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Isotopic evidence of C_4 grasses in southwestern Europe during the Early Oligocene–Middle Miocene

Michael A. Urban[1], David M. Nelson[2*], Gonzalo Jiménez-Moreno[3], Jean-Jacques Châteauneuf[4], Ann Pearson[5], and Feng Sheng Hu[1,6*]

[1]Program in Ecology, Evolution and Conservation, University of Illinois, Urbana, Illinois 61801, USA
[2]University of Maryland Center for Environmental Science, Appalachian Laboratory, Frostburg, Maryland 21532, USA
[3]Departamento de Estratigrafia y Paleontologia, Universidad de Granada, Granada 18002, Spain
[4]French Geological Survey Consultant, BRGM (Bureau de Recherche Géologiques et Minières), Avenue de Concyr, 45100 Orléans, France
[5]Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts 02138, USA
[6]Departments of Plant Biology and Geology, University of Illinois, Urbana, Illinois 61801, USA

ABSTRACT

C_4 plants are widely successful in the grass-dominated ecosystems of tropical, subtropical, and warm-temperate regions, largely as a result of their ability to limit photorespiration and improve water-use efficiency. A widely held paradigm is that low (<~400 ppm) atmospheric CO_2 concentrations were an important factor selecting for the origin of C_4 plants, although support in geological records is limited. We determined the carbon isotopic composition of 686 individual grass-pollen grains preserved in eight samples of lacustrine and shallow-marine sediments from three basins spanning the Early Oligocene to Middle Miocene in southwestern Europe. Grasses composed <15% of the total abundance of terrestrial pollen grains, and 26%–62% of the grass pollen was from C_4 grasses. Thus C_4 grasses occurred on the landscape as early as the earliest Oligocene, ~14 m.y. earlier than previous isotopic evidence of first C_4 plants and before pCO_2 fell during the Oligocene.

INTRODUCTION

C_4 plants compose only 3% of extant species of flowering plants, yet they account for ~25% of global terrestrial primary productivity, dominate tropical, subtropical, and warm-temperate grasslands, and include important crops and weeds. The success of C_4 plants primarily results from enhanced carbon fixation under conditions promoting photorespiration and improved water-use efficiency during drought (Sage, 2004). C_4 photosynthesis first arose in the grass family (Poaceae); a lag of millions of years ensued before the subsequent rise of C_4 grasses to ecological dominance during the Late Miocene–Pliocene (Edwards et al., 2010). Variability in the timing of this worldwide expansion of C_4 grasslands indicates the importance of regional (e.g., climate, fire), rather than global (e.g., pCO_2) controls on their spread (Osborne and Beerling, 2006; Tipple and Pagani, 2007). Nevertheless, it remains a prominent hypothesis that declining pCO_2 during the Oligocene was a driver and/or precondition for origin of C_4 photosynthesis (e.g., Edwards et al., 2010; Sage, 2004). However, support for this supposition from paleorecords is limited (Tipple and Pagani, 2007).

Detecting C_4 grasses within predominantly C_3 plant communities in geological records is challenging. Grass pollen is morphologically indistinct below the family level, and thus palynological analysis is unsuitable for distinguishing C_3 and C_4 grasses. The oldest known grass macrofossil with diagnostic C_4 anatomy dates to ca. 13 Ma (MacGinitie, 1962), but the distinct $\delta^{13}C$ signature of C_4 plants is detected in paleosols and *n*-alkanes beginning ca. 18 Ma (Tipple and Pagani, 2007), and the earliest likely C_4 phytoliths are from ca. 19 Ma (Strömberg, 2005). Molecular phylogenies of Poaceae place the origin of C_4 ca. 25–37 Ma, which encompasses a precipitous drop in pCO_2 from >1000 to <500 ppm (e.g., Pagani et al., 2005), arguably supporting the C_4-pCO_2 hypothesis (Bouchenak-Khelladi et al., 2009; Christin et al., 2008; Vicentini et al., 2008). However, this range is too large to definitively attribute C_4 origin to declining pCO_2. In addition, recently discovered grass phytoliths in Late Cretaceous coprolites (Prasad et al., 2005) suggest a significantly older, Paleocene–Eocene (ca. 65–34 Ma), origin of C_4 grasses (Vicentini et al., 2008).

Carbon isotopic analysis of grass-pollen grains provides a novel approach for rigorously detecting C_4 grasses among C_3 plant biomass (Amundson et al., 1997). Using a spooling-wire microcombustion device interfaced with an isotope ratio mass spectrometer (SWiM-IRMS), Nelson et al. (2007) showed that the population distribution of individual pollen grains from known C_3 and C_4 grasses could be distinguished using a threshold $\delta^{13}C$ value. This approach was validated by comparing $\delta^{13}C$-based estimates of C_4 grass pollen abundance in lake-sediment surface samples with the abundance of C_4 grasses on the surrounding landscape (Nelson et al., 2008). Because this approach does not use an isotope-mixing model, it enables the detection of C_4 grasses at lower abundances and higher confidence limits than bulk-phase isotopic approaches. Here we report $\delta^{13}C$ analyses of fossil grass-pollen grains extracted from Early Oligocene to Middle Miocene deposits to assess the hypothesis that low pCO_2 was required for the origin of C_4 grasses.

STUDY SITES AND PALEOENVIRONMENTS

The samples come from three distinct sites in southwestern Europe (Fig. 1). The proportions of different terrestrial pollen types in sediment records from these sites indicate the relative abundances of different plants on the landscape, albeit imperfectly because of differences in pollen production, dispersal, and preservation (Bennett and Willis, 2001). However, we do not attempt to reconstruct temporal variations in C_4 grass pollen abundance because of potential basin-specific controls on vegetation composition, lack of contiguous paleorecords spanning the Oligocene–Miocene, and the small number of samples per site.

Rubielos de Mora

Four samples come from a 320-m-long core from the Rubielos de Mora Basin in northeast Spain (40°10′60″N, 0°39′0″W). Mammal assemblages and magnetostratigraphy indicate that these samples date to the Burdigalian–Langhian (ca. 20–15 Ma). During the Early Miocene the basin contained a deep lake with anoxic hypolimnetic waters. Pollen assemblages indicate that in local to regional uplands during the Burdigalian–Langhian the climate was characterized by a 7–9 month dry season, as inferred from abundant xerophytic taxa. Grasses compose 10–15% of the pollen spectra, which together with the xerophytes, suggests an open landscape similar to modern warm steppe or grass-dominated woodland. Similar xerophytic pollen assemblages today occur along the coast of the

*E-mails: dmnelson@al.umces.edu; fshu@life.illinois.edu.

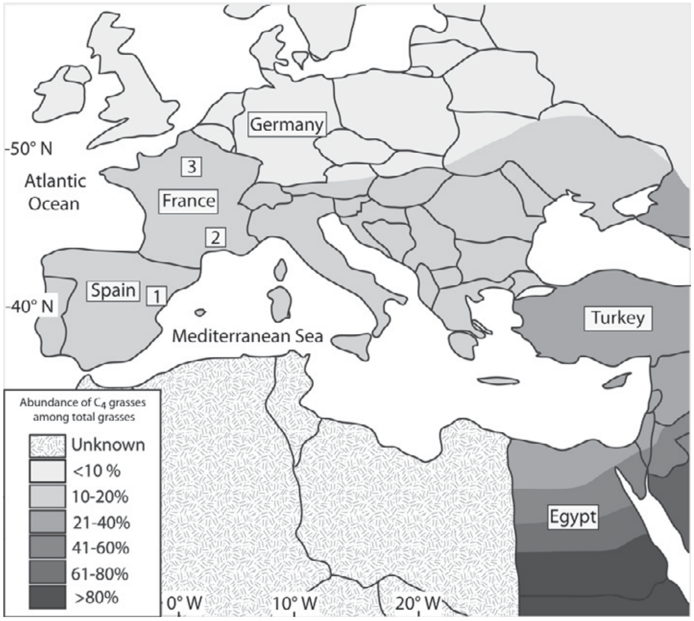

Figure 1. Sample localities: 1—Rubielos de Mora Basin (15–20 Ma); 2—Provence Basin (24–26.5 Ma); 3—Paris Basin (32–34 Ma). Shading indicates modern abundances of C_4 grasses in grass floras (from Sage et al., 1999).

Red Sea, the shores of the Arabian Peninsula, and in North Africa. Mean annual air temperatures were 18–20 °C (Jiménez-Moreno et al., 2007).

Provence Basin

Two samples come from an ~80-m-long section from the Provence Basin (43°31′N, 5°25′70″W). The core was dated using mammal, palynomorph, and gastropod assemblages, which indicate that these samples date to the Chattian (ca. 26.5–24 Ma). Sedimentary evidence indicates that the deposits were of lacustrine and shallow-marine origin and preserved in anoxic conditions. The pollen assemblages indicate abundant thermophilous plants and a subtropical and/or subarid climate. Grasses compose <10% of the pollen spectra. Today similar pollen assemblages occur in tropical-subtropical America, Africa, and Asia (Châteauneuf and Nury, 1995).

Paris Basin

Two samples come from the Paris Basin: one from the Caillasse d'Orgemont Formation in the Bois d'Automne quarry (49°00′N, 2°51′W) and one from the Mezieres S 55A well in the Brenne area (46°44′20″N, 1°17′70″W) in red clays correlated with upper Ludian Formation of the Paris Basin: palynomorphs indicate that these samples date to the lower Rupelian (ca. 33–32 Ma) and near the Priabonian-Rupelian boundary (ca. 34–33 Ma), respectively. These lacustrine to shallow-marine sediments were preserved in anoxic conditions. Pollen and macrofossils indicate abundant thermophilous taxa and a subtropical climate. Several xerophytes in the pollen spectra indicate marked seasonality of precipitation. Grasses compose ~10% of the pollen spectra, typical of modern landscapes in tropical-subtropical America, Africa, and Asia (Châteauneuf, 1980).

MATERIALS AND METHODS

We extracted pollen from 10–30 cm^3 of sediment using standard techniques (including an acid treatment to remove carbonate) modified to exclude carbon-containing chemicals (Nelson et al., 2006). Single grains of grass pollen were isolated from the resulting pollen-water slurry at 200× magnification on a microscope slide, thoroughly rinsed in nano-pure water, and transferred to ~0.4 L drops of nano-pure water using a micromanipulator. The grains (and water) were then applied to a SWiM-IRMS using a steel and glass syringe (Nelson et al., 2007, 2008). The number of individual grains of grass pollen applied to the SWiM device ranged from 139 to 222 per sample (see the GSA Data Repository[1]).

Following the methods of Nelson et al. (2008), blanks (nano-pure water to which single grains of grass pollen were added and then removed) were analyzed concomitantly with samples from each site. A 2σ threshold of blank CO_2 yields was used to set the minimum size threshold; samples below this threshold were excluded from further analysis. The final $\delta^{13}C$ data were blank-corrected using isotopic mass balance. A threshold of −19.2‰ was used to distinguish C_3 (more negative) from C_4 grass pollen (more positive), except that in our study we accounted for variations in the threshold value that likely occurred in response to variations in $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C$-CO_2) over the Cenozoic (Zachos et al., 2001). Variations in $\delta^{13}C$-CO_2 were estimated by assuming long-term isotopic equilibrium between $\delta^{13}C$-CO_2 and $\delta^{13}C$ of marine carbonate ($\delta^{13}C_{mc}$), and offsets of −7‰ (Mora et al., 1996) and −9‰ (Koch et al., 1995) between these pools. The resulting variation in $\delta^{13}C$-CO_2 was used to adjust the −19.2‰ threshold (Fig. 2), and to plot errors on estimates of C_4 grass pollen abundance (Fig. 3). Thresholds adjusted using an offset of −7‰ (−9‰) between $\delta^{13}C$-CO_2 and $\delta^{13}C_{mc}$ ranged from −19.1‰ to −18.4‰ (−21.1‰ to −20.4‰).

RESULTS AND DISCUSSION

The number of grains of grass pollen applied to the wire with peak areas exceeding the 2σ range of blanks ranged from 63 to 100 (see the Data Repository). An average of 45% of applications were above the minimum size threshold for analysis, which is similar to the percent (47%) obtained from grass pollen in contemporary lake surface sediments (Nelson et al., 2008). Consistent with previous studies (Nelson et al., 2007, 2008), the majority (79%) of $\delta^{13}C$ data points (Fig. 2) are within or between the typical range of $\delta^{13}C$ values expected for C_3 (−33‰ to −22‰) and C_4 (−15‰ to −10‰) plants. Values more negative than −33‰, or more positive than −10‰, likely occur because of poor analytical precision and uncertainty in the isotopic composition and variability of the subtracted analytical blanks (Nelson et al., 2007). In addition, samples of pollen from known C_3 (*Bromus thominii*, *B. viomozorius*, and *Koeleria capensis*) and C_4 (*Zea mays* and *Ehrharta erecta var. natalensis*) grasses that were treated and analyzed concomitantly with fossil samples yielded accurate classification, on average, 87% of the time for C_3 and 88% of the time for C_4, when using a cutoff threshold of −19.2‰.

The $\delta^{13}C$ threshold values modified for variations in $\delta^{13}C$-CO_2 indicate that the sediment samples contain 26%–62% C_4 grass pollen (Figs. 2 and 3). Artificial mixtures of 50 grains of grass pollen that authentically contained 0% C_4 grass pollen were classified by our methods as containing 0%–20% C_4 pollen, whereas samples of 100 grains were classified as 0%–15% C_4 (2σ, $p < 0.05$), providing an estimated boundary for the expected rate of false positives (Nelson et al., 2007). Assuming that the false positive rate is comparable for grains from well-preserved sediments, the percentages of C_4 grass pollen in our samples exceed these thresholds.

Diagenesis could influence the measured $\delta^{13}C$ values of grass pollen, potentially leading to isotopic biases. However, a previous calibration study (Nelson et al., 2008) suggested that early diagenesis of pollen does not significantly influence the measured $\delta^{13}C$ values of grass pollen. The false detection rate for Oligocene–Miocene sediments is unlikely to be

[1]GSA Data Repository item 2010297, table of detailed sample information, is available online at www.geosociety.org/pubs/ft2010.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

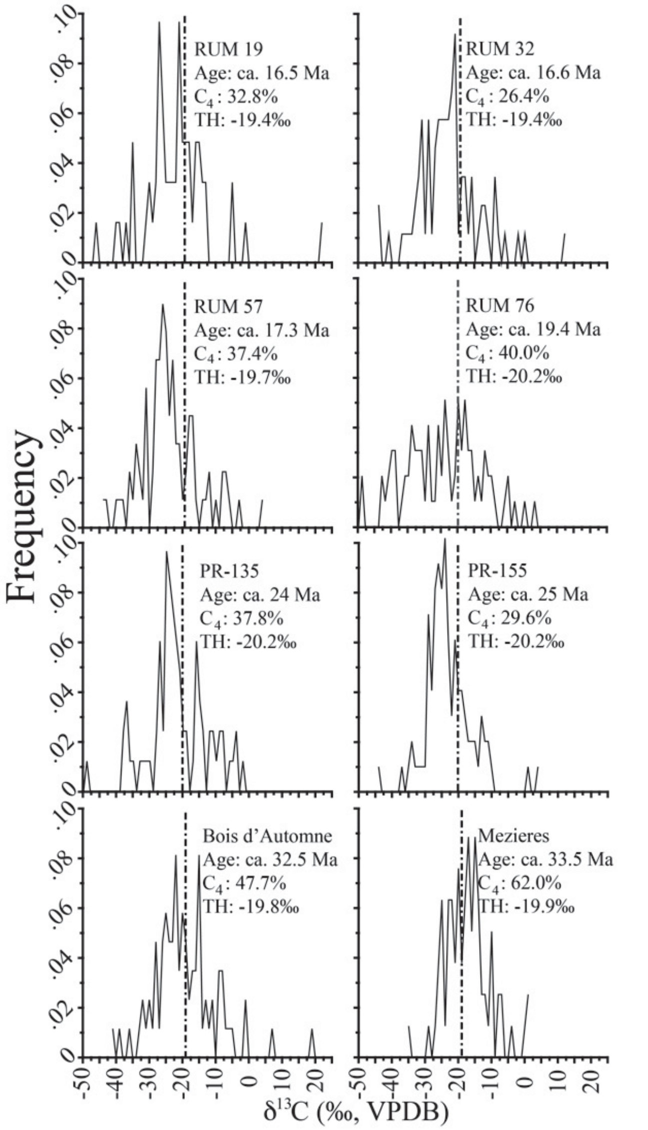

Figure 2. Histograms of $\delta^{13}C$ values of grass pollen samples analyzed for this study. Black lines represent proportion of each sample within 1‰ bins. Vertical dashed lines signify thresholds (TH) for distinguishing C_3 from C_4 grass pollen using –8‰ offset between $\delta^{13}C$-CO_2 and $\delta^{13}C_{mc}$ (–8‰ being average of offsets indicated by Mora et al. [1996] and Koch et al. [1995]; mc—marine carbonate). VPDB—Vienna Peedee belemnite.

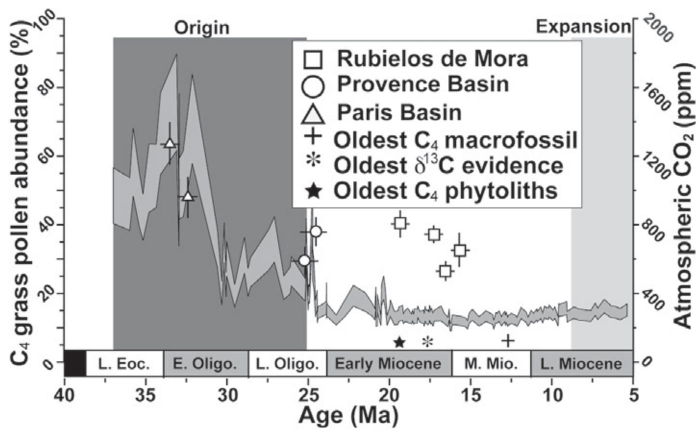

Figure 3. C_4 grass pollen abundances in this study as percent of total grass pollen using thresholds from Figure 2. Error bars on estimates of C_4 grass pollen abundance indicate uncertainty in offset between $\delta^{13}C$-CO_2 and $\delta^{13}C_{mc}$ (–7‰ to –9‰, as described in text; mc—marine carbonate). Error bars on ages indicate dating uncertainties. Also shown is pCO$_2$ (Pagani et al., 2005; gray curve) record. Light gray rectangle represents approximate timing of expansion of C_4 grasslands in many regions of the world (Tipple and Pagani, 2007) and darker gray rectangle represents molecular clock range for C_4 grass origin (Bouchenak-Khelladi et al., 2009; Christin et al., 2008; Vicentini et al., 2008). L. Eoc.—Late Eocene; E. Oligo.—Early Oligocene; L. Oligo.—Late Oligocene; M. Mio.—Middle Miocene. References for oldest C_4 grass macrofossil, oldest $\delta^{13}C$ evidence of C_4, and oldest C_4 phytoliths include MacGinitie (1962), Tipple and Pagani (2007), and Strömberg (2005), respectively.

significantly different, because the sporopollenin exine of pollen grains is highly resistant to physical and chemical alteration (van Bergen et al., 1993); although pollen grains are susceptible to oxidation, the sediments used in our study were preserved in anoxic conditions. Neither the sediments nor the pollen grains show signs of strong diagenetic alteration. Regardless, Jahren (2004) showed that pollen treated with 30% hydrogen peroxide for 24 h was <0.5‰ different in $\delta^{13}C$ than untreated pollen.

There are possible environmental influences on the threshold value (−19.2‰) used to distinguish C_3 from C_4 pollen that exceed the adjustments for variations in $\delta^{13}C$-CO_2. Large changes in pCO$_2$, such as occurred during the Oligocene (Fig. 3), might affect the threshold by altering the ratio of intercellular to atmospheric CO_2 and thus the isotopic composition of C_3 plants. However, variations in pCO$_2$ over the range that occurred during the Oligocene–Miocene do not significantly influence plant $\delta^{13}C$ values (e.g., Arens et al., 2000). Another possible influence is semiarid climatic conditions in southwestern Europe during the Oligocene–Middle Miocene (e.g., Jiménez-Moreno et al., 2007). Under conditions of low water availability, $\delta^{13}C$ values of C_3 plants have been observed to shift by as much as ~+3‰ (e.g., Ehleringer and Cooper, 1988). Thus a threshold $\delta^{13}C$ value that does not account for aridity could potentially cause misclassification of C_3 grass pollen grains as C_4. To investigate the potential effect of aridity, we shifted the threshold values for distinguishing C_3 and C_4 grass pollen by 3‰ in the positive direction. After this shift, our estimates for C_4 grass pollen abundance for 5 of the 8 samples, including 3 of the 4 Oligocene samples, remain above a conservative analytical error of 20% misclassification (see the Data Repository).

Because the existence of C_4 grass pollen in our samples cannot be explained by diagenesis and/or environmental influences on threshold values, our data provide unequivocal evidence for C_4 grasses in southwestern Europe by the Early Oligocene. This conclusion is in accord with those from recent molecular results (e.g., Vicentini et al., 2008) indicating that C_4 grasses originated earlier than has been deduced from previous geochemical approaches.

The likely greater proportions of C_4 grasses during the Oligocene–Middle Miocene than at present in southwestern Europe (Fig. 1) imply that temperatures and/or moisture availability were favorable for C_4 plants. Consistent with this notion, the closest modern analogs of Oligocene–Middle Miocene pollen assemblages from southwestern Europe are from regions where today C_4 grasses dominate grass floras (e.g., northern Egypt and the northern Sinai Peninsula) and where climates are warmer and lacking the seasonal Mediterranean climates that became established in Europe during the Pliocene (Suc, 1984). Climate reconstructions from Central Europe indicate that mean annual temperatures were lower in the Late Eocene−Early Oligocene than in the Late Oligocene (Mosbrugger et al., 2005). However, the lower annual temperatures during the Late Eocene−Early Oligocene resulted from colder winters, and warm-month (i.e., growing season) temperatures remained >25 °C during the Eocene–

Middle Miocene (Mosbrugger et al., 2005), consistent with temperature inferences from pollen assemblages at our sites (e.g., Jiménez-Moreno et al., 2007). Thus subtropical to warm-temperate conditions during the Oligocene–Middle Miocene in western Europe may have promoted photorespiration in C_3 plants and conferred a competitive advantage to C_4 grasses despite high pCO_2. Aridity may also have favored C_4 grasses. Vegetation reconstructions at our sites suggest semiarid climates (e.g., Jiménez-Moreno et al., 2007), as does increased hypsodonty in the teeth of terrestrial plant-eating mammals after 18 Ma in southwestern Europe (Fortelius et al., 2002).

A recent vegetation-climate modeling study (Lunt et al., 2007) that estimated the worldwide distribution of C_4 grasses found a high relative extent and abundance of C_4 grasses during the Late Oligocene, despite simulated pCO_2 up to 838 ppm. Lunt et al. (2007) suggested that the competitive advantage that high temperatures confer to C_4 grasses outweighs the competitive advantage of high pCO_2 for C_3 grasses. Our data support this conclusion by showing that C_4 grasses existed before pCO_2 dropped below 800 ppm (Fig. 3). The evidence refutes the idea that low pCO_2 was an important driver and/or precondition for development of C_4 photosynthesis (e.g., Sage, 2004; Vicentini et al., 2008).

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