American Journal of Botany 87(8): 1211–1215. 2000.

CLIMATE AND THE U.S. DISTRIBUTION OF C_4 GRASS SUBFAMILIES AND DECARBOXYLATION VARIANTS OF C_4 PHOTOSYNTHESIS[1]

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I compared the C_4 grass flora and climatic records for 32 sites in the United States. Consistent with previous studies, I found that the proportion of the grass flora that uses the NADP malic enzyme (NADP-ME) variant of C_4 photosynthesis greatly increases with increasing annual precipitation, while the proportion using the NAD malic enzyme (NAD-ME) variant (and also the less common phosphoenolpyruvate carboxykinase [PCK] variant) decreases. However the association of grass subfamilies with annual precipitation was even stronger than for the C_4 decarboxylation variants. Analysis of the patterns of distribution by partial correlation analysis showed that the correlations between the frequency of various C_4 types and rainfall were solely due to the association of the C_4 types with particular grass subfamilies. In contrast, there was a strong correlation of the frequency of the different subfamilies with annual precipitation that was independent of the influence of the different C_4 variants. It therefore appears that other, as yet unidentified, characteristics that differ among grass subfamilies may be responsible for their differences in distribution across natural precipitation gradients.

Key words: climate; C_4 photosynthesis; grasses; NAD-ME photosynthesis; NADP-ME photosynthesis; PCK photosynthesis; Poaceae; precipitation.

Differences among major taxonomic groups of grasses in distribution across continental scale gradients of temperature and precipitation have been noted at least since the 1950s (Hartley, 1958a, b; Hartley and Slater, 1960). At the time these observations were first made, there was little knowledge that could suggest possible physiological bases for the observed distributional differences among the grass subfamilies. Following the discovery of the C_4 photosynthetic pathway in the 1960s (Hatch and Slack, 1966), it became clear that different photosynthetic pathways were associated with different grass subfamilies (Hattersley, 1987). Teeri and Stowe (1976) for North America, Hattersley (1983) for Australia, and Vogel, Fuls, and Ellis (1978) for South Africa demonstrated that C_4 grass species were relatively most abundant in areas of high temperature. Along with an understanding of mechanistic reasons for a higher temperature optimum for photosynthesis of C_4 vs. C_3 species (Ehleringer, 1978) this has provided a convincing explanation for some of the differences in distribution among grass clades that were first noticed by Hartley (1958a, b, 1973; Hartley and Slater, 1960).

Some of the patterns noted by Hartley cannot be explained by the C_3 vs. C_4 distinction. Hartley found that the subfamily Eragrostoideae (= Chloridoideae) is distributed in drier areas than the tribe Andropogoneae (of the subfamily Panicoideae), although both taxa are exclusively C_4 (Hartley, 1958a; Hartley and Slater, 1960). One potential explanation lies in the variation that is present within C_4 photosynthesis. There are three distinct biochemical variants of C_4 photosynthesis, with different C_4 species using any one variant nearly exclusively. The three varieties of C_4 photosynthesis are termed NAD malic enzyme (NAD-ME), NADP malic enzyme (NADP-ME), and phosphoenolpyruvate carboxykinase (PCK) after the bundle sheath decarboxylation enzyme used in each pathway (Hatch, Kagawa, and Craig, 1975). There is a strong association of C_4 variants with particular grass subfamilies. In the subfamily Chloridoideae, virtually all C_4 species are of either the NAD-ME or PCK type (Table 1). In the subfamilies Arundinoideae and Panicoideae, the great majority of C_4 species are NADP-ME (Table 1).

Hattersley (1992) in Australia and Ellis, Vogel, and Fuls (1980) in Namibia have found strong correlations between precipitation and the percentage of the C_4 grass flora in an area with a particular C_4 variant. Species with the NAD-ME variety of decarboxylation are predominantly found in the driest habitats, and the percentage of the C_4 grass flora using the NADP-ME variety of decarboxylation increases with annual rainfall.

As neither study distinguished the role of C_4 pathway variation from that of subfamily membership, these observed patterns of distribution suggest two different hypotheses. One possibility is that the variants of C_4 photosynthesis differ functionally, so that the NAD-ME pathway itself is more adaptive than the NADP-ME pathway to life in arid environments. The differences in the distributions of panicoid and chloridoid grasses noted by Hartley would result from the different C_4 pathways that they use. Alternatively, the NAD-ME and NADP-ME C_4 pathways may be functionally equivalent, and other characteristics of the grass subfamilies may be responsible for their differences in distribution across precipitation gradients. The differences in the distributions of the NAD-ME and NADP-ME pathways would then result from the chance evolutionary association of the pathways with these other, adaptively important characteristics of the grass subfamilies in which they occur.

If there were a perfect association of grass subfamilies with particular C_4 variants, it would be impossible to distinguish between these two alternatives on the basis of geographic surveys of C_4 grass distribution. However, ~26% of the worldwide C_4 grass flora consists of species that are neither NAD-

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[1] Manuscript received 4 January 2000; revision accepted 18 April 2000.

The author thanks Jessica Gurevitch and Daniel Sims and the anonymous reviewers for comments on drafts of this manuscript and Toby Kellogg and many others for discussion of this work. This is contribution 1069 in Ecology and Evolution, State University of New York, Stony Brook.

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TABLE 1. Rough estimates of the number of grass species worldwide that use each biochemical variant of C_4 photosynthesis. Genera that have not yet been biochemically typed are assumed to have the same proportions of the different C_4 variants as the genera within their tribe that have been biochemically typed. In genera in which there is known variation for C_4 pathway, it is assumed that the proportion of species using each variant is the same among species not yet biochemically typed as among species that have been. This table does not include C_3 species, which are also found in each subfamily. Information was compiled from Willis and Airy Shaw (1966), Brown (1977), Hattersley (1987), Hattersley and Watson (1992), Sage, Li, and Monson (1999), and Watson and Dallwitz (1999).

Grass subfamily	NADP-ME	NAD-ME	PCK
Arundinoideae	372	4	0
Chloridoideae	9	957	431
Panicoideae	2476	250	167

ME chloridoids nor NADP-ME panicoids (Table 1). It is therefore possible to consider the relationships of climatic variables with subfamily membership and C_4 pathway variants separately, in an attempt to distinguish the roles of C_4 pathway variation from that of subfamily membership in determining the geographic distributions of C_4 grasses.

To address this question, I surveyed the C_4 grass flora and climate of 32 sites from across the United States, using data assembled from a variety of sources (listed in the Appendix). The United States was chosen for this study because it provides a test of the relationships observed in Australia and Namibia on an additional continent, and because the extensive network of weather stations across the United States makes it possible to find weather data from locations near the floristic survey sites. Unlike previous studies, I statistically tested relationships with climate both on a C_4 variant basis and on a grass subfamily basis.

MATERIALS AND METHODS

Sources for grass floras and climatic data are given in the Appendix. All flora sites in the databases used for this study were included in the analyses if they had at least 18 C_4 grass species and were <600 km^2 in area. A minimum number of C_4 grass species was desired so that percentages of the flora at a site (for example, the percentage of C_4 grass species which use the NAD-ME pathway) would not be excessively influenced by the recorded presence or absence of a single species. A maximum area for sites was desired to minimize the amount of climate variation likely to be present within a site.

Because the areas surveyed for flora lists varied greatly (1–593 km^2), comparisons among sites in absolute numbers of species would depend largely on the size of the areas, rather than on the botanical characteristics of the sites. Data are therefore presented on the basis of percentages of the C_4 flora rather than on absolute numbers of species.

Weather stations were located at an average distance of 23 km (maximum = 55 km) from the geographic center of their corresponding flora site. The average difference in altitude between weather stations and the average altitude for their corresponding flora site was 83 m (maximum = 357 m).

The variety of C_4 photosynthesis used by each species was determined from several sources (primarily Table 2.2 in Hattersley and Watson, 1992), using the established associations of leaf anatomy with C_4 variants. Genera with Hattersley and Watson's type 1 or 6 leaf anatomy were identified as NADP-ME, and genera with type 2 or 7 leaf anatomy were identified as NAD-ME. Species of *Panicum* were identified to C_4 variant based on leaf anatomy using Table 6 of Brown (1977), and by reference to Zuloaga (1987). The only leaf anatomy type found in this survey for which pathway cannot be confidently identified is Hattersley and Watson's leaf anatomy type 3; species with this type of leaf anatomy can be either PCK or NAD-ME. Some of these genera have been biochemically typed; these were identified from Hattersley (1987). Species which could not be resolved as to whether they were of the PCK or NAD-ME variants (e.g., a number of species in the genera *Bouteloua* and *Sporobolus*) were included in analyses based on grass subfamilies, but excluded from those which compared C_4 variants.

Ten climatic variables were included in this study, including normal annual precipitation and nine temperature variables (Table 2). These climatic variables were chosen because previous studies have found both annual precipitation and mid-summer temperatures to be correlated with the proportion of NAD-ME and NADP-ME species in local and regional floras (Ellis, Vogel and Fuls, 1980; Hattersley, 1992).

RESULTS

The strongest relationships of grass subfamily and C_4 variant frequency with climatic factors were with normal annual precipitation. The distributions of the Panicoideae and Chloridoideae subfamilies along precipitation gradients were diametrically opposed, with the Panicoideae positively ($r = 0.89$) and the Chloridoideae negatively ($r = -0.90$) correlated with normal annual precipitation (Fig. 1, Table 2). All three C_4 variants were also highly significantly correlated with normal annual precipitation, the NADP-ME pathway positively ($r = 0.83$), and the PCK and NAD-ME pathways negatively ($r = -0.75$ and $r = -0.68$, respectively; Fig. 2, Table 2).

It is possible through the method of partial correlation analysis to statistically resolve the correlations among three intercorrelated variables (Sokal and Rohlf, 1995). This method allows consideration of the correlation that is found between two variables when the value of a third variable is mathematically held constant. In the present case, for example, partial correlation analysis allows us to consider the correlation between

TABLE 2. Correlations of C_4 grass floristic composition with climate variables across 32 U.S. sites. Climate variables are 30-yr normal values. Definitions of the climate variables are given in the climate data sources (see Appendix). Floristic variables are the percentages of the total C_4 grass flora at a site that are from particular grass subfamilies or that use a particular variant of C_4 photosynthesis.

Variable	% Chloridoideae	% Panicoideae	% Arundinoideae	% NAD-ME	% NADP-ME	% PCK
Annual precipitation	−0.90	0.89	−0.14	−0.68	0.83	−0.75
July cooling degree days	−0.14	0.16	−0.15	−0.25	0.34	−0.34
Annual cooling degree days	−0.30	0.32	−0.12	−0.44	0.49	−0.40
Annual heating degree days	0.37	−0.35	−0.03	0.48	−0.53	0.43
July daily minimum temperature	−0.45	0.46	−0.14	−0.45	0.58	−0.56
July daily mean temperature	−0.14	0.16	−0.15	−0.25	0.34	−0.34
July daily maximum temperature	0.28	−0.24	−0.12	0.07	−0.05	0.03
Annual daily minimum temperature	−0.49	0.48	−0.03	−0.54	0.61	−0.51
Annual daily mean temperature	−0.36	0.35	−0.01	−0.47	0.53	−0.43
Annual daily maximum temperature	−0.18	0.17	0.01	−0.36	0.39	−0.31

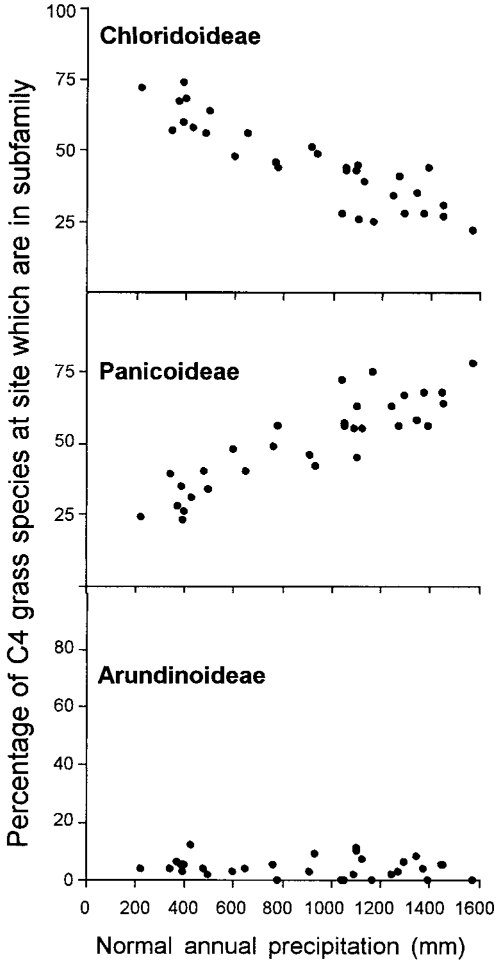

Fig. 1. Relationship between annual precipitation and the percentage of the C_4 grass flora that is in the Chloridoideae, Panicoideae, and Arundinoideae subfamilies for 32 sites in the United States.

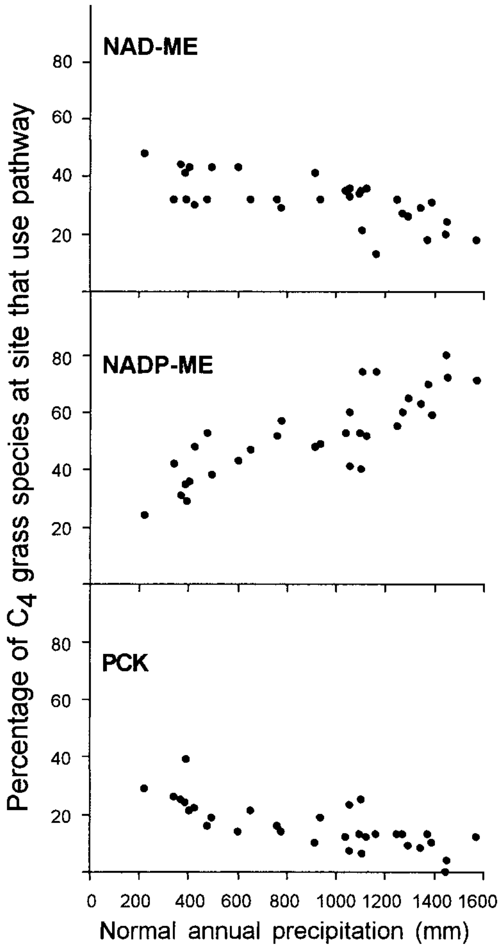

Fig. 2. Relationship between annual precipitation and the percentage of the C_4 grass flora that uses the NAD-ME, NADP-ME, and PCK biochemical variants of C_4 photosynthesis for 32 sites in the United States.

the percentage of the local C_4 flora that uses the NADP-ME pathway and annual precipitation, free of the influence of the third variable, the percentage of the C_4 flora that is in the Panicoideae.

When partial correlation analysis is performed to remove the influence of the frequency of C_4 pathway types, the correlations between the frequency of the Panicoideae and Chloridoideae subfamilies in local floras and normal annual precipitation remain large and highly significant (Table 3), with partial correlations of the frequency of the Panicoideae with annual precipitation of 0.61, and of the Chloridoideae subfamily with annual precipitation of −0.79 and −0.75 (with the frequency of the NAD-ME and PCK C_4 variants held constant, respectively). This suggests that the relationships between the frequencies of these grass subfamilies and precipitation are independent of the frequency of the C_4 decarboxylation variants.

In contrast, when partial correlation analysis is used to remove the influence of grass subfamily frequency, the correlations between the frequency of C_4 variants and annual precipitation are small and nonsignificant (partial $r = -0.01$, $r = -0.17$, and $r = 0.24$ for the NAD-ME, PCK, and NADP-ME pathways, respectively).

TABLE 3. Partial correlations of floristic variables with annual precipitation for 32 sites in the United States. Each correlation is of annual precipitation with the proportion of the C_4 grass flora at a site that is from a particular subfamily of the Poaceae, or that uses a particular biochemical variant of C_4 photosynthesis. In each case, values of a second floristic variable (highly correlated with the first floristic variable) are statistically held constant.

Correlated variables	Variable held constant	*r*
% Chloridoideae and annual precipitation	% NAD-ME	−0.79
% Chloridoideae and annual precipitation	% PCK	−0.75
% Panicoideae and annual precipitation	% NADP-ME	0.61
% NAD-ME and annual precipitation	% Chloridoideae	−0.01
% PCK and annual precipitation	% Chloridoideae	−0.17
% NADP-ME and annual precipitation	% Panicoideae	0.24

DISCUSSION

The tight relationships found here between grass subfamily / C_4 variant frequency and annual precipitation are especially striking in light of the relatively coarse nature of the analysis. Neither microsite variation nor the seasonality of precipitation have been taken into account in this analysis (or in that of Ellis, Vogel, and Fuls [1980] in Namibia). Nonetheless, this study found, as have previous studies (Ellis, Vogel, and Fuls, 1980; Hattersley, 1992), a very strong and significant relationship between annual precipitation and the prevalence of the NAD-ME and NADP-ME photosynthetic pathways in local grass floras.

At least for the United States this seems to be due to a shared correlation of these variables with the frequencies of the Panicoideae and Chloridoideae grass subfamilies. This suggests that the correlations between the frequencies of the NAD-ME and NADP-ME C_4 variants and annual precipitation found by Hattersley (1987) and Ellis, Vogel, and Fuls (1980) might also be due to the tight association between C_4 variants and grass subfamilies.

A comparison of the distributions of PCK grasses in Australia and the United States additionally suggests the importance of subfamily-level traits other than C_4 variants in structuring their distribution. The prevalence of the PCK pathway is negatively correlated with annual precipitation in the United States and positively correlated with annual precipitation in Australia (Hattersley, 1992). The explanation for these divergent patterns may lie in the different compositions of the PCK flora of these two areas. In the United States, the great majority of PCK species are from the subfamily Chloridoideae. In the sites in this sample, for example, an average of 97% of the PCK species are from the subfamily Chloridoideae. In Australia, on the other hand, a slim majority (51%) of the PCK species are members of the Panicoideae (Prendergast, 1989).

The results of the partial correlation analysis do not support the hypothesis that functional differences among the C_4 variants are responsible for the differences in distribution between C_4 panicoid and chloridoid grasses. This begs the question of what traits are responsible for the relationship between C_4 variant composition in local floras and precipitation. It may well prove that no individual trait, but a suite of interrelated traits, including aspects of physiology, anatomy, and life history differ between these subfamilies and is ultimately responsible for the striking differences in distribution along precipitation gradients seen between the C_4 members of the Chloridoideae and Panicoideae.

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APPENDIX. Sites used in this study.

Flora site	State	Weather Station	Data sources[a]
Agate Fossill Beds Natl. Monument	NE	Torrington Experimental Farm	b,v
Assateaugue Island Natl. Seashore	MD	Salisbury	a,v
Big Thicket National Preserve	TX	Port Arthur	a,y
Canaveral Natl. Seashore	FL	Titusville	a,v
Cape Cod Natl. Seashore	MA	Provincetown	a,v
Cape Lookout Natl. Seashore	NC	Moorehead City 2WNW	a,v
Chickasaw Natl. Recreation Area	OK	Ardmore	a,v
Colorado Natl. Monument	CO	Fruita	a,v
Cumberland Island Natl. Seashore	GA	Brunswick	a,v
Cuyahoga Valley Natl. Recreation Area	OH	Hiram	b,v
Devil's Tower Natl. Monument	WY	Colony	a,v
Effigy Mounds Natl. Monument	IA	Prairie Du Chien	b,v
Fort Davis Natl. Historic Site	TX	Alpine	a,v
Fort Donelson Natl. Battlefield	TN	Dover 1W	a,v
Fort Larned Natl. Historic Site	KS	Larned	b,v
Fort Scott Natl. Historic Site	KS	Fort Scott	b,v
George Washington Carver Natl. Monument	MO	Neosho	b,v
Hopewell Furnance Natl. Historic Site	PA	West Chester	a,v
Indiana Dunes Natl. Lakeshore	IN	Hobart 2WNW	a,v
Jean LafFitte Natl. Historic Park	LA	New Orleans	a,x
Lake Meredith Natl. Recreation Area	TX	Amarillo	a,w
Mammoth Cave Natl. Park	KY	Bowling Green FAA AP	a,v
Montezuma Castle Natl. Monument	AZ	Childs	a,v
Moore's Creek Natl. Battlefield	NC	Southport 5N	a,v
Ozark Natl. Scenic River	MO	Doniphan	b,v
Pipestone Natl. Monument	MN	Pipestone	b,v
Richmond Natl. Battlefield Park	VA	Hopewell	a,v
Saratoga Natl. Historic Park	NY	Saratoga Springs 4SW	a,v
Scottsbluff Natl. Monument	NE	Scottsbluff	b,z
St. Croix Natl. Scenic Riverway	WI	Ashland Experimental Farm	b,v
Wind Cave Natl Park	SD	Hot Springs	a,v
Zion Natl. Park	UT	Zion National Park	a,v

[a] Sources (see bibliography for full citations):
Flora: a = NPS (no date), b = WICPSU (no date);
Climate: v = NCDC (1994), w = NCDC (1993a), x = NCDC (1993b), y = NCDC (1993c), z = NCDC (1993d).