

C₃ and C₄ photosynthesis in *Cyperus* (Cyperaceae) in temperate eastern North America

Mei-Rong Li, David A. Wedin, and Larry L. Tieszen

Abstract: All species in the genus *Cyperus* (Cyperaceae) that occur in temperate eastern North America were assigned to either the C₃ or C₄ photosynthetic pathway using leaf anatomical characteristics and stable carbon isotope ratios. Of the 39 species in the study area, 32 had C₄ photosynthesis while 7 had C₃. The numbers of C₃, C₄, and total *Cyperus* species were significantly and positively correlated with both summer precipitation and mean annual temperature. However, the proportional abundance of C₄ species within *Cyperus* was not significantly related to either climatic variable. The highest *Cyperus* diversity was found on the Atlantic Coastal Plain, a pattern that remained after climatic differences between regions were accounted for. All the C₃ species and the majority of the C₄ species were restricted to wetlands or damp soil habitats; 13 of the 32 C₄ species occurred in dry, sandy habitats. Given that the C₃ pathway is ancestral in the genus *Cyperus*, it appears that C₄ photosynthesis evolved in a wetland context for this genus. We suggest that the high nitrogen use efficiency associated with the C₄ pathway is largely responsible for the evolution and ecological success of C₄ *Cyperus* species in infertile, temperate wetlands.

Key words: C₃ and C₄ photosynthetic pathways, *Cyperus*, Cyperaceae, eastern North America, stable carbon isotopes.

Résumé : Les auteurs ont attribué chacune des espèces du genre *Cyperus* (Cyperaceae) de la région tempérée de l'est de l'Amérique du Nord, au sentier photosynthétique C₃ ou C₄ en utilisant des caractéristiques foliaires et les proportions d'isotopes stables du carbone. Sur les 39 espèces qui se retrouvent dans cette région, 32 suivent le sentier photosynthétique C₄ alors que 7 utilisent le sentier C₃. Les quantités d'espèces en C₃, C₄ et le nombre total d'espèces de *Cyperus* montrent une corrélation positive significative avec à la fois les précipitations estivales et les températures annuelles moyennes. L'abondance proportionnelle des espèces en C₄ dans le genre *Cyperus* n'est cependant pas significativement reliée à aucune des deux variables climatiques. La plus grande diversité de *Cyperus* se retrouve sur la plaine côtière Atlantique, un patron qui persiste après avoir pris en compte les différences climatiques entre les régions. Toutes les espèces en C₃ et la majorité des espèces en C₄ sont restreintes aux terres humides et aux habitats avec sol boueux, alors que 13 des plantes en C₄ se retrouvent dans des habitats secs et sablonneux. Compte tenu que le sentier C₄ apparaît comme ancestral dans le genre *Cyperus*, il semble que dans ce genre, la photosynthèse en C₄ aurait évolué dans des conditions de terres humides. Les auteurs suggèrent que la grande efficacité d'utilisation de l'azote associée au sentier C₄ serait largement responsable de l'évolution et du succès écologique d'espèces en C₄ telles que les *Cyperus*, dans des terres humides tempérées infertiles.

Mots clés : sentiers photosynthétiques C₃ et C₄, *Cyperus*, Cyperaceae, est de l'Amérique du Nord, isotopes stables du carbone.

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Introduction

The C₄ photosynthetic pathway has apparently evolved multiple times and is found in a wide range of vascular plant families (Ehleringer and Monson 1993). It is generally ac-

cepted that the C₄ pathway is primarily an adaptation to hot, often dry, high irradiance conditions. This conclusion is based on both the distribution patterns of C₄ plants, which tend to occur in warm (tropical to temperate) and dry (arid to subhumid) regions (Stowe and Teeri 1978; Tieszen et al. 1979; Ehleringer and Monson 1993), and on a mechanistic understanding of C₄ photosynthesis. By concentrating CO₂ near the site of carboxylation (the enzyme Rubisco), C₄ species attain lower CO₂ compensation points and minimal photorespiration. This allows higher net photosynthesis for C₄ species compared with C₃ species under conditions of high leaf temperatures, moisture stress, and high irradiance (Hatch 1987; Ehleringer and Monson 1993; Ehleringer et al. 1997).

Given the polyphyletic origin of C₄ photosynthesis, however, a more diverse explanation of its evolutionary and ecological significance is merited (Hattersley 1983). Ehleringer

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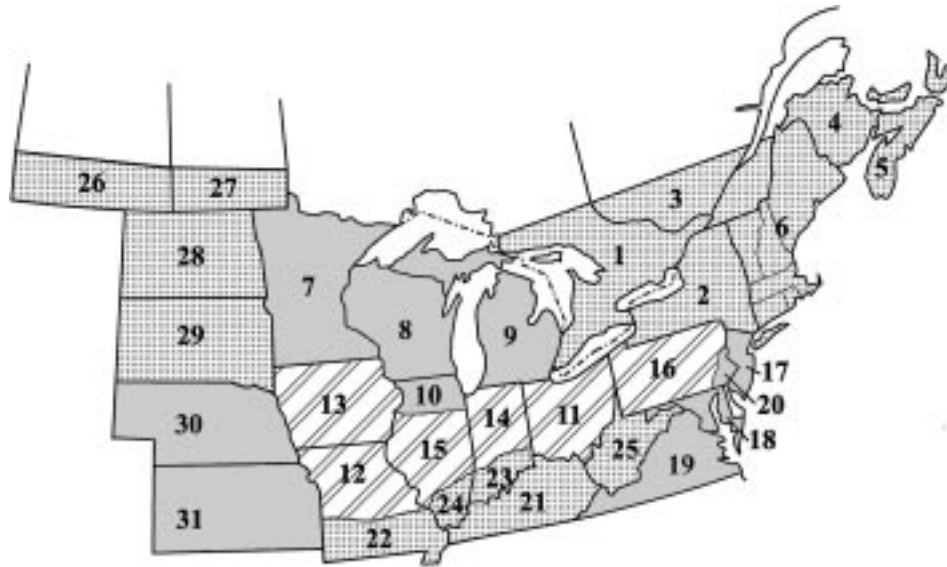
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Fig. 1. Map of regions ($n = 7$) and subregions ($n = 31$) of temperate eastern North America. See Table 2 for the *Cyperus* floras of subregions corresponding to the numbers on the map.



et al. (1991) argued that the C_4 pathway may also be an adaptation to low atmospheric CO_2 concentrations that occurred in the geologic past. Some C_4 species, particularly members of the Cyperaceae, occur predominantly in cool wet environments (Li 1993a; Li and Jones 1994, 1995). Li (1993b) concluded that the high photosynthetic nitrogen use efficiency (the rate of CO_2 fixation per unit leaf nitrogen or NUE) of C_4 members of the genus *Cyperus* was important to the success of these species in temperate European wetlands. The polyphyletic origin of C_4 photosynthesis also suggests that phylogenetic constraints may confound interpretation of biogeographic patterns of C_4 distribution, a point previously raised but not well incorporated into studies of C_4 distribution (Stowe and Teeri 1978; Teeri et al. 1980; Ehleringer and Monson 1993).

We examined the occurrence of C_3 and C_4 photosynthesis in the genus *Cyperus* in temperate eastern North America ($37\text{--}50^\circ\text{N}$, $55\text{--}104^\circ\text{W}$). The genus *Cyperus* includes approximately 700 species worldwide and is widely distributed in both wet and dry habitats in tropical and temperate regions. Approximately 90 *Cyperus* species occur in North America (Tucker 1986; G.C. Tucker, personal communication). The genus is taxonomically complex, containing both C_3 and C_4 species (Lerman and Raynal 1972; Hesla et al. 1982). Thus, it is one of a small group of genera, including *Panicum*, *Eragrostis*, *Neurachne*, and *Alloteropsis* in the Poaceae, *Eleocharis* and *Cyperus* in the Cyperaceae, and *Atriplex* in the Chenopodiaceae, that contain both photosynthetic pathways. Although the C_4 pathway evolved several times in the Cyperaceae, it probably arose only once in the genus *Cyperus* (Goetghebeur 1989).

Approximately 70 years before the C_4 photosynthetic pathway was identified in *Cyperus* by Hatch and co-workers (Hatch et al. 1967), Rikli (1895) surveyed the leaf anatomy of *Cyperus* species and divided them into two subgenera, *Eucyperus* and *Chlorocyperus*. The latter was characterized by radiate chlorenchyma (i.e., Kranz anatomy), while the former was nonradiate. Lerman and Raynal (1972) confirmed the presence of C_3 and C_4 photosynthesis in *Cyperus*

using stable carbon isotope ratios. Although the photosynthetic pathway of most *Cyperus* species could be inferred by their classification into subgenera, for many of the species their C_3 or C_4 status and the basis of that determination (e.g., leaf anatomy, carbon isotopic signature) have not been reported. Precise determination of photosynthetic pathway in these species is required for a modern classification of the genus.

The appropriate classification of the genus into subgenera is still debated, however, and is not the focus of our paper. Rather, we examined the climatic and habitat variables associated with the distribution of C_3 and C_4 *Cyperus* in temperate eastern North America. Finally, we ask if the distribution patterns of C_4 *Cyperus* in this region support current generalizations about the ecological and evolutionary significance of the C_4 pathway (Tieszen et al. 1979; Teeri et al. 1980; Ehleringer and Monson 1993).

Materials and methods

Study region and species lists

Our study area was the region covered by Gleason and Cronquist (1991), which also formed our primary species list (Fig. 1). Their flora covers the states north of 37°N latitude as well as New Brunswick, Nova Scotia, and portions of Ontario and Quebec south of 47°N . We extended the study region west to approximately 104°W and north to 50°N to include Kansas, Nebraska, South and North Dakota, and southern Manitoba and Saskatchewan. Statewide or regional species lists were compiled using 28 floras and checklists (see Table 2). Our final species total of 39 exceeds the total of 37 in Gleason and Cronquist (1991) because of the inclusion of 2 additional species (*C. setigerus* and *C. aggregatus*) listed in the regional floras. Our nomenclature follows Gleason and Cronquist (1991) except for *C. aggregatus*, which was previously treated as *C. cayennensis* (G.C. Tucker, personal communication).

The floras were also used to classify the species as to habitat moisture status (five classes: emergent aquatic, wetland, damp soil, mesic, and dry). Also noted were whether the species occurs on mineral (sand or clay) or organic soils and whether the habitat is open (including dry open woodlands) or forested. Although habitat

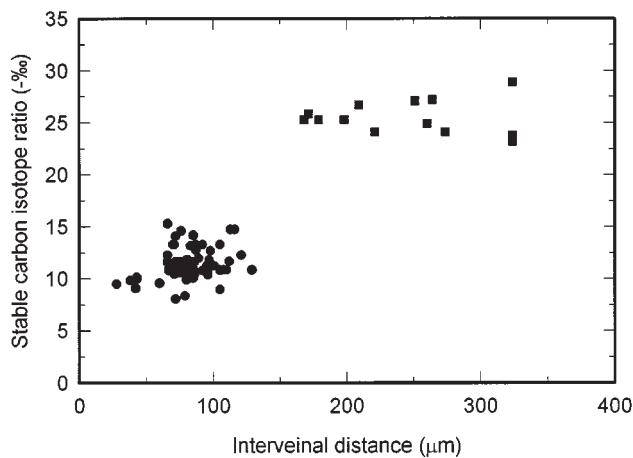
Table 1. Leaf characteristics of *Cyperus* species from temperate eastern North America.

Species	HP	K	$\delta^{13}\text{C}$	C ₃ or C ₄	Source of plant material or reference
<i>C. acuminatus</i> T. & H.	-	-		C ₃	NYB-Nee No. 25449, NYS-Bryson No. 6204, UNO No. 19553, NCU-Demaree No. 56594, GH-Baker No. 6164
<i>C. aggregatus</i> (Willd.) Endl.	-	-		C ₃	TRT No. 46265
<i>C. bipartitus</i> Torr.	+	+	-14.7	C ₄	TRT No. 241056, TRT No. 175612, NYS-Tucker No. 3570, NYB-CA-1896, TRT No. 239622, TRT No. 247898, TRT No. 239621, TRT No. 184777, CC-Delaney-82
<i>C. brevifolioides</i> T. & D.	-	+		C ₄	Takeda et al. 1985
<i>C. compressus</i> L.	+	+	-11.0	C ₄	Druyts-Voets 1970; Downton 1975; Hesla et al. 1982
<i>C. croceus</i> Vahl	-	+	-12.0	C ₄	Downton 1975
<i>C. dentatus</i> Torr.	-	-	-27.1	C ₃	TRT No. 241106, TRT No. 158535, TRT No. 7226
<i>C. diandrus</i> Torr.	+	+	-10.9	C ₄	TRT No. 175613, TRT No. 85742, NYS No. A4541, CC-Delaney- 87, NYB-Crutchfield No. 2050
<i>C. difformis</i> L.	-	-	-26.6*	C ₃	TRT No. 42469; Hesla et al. 1982; Kalapos et al. 1997
<i>C. echinatus</i> (L.) Wood	-	+	-14.0	C ₄	Li 1993a
<i>C. erythrorhizos</i> Muhl.	-	+		C ₄	TRT No. 110182, TRT No. 212724, TRT No. 182838
<i>C. esculentus</i> L.	+	+	-12.7	C ₄	TRT No. 29955, CC-Delaney-84; Hesla et al. 1982
<i>C. filicinus</i> Vahl.	+	+	-10.8*	C ₄	TRT No. 151783, TRT No. 7753
<i>C. filiculmis</i> Vahl.	-	+	-13.3	C ₄	TRT No. 240296, NCU-Nighswonger No. 1913; Downton 1975
<i>C. flavescens</i> L.	+	+	-10.1	C ₄	TRT No. 29958; Kalapos et al. 1997
<i>C. flavicomus</i> Michx.	-	+		C ₄	GH-Fernald No. 3712, NYS No. A338, NYB-Tucker No. 2995
<i>C. fuscus</i> L.	-	-	-26.5*	C ₃	Li 1993a; Kalapos et al. 1997
<i>C. grayi</i> Torr.	-	+	-12.3*	C ₄	NYS-Fernald-1918, NYB-NJ-1984, NCU Rothrock No. 1210, TRT No. 223119
<i>C. grayioides</i> Mohl.	+	+	-12.3	C ₄	NCU-Orzel No. 8626, NYB-Carter No.8251, GH-Carter No. 8263
<i>C. haspan</i> L.	-	-	-28.3	C ₃	Druyts-Voets 1970; Hesla et al. 1982
<i>C. houghtonii</i> Torr.	-	+	-11.2	C ₄	TRT No. 239652, TRT No. 242578
<i>C. hystericinus</i> Fern.	-	+		C ₄	TRT No. 218495
<i>C. iria</i> L.	+	+	-14.2	C ₄	Metcalf 1971; Downton 1975; Hesla et al. 1982
<i>C. lancastricensis</i> P. ex G.	-	+	-11.5	C ₄	TRT No. 151819
<i>C. lupulinus</i> (Spreng) Mar.	-	+	-11.0	C ₄	TRT No. 61636, TRT No. 162937, TRT No. 109921, CC-Delaney-80
<i>C. microiria</i> Steud.	-	+		C ₄	GH-URSS-1964, NYS No. A15080, NYB-Inamasu No. 425
<i>C. odoratus</i> L.	+	+	-11.3*	C ₄	TRT No. 224211, TRT No. 246235, TRT No. 247703, TRT No. 246182, TRT No. 182014, TRT No. 190566, CC-Delaney-87, CC-Delaney-86b, TRT No. 166496, TRT No. 189573, TRT No. 108573
<i>C. plukenetii</i> Fern.	-	+		C ₄	TRT No. 156610
<i>C. polystachyos</i> Rottb.	-	+	-9.6	C ₄	TRT No. 241121; Downton 1975; Takeda et al. 1985
<i>C. pseudovegetus</i> Steud.	-	-		C ₃	TRT No. 151097, NYS-Crane-1980, NCUBell No. 3616, NYB-Okrrylour No. 1898, GH-Demaree No. 29095
<i>C. refractus</i> Engelm.	-	+		C ₄	TRT No. 7780
<i>C. retrofractus</i> (L.) Torr.	-	+		C ₄	TRT No. 172713
<i>C. retrorsus</i> Champ.	-	+	-10.5	C ₄	TRT No. 151030, TRT No. 3250
<i>C. rotundus</i> L.	+	+	-11.1	C ₄	TRT No. 100649; Hesla et al. 1982
<i>C. schweinitzii</i> Torr.	-	+	-11.8	C ₄	TRT No. 221239, TRT No. 199979, TRT No. 181316, CC-Delaney-85
<i>C. setigerus</i> Toor. & Hook.	+	+		C ₄	NCU-Lipscomb No. 1949, GH-Bush No.7644, GH-Cory No. 25388, NYS-1891, NYB-Bush No. 7644a
<i>C. squarrosus</i> L.	-	+	-10.9*	C ₄	TRT No. 224891, TRT No. 68543
<i>C. strigosus</i> L.	-	+	-10.1*	C ₄	TRT No. 240484, TRT No. 83143, CC-Delaney-88
<i>C. tenuifolius</i> (Steud.) Dan.	-	+		C ₄	TRT No. 151853

Note: Leaf characteristics are as follows: HP, presence of hypodermis; K, Kranz anatomy; $\delta^{13}\text{C}$, leaf stable carbon isotope ratio in ‰; C₃ or C₄, photosynthetic pathway. Sources of plant material are as follows: CC, Cedar Creek Natural History Area, University of Minnesota; GH, Gray Herbarium; NCU, North Carolina University Herbarium; NYS, Herbarium of New York State Museum; NYB, Herbarium of New York Botanical Garden; TRT, Herbarium of Royal Ontario Museum; UNO, Herbarium of the University of Nebraska at Omaha.

*Mean from multiple samples.

Fig. 2. The relationship between stable carbon isotope ratio ($\delta^{13}\text{C}$) and interveinal distance in C_3 (■) and C_4 (●) *Cyperus* species. Values represent individual specimens from this study (see Table 1) as well as data on European *Cyperus* species (Li 1993a; Li and Jones 1994).



descriptions are often vague in floras, multiple floras were used for each species and in almost all cases there was general agreement across sources.

We divided the 25 states and six provinces in the study into seven regions and 31 subregions (Table 2; Fig. 1). Long-term averages (in most cases >30 years) for mean annual temperature, minimum daily temperature in July, total annual precipitation, and summer (June to August) precipitation were obtained for a centrally located long-term weather station in each subregion (Williams 1995).

Plant materials

Herbarium specimens from the Royal Ontario Museum (Toronto, Ont.) were the major source of plant material. Specimens from seven other herbaria were also included. Ninety-four specimens were examined. About 2 cm of mature leaf from each specimen was removed for anatomical and isotopic analysis. Leaves were hand-sectioned after soaking in distilled water for 48 h and examined by light microscopy at 100–400× magnification. Sections were stained with iodine to show starch grains in the vascular bundle sheath cells for C_4 species. A species was classified as C_4 if its leaves showed Kranz-type anatomy (Laetsch 1974; Hatch 1987).

Interveinal distances (the distance between vein centers) were measured using an optical micrometer. For each sample, the mean interveinal distance is based on values from several vascular bundle pairs in each of three to six fields of view. Previous work with *Cyperus* indicated that a species is C_4 if its leaf interveinal distance is less than 130 μm (Takeda et al. 1980; Li and Jones 1994).

The presence of a hypodermal layer was determined with a light microscope on leaf cross sections. The hypodermis consists of at least one and sometimes more layers of translucent cells (with few or no chloroplasts) between the adaxial epidermis and the chlorenchyma (Metcalf 1971; Li and Jones 1994). The presence of a hypodermis in some species was also confirmed by comparison with Druyts-Voets (1970) and Metcalf (1971).

The stable carbon isotope ratio ($\delta^{13}\text{C}$) was measured on leaf sections from approximately 30 of the specimens. Samples were chosen for isotopic analysis if the anatomical observations were not definitive (i.e., clear Kranz anatomy, bundle sheath starch granules, and short interveinal distances in the case of C_4 species) or if different specimens of the same species gave conflicting classifications. Tissue samples were combusted, cryogenically purified, and analyzed by a continuous flow system consisting of a Carlo-Erba

CHN analyzer and a VG Micromass SIRA 10 isotope ratio mass spectrometer. The $\delta^{13}\text{C}$ value was calculated as

$$[1] \quad \delta^{13}\text{C} = \frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}_{\text{sample}}} - \frac{^{13}\text{C}}{^{12}\text{C}_{\text{reference}}} \right)}{\left(\frac{^{13}\text{C}}{^{12}\text{C}_{\text{reference}}} \right)} \times 1000$$

Values are referenced to the PDB standard and presented on a per mil (‰) basis. Plants with the C_3 photosynthetic pathway are consistently more depleted in ^{13}C ($\delta^{13}\text{C}$ generally –24 to –30‰) than C_4 plants ($\delta^{13}\text{C}$ generally –10 to –15‰) (Hesla et al. 1982; Farquhar 1983).

Results

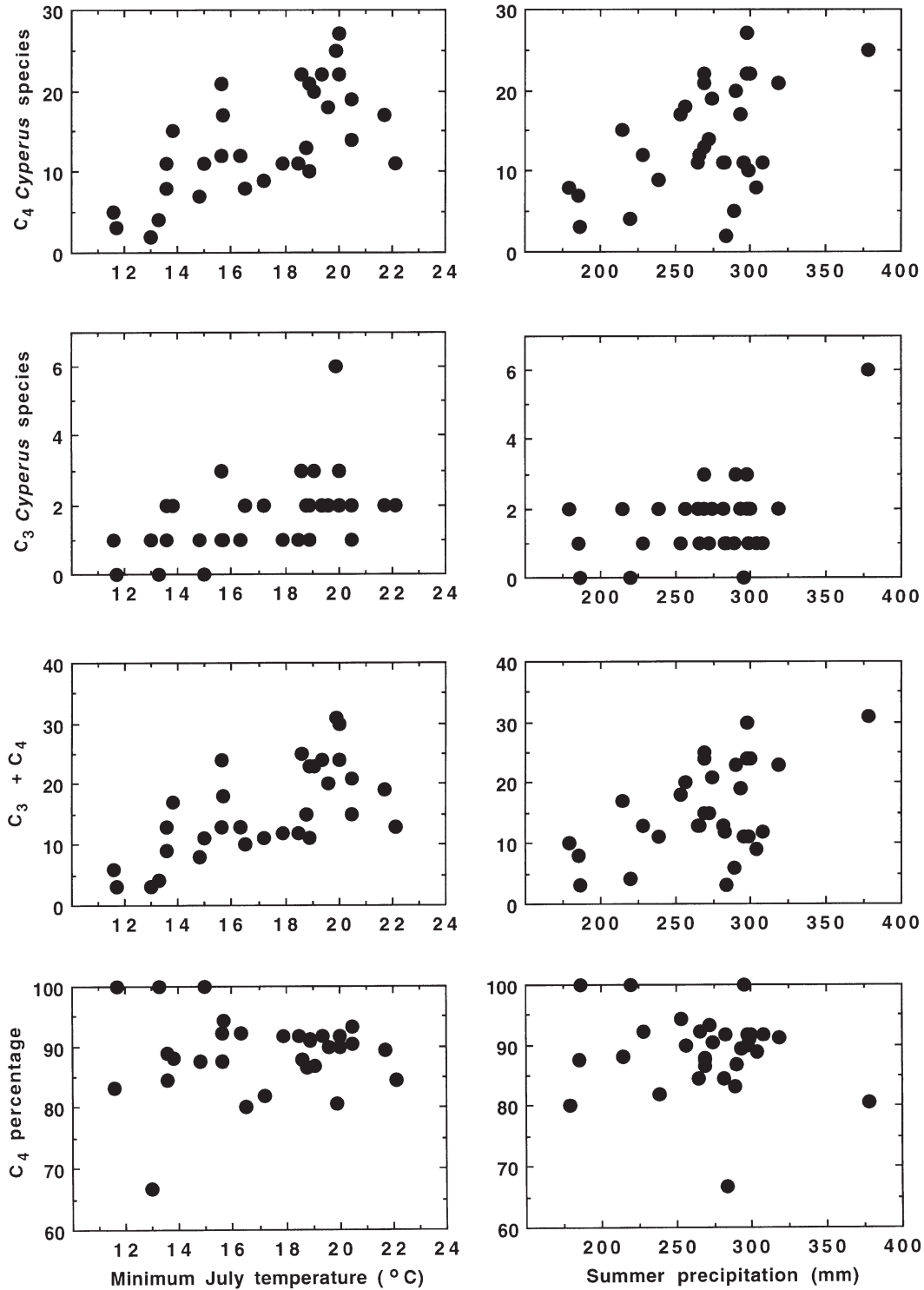
Photosynthetic pathway

Of the 39 *Cyperus* species found in temperate eastern North America, 32 species (82%) had the C_4 photosynthetic pathway while 7 species (18%) had C_3 (Table 1). For 19 species, photosynthetic pathway is reported for the first time. Assignment to a photosynthetic pathway was based on the presence of Kranz-type anatomy, and for 19 species, on stable carbon isotope ratios. Isotopic signatures ($\delta^{13}\text{C}$) for C_4 species ranged from –9.6 to –14.7‰ (mean = –11.7 ‰), while for C_3 species $\delta^{13}\text{C}$ ranged from –26.5 to –28.3‰ (mean = –27.1‰). These $\delta^{13}\text{C}$ values are comparable to previously published values for C_3 and C_4 plants (Hesla et al. 1982; Farquhar 1983; Li 1993a).

The analyses also supported the previous conclusion that within *Cyperus*, interveinal distance differs between C_3 and C_4 species (Takeda et al. 1980; Li and Jones 1994). When our data on temperate North American *Cyperus* were combined with data on European *Cyperus* species (Li 1993a; Li and Jones 1994), distinct ranges for both interveinal distance and stable C isotope ratio were found for C_3 and C_4 species (Fig. 2). We also examined leaves of all species for the presence of a hypodermis as a possible diagnostic tool in distinguishing C_3 versus C_4 or dryland versus wetland species (Table 1; see below for a discussion of habit distribution patterns). None of the C_3 species had a hypodermis, while 11 of 22 C_4 species did. Within the C_4 species, a hypodermis was found in roughly half of the wetland species (6 out of 14), but in only 1 of 13 dry habitat species. Although these patterns merit further examination, the presence or absence of a hypodermis seems not to be diagnostic for either photosynthetic pathway or habitat within the genus *Cyperus*.

The occurrence of the two photosynthetic pathways in our subset of 39 species is generally consistent with classifications of the genus *Cyperus* (Goetghebeur 1989; Gleason and Cronquist 1991; Tucker 1983, 1994). Within the subgenus *Eucyperus*, the section *Choristachys* contained 15 C_4 species while the section *Pycnostachys* contained six C_3 species. The subgenus *Mariscus* contained 15 C_4 species and one C_3 (*C. aggregatus*). The other 10 species, all C_4 , were in the subgenera *Pycneus* (7 species), *Kyllinga* (2 species), and *Torulium* (1 species).

Fig. 3. The numbers of C_3 , C_4 , and total *Cyperus* species in 31 subregions of temperate eastern North America versus either summer (June–August) precipitation or average minimum July daily temperature. Regression results are given in Table 3.



Distribution

Consistent with the strong tropical affiliation of the genus *Cyperus* (Tucker 1983, 1994), most of the species found in temperate eastern North America also occur in subtropical North America or in tropical Central America (25 of 39 species). The remaining 14 species appear to be primarily temperate. Six of the species in our checklist were introduced from Eurasia: *C. brevifolioides* (C_4), *C. iria* (C_4),

C. microiria (C_4), *C. aggratus* (C_3), *C. fuscus* (C_3), and *C. difformis* (C_3).

Within our study area, 37 of the *Cyperus* species were found on the Atlantic coastal plain region (i.e., from Virginia north to the coastal plain region of eastern Pennsylvania; Fig. 1). Only two (*C. grayoides* and *C. setigerus*) were absent from this region. West of the coastal plain, the number of *Cyperus* species decreased to 31 in the southern region

Table 2. A checklist of *Cyperus* species found in temperate eastern North America.

Habitat	Northeast					North				Central					Atlantic coastal plain				South					Northern Great Plains				Central Great Plains				
	ON	NY	QB	NB	NS	NE	MN	WI	MI	n.IL	OH	n.MO	IA	n.IN	c.IL	w.PA	NJ	MD	VA	e.PA	KY	s.MO	s.IN	s.IL	WV	SA	MT	ND	SD	NA	KS	
C₄ species																																
<i>C. bipartitus</i>	2, 3	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	
<i>C. brevifolioides</i>	2, 3	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	
<i>C. compressus</i>	3, 4	-	+	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	
<i>C. croceus</i>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	
<i>C. diandrus</i>	2, 3	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	
<i>C. echinatus</i>	5	-	+	-	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	
<i>C. erythrorhizos</i>	2, 3	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	
<i>C. esculentus</i>	2, 3, 4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	
<i>C. filicinus</i>	2, 3	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	
<i>C. filiculmis</i>	5	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	
<i>C. flavescens</i>	2, 3	+	+	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+
<i>C. flavicomus</i>	2, 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	
<i>C. grayi</i>	5	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
<i>C. grayioides</i>	4, 5	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. houghtonii</i>	4, 5	+	+	+	-	-	+	+	-	+	+	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
<i>C. hystricinus</i>	4, 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	
<i>C. iria</i>	2, 3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	
<i>C. lancastricensis</i>	3, 4	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	
<i>C. lupulinus</i>	5	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	+	+	+	-	-	+	-	-	-	-	-	-	+	+	+	
<i>C. microiria</i>	2, 3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	
<i>C. odoratus</i>	2, 3	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	
<i>C. plukenetii</i>	5	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	
<i>C. polystachyos</i>	2, 3	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	
<i>C. refractus</i>	4, 5	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	
<i>C. retrofractus</i>	4, 5	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	
<i>C. retrorsus</i>	4, 5	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
<i>C. rotundus</i>	3, 4	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	+	
<i>C. schweinitzii</i>	4, 5	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	-	-	-	+	
<i>C. setigerus</i>	2, 3, 4	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+		
<i>C. squarrosus</i>	2, 3	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
<i>C. strigosus</i>	1, 2, 3	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	
<i>C. tenuifolius</i>	2, 3	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+
C₄ total		11	21	8	5	2	15	12	11	12	13	17	17	10	11	14	22	20	22	25	27	21	22	18	11	11	3	4	7	8	9	19

Table 2. (concluded).

Habitat	Northeast			North			Central			Atlantic coastal plain			South			Northern Great Plains			Central Great Plains																	
	ONNY	QBNB	NS NE	MNW	MI n.	IL	OH n.	MO	IA	n.	IN	c.	IL	w.	PA	NJ	MD	VA	e.	PA	KY	s.	MO	s.	IN	s.	IL	WV	SA	MT	ND	SD	NA	KS		
<i>C. acuminatus</i>	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	
<i>C. aggregatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. dentatus</i>	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. difformis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. fuscus</i>	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. haspan</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. pseudovegetus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C₃ total</i>	2	3	1	1	2	1	2	1	2	1	1	1	3	3	2	6	3	2	6	3	2	2	2	2	2	2	1	0	0	0	1	2	2	2	2	2
No. on map (Fig. 1)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	31	31	31	31	

Note: Habitat codes are as follows: 1, emergent aquatic; 2, wetland; 3, damp soil; 4, mesic; 5, dry soil. Abbreviations for the subregions are as follows: IA, Iowa; IL, Illinois; IN, Indiana; KS, Kansas; KY, Kentucky; MD, Maryland; MI, Michigan; MN, Minnesota; MO, Missouri; MT, Manitoba; NA, Nebraska; NB, New Brunswick; ND, North Dakota; NE, New England; NJ, New Jersey; NS, Nova Scotia; NY, New York; OH, Ohio; ON, Ontario; PA, Pennsylvania; QB, Quebec; SA, Saskatchewan; SD, South Dakota; VA, Virginia; WI, Wisconsin; WV, West Virginia. The references for floras in all subregions following the numbers on the map (Fig. 1) are as follows: 1–31, Gleason and Cronquist (1991); 1, Morton and Venn (1990); 2, Mitchell (1986); Taylor (1915); 3, Marie-Victorin (1964); 4, Hind (1986); 5, Roland and Smith (1966); 6, Ahmadjian and Moser (1979); Seymour (1969); 7, Ownbey and Morley (1991); 8, Marcks (1974); Seymour (1960); 9, Voss (1972); 10, Swink and Wilhelm (1994); 11, Braun (1967); 12 and 22, Steyermark (1964); 14, Peattie (1930); 15 and 24, Mohlenbrock (1976); 17, Stone (1973); 18, Tattall (1946); 16 and 20, Wherry et al. (1979); 21, Browne and Athey (1992); 25, Strausbaugh and Core (1970); 26 and 27, Looman and Best (1987); Scoggan (1957); 31, Barkley (1968); 26–31, Barkley (1977, 1986).

(north of 37°N latitude and excluding the coastal plain of Virginia) and 32 in the central region. Further west, 22 species occurred in the central Great Plains, 18 in the north central states, and 14 in the northern Great Plains. North of the coastal plain, the number dropped to 26 species in the north-east region.

When analyzed using climatic data for the 31 subregions, the number of *Cyperus* species was strongly and positively correlated with mean annual temperature ($r^2 = 0.513$, Table 3). This pattern also held for the number of either C_3 or C_4 species. Thus, the proportional abundance of C_4 species was not significantly dependent on mean annual temperature ($r^2 = 0.011$). Teeri and Stowe (1976) and Teeri et al. (1980) suggested that the average minimum daily temperature in July was well correlated with the abundance of C_4 species. In our case this parameter gave comparable, although slightly weaker, results to mean annual temperature (Fig. 3). The pattern was the same: the numbers of C_3 , C_4 , or total *Cyperus* species were positively correlated with minimum July temperature, while the proportional abundance of C_4 species showed no relationship.

The number of *Cyperus* species was also positively correlated with summer precipitation ($r^2 = 0.268$, Table 3; Fig. 3). Because this pattern held for both C_3 and C_4 species, the proportional abundance of C_4 species was independent of summer precipitation. The number of *Cyperus* species formed a nonlinear relationship with annual precipitation, but because this pattern was driven by unusually high winter precipitation in the Maritime region of Canada, we disregarded it. When both minimum July temperature and summer precipitation were considered together in a multiple regression ($r^2 = 0.505$), temperature was a highly significant predictor of number of *Cyperus* species (partial $F = 13.41$, $P = 0.001$), while precipitation was almost significant (partial $F = 3.79$, $P = 0.062$). For both the number of C_3 species and the number of C_4 species, temperature was a significant predictor in multiple regression, whereas precipitation was not.

To test if strong regional differences in the number of *Cyperus* species simply reflected climatic gradients, we performed an analysis of covariance with region as a categorical predictor and minimum July temperature as a continuous predictor (summer precipitation was insignificant and dropped from the model). Region remained a significant predictor of species number (partial $F = 2.94$, $P = 0.028$) with temperature (partial $F = 4.36$, $P = 0.048$) in the model. When species numbers were corrected for temperature differences (i.e., least squares means), the average number of *Cyperus* species per subregion was 24 in the Atlantic coastal plain, 17 in the northeast, and 15 in the south. The central, north central, and southern Great Plains regions all had, on average, 14 species per subregion when corrected for temperature differences, while the northern Great Plains had 10.

Habitat

Although our coding with respect to habitat was qualitative and based on comments in various floras, several patterns are clear. All the C_3 *Cyperus* species were restricted to either wetlands or damp soils (Table 2). In contrast, 13 of 32 C_4 species occurred in a dry habitat, and 18 of 32 C_4 species occurred in habitats coded as mesic or dry. In almost all

Table 3. Regression analyses comparing the dependent variables of the numbers of C₃ species, C₄ species, total *Cyperus* species, and the percentage of C₄ species in 31 regions of temperate eastern North America with the independent variables of mean annual temperature (°C), average minimum July daily temperature (°C), and June to August precipitation (mm).

Dependent variable	Intercept	Slope	r ²	P
Mean annual temperature				
No. of C ₄ species	1.271	1.287	0.510	<0.001
No. of C ₃ species	0.019	0.173	0.307	<0.001
No. of C ₃ + C ₄	1.291	1.460	0.513	<0.001
% C ₄ species	90.75	-0.187	0.011	0.567
Average minimum July daily temperature				
No. of C ₄ species	-11.712	1.487	0.441	<0.001
No. of C ₃ species	-1.559	0.191	0.240	0.005
No. of C ₃ + C ₄	-13.272	1.678	0.438	<0.001
% C ₄ species	88.16	0.044	0.000	0.914
June to August precipitation				
No. of C ₄ species	-7.803	0.081	0.255	0.004
No. of C ₃ species	-1.787	0.013	0.221	0.008
No. of C ₃ + C ₄	-9.59	0.093	0.268	0.003
% C ₄ species	94.59	-0.021	0.019	0.464

cases, these dry habitats were dry sandy soils, beaches, or dunes. The remaining C₄ species are restricted to wetlands or damp soils. One of the C₄ species, *C. strigosus*, sometimes occurs as an aquatic emergent. Several weedy C₄ species, including *C. esculentus*, *C. setigerus*, and *C. rotundus*, have a broad habitat tolerance that ranges from wetlands to mesic habitats. The proportion of C₄ species in a subregion coded as dry averaged 33% and was not significantly related to precipitation, temperature, or region. All the dry-soil C₄ species were in the subgenus *Mariscus*. The other four subgenera with C₄ species (*Eucyperus*, *Pycrus*, *Kyllinga*, and *Torulinium*) lacked dry-habitat species.

Approximately two-thirds of the *Cyperus* species (both C₃ and C₄) were described as occurring on sandy soils (either wet or dry), a pattern consistent with the high species numbers on the Atlantic Coastal Plain, where sandy habitats are widespread. None of the species were described as occurring in closed canopy or forested habitats, although about 15% were described as occurring in woods, dry woodlands, or barrens.

Discussion

Across the angiosperms, most C₄ species are generally thought to occur in hot, semi-arid to arid, high-light environments. However, the evolutionary background and ecological significance of the C₄ pathway is diverse (Stowe and Teeri 1978; Moore 1982; Ehleringer et al. 1991, 1997). The approximately 8000 C₄ species occur across 20 dicotyledon and 5 monocotyledon families (Sage et al. 1999a). Within temperate eastern North America, the genus *Cyperus* contains predominantly C₄ species (82%), which is similar to the worldwide pattern (e.g., 70% C₄ in East Africa; Hesla et al. 1982). In spite of this C₄ dominance in *Cyperus*, the C₃ photosynthetic pathway appears to be ancestral in the genus. Goetghebeur (1989) identified the subgenus *Pycnostachys*, which contains most of the C₃ *Cyperus* species, as the most primitive section. In an alternative taxonomic treatment of

the family, Tucker (1983) described the ancestral group *Protocyperus* as the only subgenus with C₃ photosynthesis. Considering that almost all C₃ *Cyperus* species and the majority of C₄ species occur in wetland or moist soil habitats (Hains and Lye 1983; Li 1993a; Ueno and Takeda 1992), it is reasonable to conclude that the C₄ photosynthetic pathway evolved in a wetland habitat within the genus *Cyperus*.

Jones et al. (1981) noted that certain *Cyperus* species are some of the most cold-tolerant of all C₄ plants. The presence of C₄ species that are primarily adapted to moist, temperate environments calls into question common conclusions about selection pressures leading to the evolution and radiation of the C₄ photosynthetic pathway (Beale and Long 1995). What factors might account for the success of *Cyperus* in these relatively atypical habitats for C₄ species? If we assume that sandy soils are generally infertile, we can conclude that in temperate eastern North America, C₄ species in the genus *Cyperus* are generally found in high light, low nutrient environments that range from wet to dry conditions. Although the high leaf level NUE of C₄ plants is well established (Brown 1978; Sage and Pearcy 1987; Li 1993b), the evolutionary advantages of the C₄ photosynthetic pathway are generally accepted to be greater stomatal control, lower CO₂ compensation points, and the avoidance of photorespiration under hot or high irradiance conditions (Hatch 1987; Ehleringer et al. 1991; Ehleringer and Monson 1993). This generalization implicitly assumes that the high NUE of C₄ plants is a correlate rather than a cause of the evolution of the C₄ pathway. We suggest that at least for the genus *Cyperus*, the high NUE resulting from efficient use of the key photosynthetic enzyme Rubisco may have had significant adaptive value (Sage et al. 1999b). In a greenhouse study of nutrient use in 41 Ontario wetland species (McJannet et al. 1995), species were ranked according to leaf tissue N concentration, an index of NUE. Only 4 of the 41 species were C₄ (including *C. squarrosus* and *C. bipartitus*), but these species represented 4 of the 6 species with the lowest tissue nitrogen concentrations. Although

C_4 species, including *Cyperus*, comprise a small share of the flora in cool temperate wetlands, they may have a competitive advantage in low nutrient and, in particular, sandy environments.

The distribution and proportional abundance of C_4 species worldwide appears to be strongly related to climate. Surveys of various families suggest that minimum July daily temperatures are strongly correlated with the abundance of C_4 species (Teeri and Stowe 1976; Teeri et al. 1980). Our study supports this: no C_4 *Cyperus* species occurred in regions with minimum July daily temperatures lower than 10°C, the threshold proposed for both the Cyperaceae and Gramineae (Teeri and Stowe 1976; Teeri et al. 1980). The number of *Cyperus* species (both C_3 and C_4) was strongly correlated with either mean annual temperature or minimum July daily temperature across eastern North America (Table 3).

Teeri et al. (1980) also emphasized that the proportional abundance of C_4 species within the family Cyperaceae was positively correlated with temperature across North America. Within the genus *Cyperus*, we found no such pattern. The previously reported pattern was at least partly driven by an increase in the number of *Carex* species (an entirely C_3 genus; Hesla et al. 1982) and a decrease in the number of *Cyperus* species (a predominantly C_4 genus) with increased latitude. Both of these genera appear to have biogeographic constraints on their distribution independent of climate. In the case of *Cyperus*, the genus has a high species diversity along the Atlantic coastal plain, even after regional differences in climate are corrected for, and becomes progressively more species poor as one moves west across North America. For example, California, a region with a surprisingly low proportion of C_4 Cyperaceae (Teeri et al. 1980), has only 16 *Cyperus* species, 6 of which are introduced (Hickman 1993). Thus, although temperature has certainly played a key role in the evolution and distribution of the C_4 pathway, strong biogeographic and phylogenetic constraints complicate interpretation of patterns such as those reported by Teeri et al. (1980).

Phylogenetic constraints appear to be particularly important in understanding the relationship between C_4 abundance and precipitation (Stowe and Teeri 1978; Ehleringer and Monson 1993). While the number of C_4 *Cyperus* species was positively correlated with precipitation across our study region (Table 3), a pattern similar to that for Australian C_4 grasses (Hattersley 1983), the number of North American C_4 dicot species was negatively correlated with precipitation (Stowe and Teeri 1978). These inconsistencies reinforce the conclusion that diverse selection pressures, including both moisture and nitrogen limitation, were probably important in the repeated evolution of C_4 photosynthesis. We suggest that a high NUE rather than a high water use efficiency may be critical in explaining both the evolution of the C_4 pathway and the ecological success of C_4 *Cyperus* species in infertile, temperate wetlands and moist, sandy habitats.

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