic data available due to <10 % presence.

Table 2. Analysis of variance results for δ^{13}C among species of Restionaceae at New Years Peak and Riverlands

Site	**Source**	**df**	**MS**	**F**	**p**
New Years Peak	Species	10	6.80	5.71	**<0.001**
	Error	89	1.19		
Riverlands	Species	9	3.54	3.58	**<0.001**
	Error	90	0.99		

Table 3. Two-way analysis of variance of δ^{13}C for Restionaceae by species and gender at New Years Peak and Riverlands

Site	Source	df	MS	F	p
New Years Peak	Species	7	3.94	4.13	**<0.002**
	Gender	1	4.52	4.73	**0.04**
	Species x Gender	7	0.98	1.03	0.43
	Error	37	0.96		
Riverlands	Species	4	3.40	2.98	**0.03**
	Gender	1	1.42	1.24	0.27
	Species x Gender	4	2.78	2.43	0.06
	Error	40	1.14		



Phylogenetically independent contrasts of mean water table depth and δ13C for species sampled at New Years Peak and Riverlands.
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