Plant DNA: A new substrate for carbon stable isotope analysis and a potential paleoenvironmental indicator

A. Hope Jahren*
Department of Earth and Planetary Sciences, Johns Hopkins University, Baltimore, Maryland 21218, USA
Gitte Petersen
Ole Seberg
Botanical Institute, Department of Evolutionary Botany, University of Copenhagen, Gothersgade 140, DK-1123 Copenhagen K, Denmark

ABSTRACT

The $\delta^{13}C$ value of fossil plant materials can be used to gain insight into the dominant photosynthetic pathway, as well as other environmental attributes, of ancient plant ecosystems. Nucleotide sequences from land plant nucleic acids extracted from 400 ka fossil sediments have been recognized as the oldest authenticated fossil deoxyribonucleic acid (DNA), making the inference of plant taxonomy possible in substrates devoid of plant macrofossils and microfossils. If the C isotope relationship between bulk plant tissue and associated plant nucleic acids were known, fossil plant nucleic acids could be analyzed for $\delta^{13}C$ value and used as land plant isotopic substrates within mixed organic material. Toward this end, we present $\delta^{13}C$ analyses of nucleic acids isolated from 12 higher plant species that span the full phylogenetic diversity of seed plants. Extracted nucleic acids were dominated by double-stranded DNA containing fragments of *rbcL* gene ~350 base pairs in length. The C isotope compositions of plant nucleic acids were found to be enriched in ^{13}C relative to bulk plant tissue by a constant value = 1.39‰. This study represents the first comparison of the $\delta^{13}C$ value of nucleic acids to the $\delta^{13}C$ value of bulk tissue for multicellular organisms; our results contrasted with the minimal fractionations reported for microorganisms. Because the isotopic enrichment is constant across tracheophytes, the $\delta^{13}C$ value of fossil plant DNA can be used as a paleoenvironmental indicator, eliminating the need for morphological recognition of fossil plant material in paleoenvironmental studies.

Keywords: carbon isotope, nucleic acid, DNA, land plant.

INTRODUCTION

Nucleic acids are macromolecules of nucleotide monomers, known as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Sequences of nucleotides from portions of the genome can be used to indicate the taxonomic position of organisms and to establish their phylogenetic relationships. Willerslev et al. (2003) successfully isolated land plant nucleic acids from Holocene and Pleistocene sediments, and used them to taxonomically characterize the ancient plant community, thus yielding a detailed picture of the ancient terrestrial ecosystem from a substrate that was otherwise devoid of fossil information. We sought to determine if such extracted nucleic acids could also be used as a substrate for $\delta^{13}C$ analysis, making ancient plant DNA a potential paleoenvironmental indicator.

Studies of modern plants have revealed that plant tissue $\delta^{13}C$ values are highly sensitive to environmental parameters (reviewed in Dawson et al., 2002). There is broad application of $\delta^{13}C$ analysis of plant tissues to problems in paleoclimatology; plant fossil material $\delta^{13}C$ value has been used to determine the shifts from C3 to C4 vegetation in ancient environments (Scott, 2002) and to infer climate change (Boom et al., 2002). Changing $\delta^{13}C$ value of fossil plant organic material has also been used to suggest changes in the carbon isotope composition of atmospheric CO_2 (Jahren, 2002), and to identify what type of photosynthetic organism a fossil once was (Jahren et al., 2003). If nucleic acids could be isolated from mixed fossil organic materials, and verified molecularly to be of land plant origin, knowledge of the relationship between the $\delta^{13}C$ value of plant nucleic acids and the $\delta^{13}C$ value of bulk plant tissue would expand the application of plant tissue $\delta^{13}C$ analysis to a plethora of geologic substrates.

MATERIALS AND METHODS

Plant leaf tissues were collected from 12 species selected to reflect the full phylogenetic diversity of seed plants (Table 1). The data set contained three gymnosperms (all conifers) and nine angiosperms (all dicots). Within the angiosperms, the magnoliid group as well as the core eudicots (including the rosids and asterids) were represented, effectively sampling across the dicots. This study focused on trees, which have been the main subjects of plant C isotope studies (Dawson et al., 2002). Because all sampled plants were under cultivation, they were largely shielded from stress. In order to eliminate intraspecific variation, each sample contained material from between 2 and 10 individuals. Leaf tissues from each species were clipped and packed as tightly as possible into 10 22 mL borosilicate vials. In the laboratory, samples were lyophilized for 24 h, ground to a fine uniform powder, then returned to refrigeration.

All living plant cells contain nucleic acids, as well as a large amount of membrane material, proteins, carbohydrates, and other substances. We sought to extract plant nucleic acids (particularly genomic DNA) from cells, and to isolate them from all other cell substances, as well as from all chemicals added during the process. The literature is devoted

*E-mail: jahren@jhu.edu.

TABLE 1. $\delta^{13}C$ VALUES OF PLANT SPECIES AND EXTRACTED NUCLEIC ACIDS

Taxonomic group	Site of collection*	Latin name	Common name	A_{260}/A_{280}	$\delta^{13}C$ of bulk plant tissue $\pm\sigma$ (‰)†	$\delta^{13}C$ of nucleic acids $\pm\sigma$ (‰)†
Conifers	STR	*Cupressus sempervirens* L.	Italian cypress	1.38	−28.54 ± 0.59	−29.68 ± 0.07
	UCD	*Pinus canariensis* C. Smith	Canary Island pine	1.27	−26.11 ± 0.40	−27.40 ± 0.14
	UCR	*Pinus halepensis* P. Mill.	Aleppo pine	1.60	−27.90 ± 0.29	−28.97 ± 0.06
Angiosperms						
Magnoliids	UCR	*Laurus nobilis* L.	Sweet bay	1.15	−27.67 ± 0.06	−28.25 ± 0.17
	ATL	*Magnolia virginiana* L.	Umbrella tree	1.61	−27.38 ± 0.06	−29.19 ± 0.11
Rosids	ATL	*Carya alba* L.	Mockernut hickory	1.60	−26.29 ± 0.10	−28.15 ± 0.04
	UCR	*Cneoridium dumosum* Nutt.	Bush rue	1.47	−29.41 ± 0.24	−30.74 ± 0.06
	ATL	*Acer campestre* L.	Hedge maple	1.71	−29.25 ± 0.08	−31.31 ± 0.05
	ATL	*Aesculus sylvatica* Bartr.	Painted buckeye	1.59	−29.79 ± 0.07	−31.85 ± 0.14
Asterids	ATL	*Kalmia latifolia* L.	Mountain laurel	1.26	−28.91 ± 0.04	−29.83 ± 0.11
	FTG	*Manilkara zapota* L.	Sapodilla	1.60	−29.25 ± 0.04	−30.25 ± 0.13
Other Eudicots	ATL	*Hamamelis virginiana* L.	American witch hazel	1.47	−28.75 ± 0.47	−30.26 ± 0.06

*Samples were collected at the following arboreta and botanical gardens during 2001: Atlanta Botanical Gardens (ATL), Fairchild Tropical Gardens (FTG), Strybing Arboretum and Botanical Gardens (STR), University of California at Davis Arboretum (UCD), University of California at Riverside Botanical Garden (UCR).
†σ is the standard deviation seen in three replicates of the sample.

to the development of methods that extract the small amount of nucleic acids sufficient for amplification by the polymerase chain reaction (PCR). For example, Burr et al. (2001) extracted between 50 and 200 ng of DNA from a portion of leaf only 550 μm × 550 μm in size. This yield contrasts sharply with the 14,300 ng of DNA required for one carbon stable isotope measurement using an automated combustion system or a dual inlet introduction to the mass spectrometer. For this reason, we turned to the classic literature (e.g., Doyle and Doyle, 1987) to develop a method suitable for large-sized samples of plant tissue. We note that gas chromatography (GC) in combination with stable isotope mass spectrometry could require as little as 2900 ng of DNA for $\delta^{13}C$ analysis; another group has pursued the analysis of organismal 16S rRNA and other nucleic acids using this approach (Pearson et al., 2002; Sessions et al., 2002).

Methods to extract nucleic acids from plant material consist of three main steps: homogenization of plant cells, deproteinization of DNA, and precipitation of nucleic acids. During homogenization, lipid membranes and plant cell walls are split, liberating nucleic acids from nuclei, mitochondria, chloroplasts, and ribosomes. We performed homogenization by digesting samples in an extraction buffer based on the solution described in detail by Lodhi et al. (1994). The extraction buffer was made by combining 20 m*M* sodium ethylenediaminetetraacetic acid (EDTA) with 100 m*M* Tris-HCl and then adjusting the pH to 8.0 using HCl. After this adjustment, 1.4 *M* NaCl and 2.0% (by weight/volume, w/v) cetyltrimethylammonium bromide (CTAB) were added. The solution was kept at 60 °C in order to dissolve the CTAB, and 0.2% (by w/v) β-mercaptoethanol was added just before use. Previous studies led us to expect that 1 g of dried plant leaf tissue would yield ~10 μg of nucleic acids (Zhang and Stewart, 2000). Therefore, we began by digesting 1.5 g of lyophilized, ground leaf material in 10 mL of extraction buffer for 1 h at 60 °C. After digestion, the resultant cell slurry was centrifuged for 5 min at 13,000 rpm and the clear supernatant was drawn off and isolated in a new centrifuge tube. Nucleic acids were separated from soluble proteins and carbohydrates via extraction with dichloromethane after the procedure described by Petit et al. (1993). In this step, 5 mL of dichloromethane was added to the supernatant, gently mixed, and centrifuged at 8000 rpm. The top, clear layer was drawn off, placed in a new centrifuge tube, and the extraction step was repeated. We then added 2× this volume of cold (−20 °C) ethanol, mixed by inversion, and placed the solution in the freezer for 10 min after the method described by Porebski et al. (1997) in order to initiate the precipitation of nucleic acids. The resulting precipitate was centrifuged at 3000 rpm, followed by 2 applications of a wash solution containing 76% ethanol and 0.2 *M* NH_4OAc (by w/v) after the method of Doyle and Doyle (1987). Finally, the precipitated white, stringy nucleic acids were thoroughly washed in ethanol, and spooled out with a glass hook (Fig. 1).

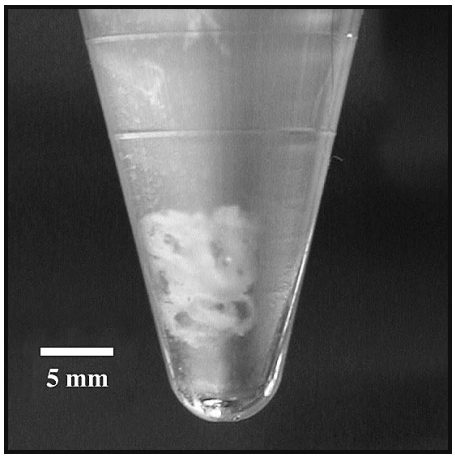

Figure 1. Precipitated nucleic acids of *Cneoridium dumosum* suspended in ethanol. Spectrophotometric scans revealed nucleic acid samples to be dominated by double-stranded deoxyribonucleic acid (dsDNA).

Before extracted nucleic acid samples were analyzed for $\delta^{13}C$ value, nucleic acid content was verified two ways: by spectrophotometry and through amplification of PCR products. For both approaches, extracts were redissolved in TE (1 m*M* Tris-HCl and 0.1 m*M* EDTA buffered to pH = 8.0) at a concentration of 50–100 ng/μL. The results of absorbance measurements across wavelengths 220 to 320 nm performed on all samples are summarized in Table 1. Absorbance scans of samples and of pure double-stranded DNA (dsDNA) show a symmetric peak centered at ~260 nm. The ratio of absorbance at 260 nm to the absorbance at 280 nm (A_{260}/A_{280}) is commonly used as an indicator of the purity of dsDNA in a sample. Values of $A_{260}/A_{280} \geq 1.0$ are found for solutions sufficiently pure in dsDNA for PCR amplification; preparations of absolutely pure dsDNA show $A_{260}/A_{280} \geq 1.70$. Values of A_{260}/A_{280} determined for samples indicated that extracts were dominated by dsDNA: A_{260}/A_{280} ranged from 1.15 to 1.71 with a mean value of 1.48 (Table 1).

Workers often perform PCR to demonstrate that a nucleic acid extraction method has successfully isolated DNA (e.g., Burr et al., 2001). Nucleic acids suspended in TE were amplified in a total volume of 50.2 μL consisting of 1 μL sample, 20 μL deoxynucleotide triphosphates, 5 μL of *Taq* buffer, 0.2 μL of *Taq* polymerase, 14 μL of sterilized distilled deionized water, and 10 μL of primer. The following primers were used: Hv890 (5′-TGCATGCAGTTATTGATAGAC-3′) and Z1204RS (5′-CCCTAAGGGTGTCCTAAAGTT-3′).

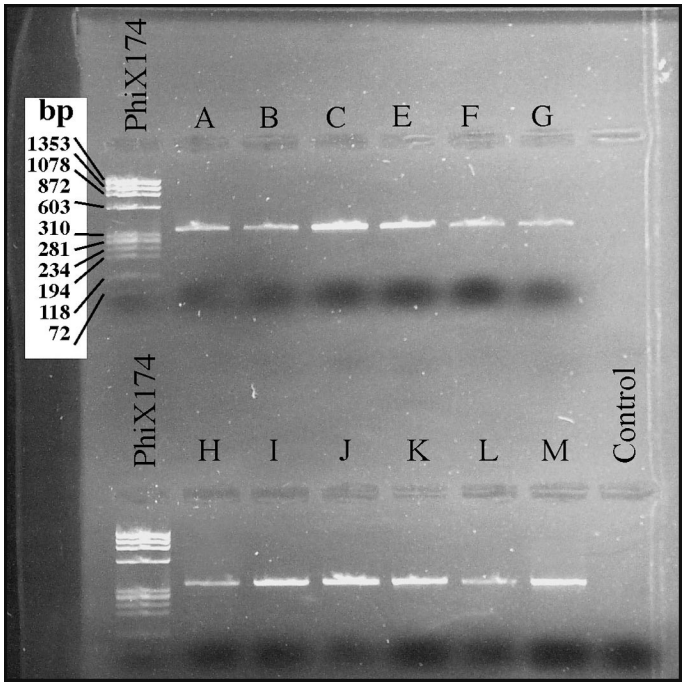

Figure 2. Amplification of ~350 bp fragment from *rbcL* gene within plant nucleic acids also analyzed for $\delta^{13}C$ value; aliquots were stained with ethidium bromide and resolved by gel electrophoresis in 3.0% agarose gels. Resulting polymerase chain reaction products were visualized under 302 nm light at intensity of 800 μW/cm². A—*Acer campestre*; B—*Aesculus sylvatica*; C—*Carya alba*; E—*Cneoridium dumosum*; F—*Cupressus sempervirens*; G—*Hamamelis virginiana*; H—*Kalmia latifolia*; I—*Laurus nobilis*; J—*Magnolia virginiana*; K—*Manilkara zapota*; L—*Pinus canariensis*; M—*Pinus halepensis*. Size marker is PhiX174, and last lane is negative control (blank).

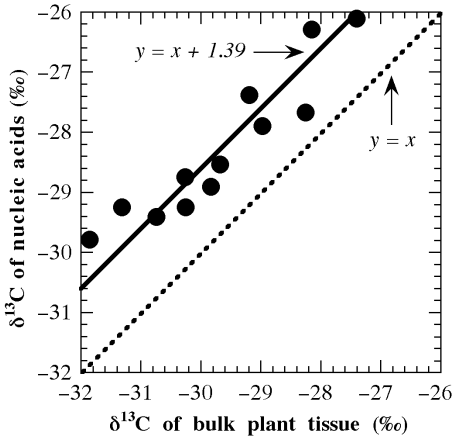

Figure 3. $\delta^{13}C$ value of bulk leaf tissue vs. $\delta^{13}C$ value of nucleic acids extracted from same plant species.

These amplified the fragment between 890 and 1204 base pairs upstream from the *rbcL* start codon (Petersen and Seberg, 2003). The gene *rbcL* codes for a large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and is shared by all photosynthetic organisms. Sequences of *rbcL* have been extensively used to investigate the phylogeny of land plants (e.g., Chase et al., 1993); a 130 bp fragment of *rbcL* was recovered from 400-k.y.-old sediments and used to infer the taxonomic status of ancient plants (Willerslev et al., 2003). We performed amplification using 35 cycles, each consisting of 1 min at 94 °C, 1 min at 57 °C, and 3 min at 72 °C. All of the DNA templates produced clear, sharp, and reproducible bands ~350 bp in length (Fig. 2), confirming the presence of nucleic acids in each sample that can potentially be sequenced.

Plant nucleic acid samples and bulk leaf tissues were analyzed for $\delta^{13}C$ value using a Eurovector automated combustion system in conjunction with an Isoprime stable isotope mass spectrometer at the Johns Hopkins University. Table 1 presents the $\delta^{13}C$ values of all plant tissues sampled and of the nucleic acids extracted from these tissues in triplicate. Stable isotope values are reported in standard delta-notation: $\delta = (R_{sample} - R_{standard}/R_{standard}) \times 1000$ (‰). The reporting standard is the Peedee belemnite (PDB) with $R = {}^{13}C/{}^{12}C = 0.011237$. Analytical uncertainty associated with each measurement was ±0.05‰. Combustion also resulted in a quantification of percent C in each sample (±1% C analytical uncertainty). Bulk leaf tissue samples had a mean value = 46.0% C, which agreed with the well-established range seen in plant tissues (45%–50% C; Reichle et al., 1973). Extracted nucleic acids had a mean value = 30.0% C, which was close to the theoretical C calculated for the nitrogen bases (adenine = 38.45% C; cytosine = 37.50% C; guanine = 36.60% C; thymine = 39.60% C).

RESULTS AND DISCUSSION

The $\delta^{13}C$ value of the bulk leaf tissue ranged from −31.85‰ to −27.40‰ with a mean value of −29.66‰ (n = 12); these values coincided with $\delta^{13}C$ values classically reported for C3 plants (Smith and Epstein, 1971). Because these plants all shared the C3 photosynthetic pathway and were subject to approximately the same value of $\delta^{13}CO_2$, species differences in bulk leaf tissue $\delta^{13}C$ value were likely a reflection of species differences in the ratio of intercellular to atmospheric pCO_2 (Ehleringer, 1993). The $\delta^{13}C$ value of nucleic acids extracted using the methods described here ranged from −29.79‰ to −26.11‰, with a mean value of −28.27‰ (n = 12). Figure 3 presents the $\delta^{13}C$ value of bulk leaf tissue plotted against the $\delta^{13}C$ value of nucleic acids extracted from the same plant species. The carbon isotope difference between bulk leaf tissue and nucleic acids ($\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids}$) seen in the species studied ranged from −2.06‰ to −0.58‰ with a mean value of −1.39‰ (n = 12; σ = 0.48‰). The data showed a strongly linear trend: $\delta^{13}C_{nucleic\ acids} = 0.85 \cdot \delta^{13}C_{plant} - 3.10$ (‰; $R^2 = 0.87$). However, since the nucleic acids resulted from identical biochemical pathways in each plant species, a standard isotopic offset between bulk leaf tissue and nucleic acids is more likely than $\delta^{13}C_{nucleic\ acids}$ being dependent on $\delta^{13}C_{plant}$. From the data displayed in Table 1 and Figure 3, this isotopic offset is best characterized by $\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids} = -1.39‰$.

Carbon stable isotopic offsets have been reported between a variety of extracted compounds and higher plant bulk tissue. Cellulose is the main polysaccharide found in plant tissues; $\delta^{13}C$ values to 4‰ higher than bulk tissue have been reported for isolated cellulose (Marino and McElroy, 1991). In contrast, lignin has been found to have lower $\delta^{13}C$ value than the bulk tissue from which it was isolated: $\delta^{13}C$ values to 4.2‰ lower than bulk tissue have been reported for isolated lignin (Benner et al., 1987). Chloroplasts isolated from *Fagus* sp. were found to have a $\delta^{13}C$ value 0.7‰ higher than the bulk plant tissue (work of Takigiku, 1987; discussed in Hayes, 2001). The carbon isotope compositions of mixed plant lipids were found to range from 5‰ to 10‰ less than bulk tissue $\delta^{13}C$ values (Park and Epstein, 1961), and subsequent investigations of specific plant lipids have reinforced the finding that lipids have lower $\delta^{13}C$ value than bulk plant tissue (e.g., Lockheart et al., 1997). Plant lignin and cellulose are much more stable molecules than DNA and can therefore be isolated in large amounts from older sediments; DNA, however, carries with it the advantage of specific taxonomic information that cannot be gained from plant lignin or cellulose.

The nucleotides that comprise nucleic acids each consist of a phosphate group, a pentose sugar and a nitrogen base (a purine or a pyrimidine). The biosynthetic processes that give rise to nucleic acids are known in detail (e.g., Sugiura and Takeda, 2000), but little has been determined about associated isotopic fractionations (discussed in Hayes, 2001). The only

published studies that assess the $\delta^{13}C$ values of nucleic acids report on heterotrophic bacteria, which were found to closely reflect the $\delta^{13}C$ values of bulk cells: $\Delta = \delta^{13}C_{cell} - \delta^{13}C_{nucleic\ acids} = -0.6‰$ to $-0.3‰$ (Blair et al., 1985; Coffin et al., 1990). Our results suggest that a different relationship applies for higher plants, probably because bacterial cells lack nuclear compartmentalization, whereas tracheophyte cells and their included nucleic acids are highly compartmentalized. The fractionation resulting from the biosynthesis of higher plant nucleic acids is consistently reflected in the relatively low values of $\Delta = \delta^{13}C_{plant} - \delta^{13}C_{nucleic\ acids}$ we observed in this study (Fig. 3), compared to the minimal fractionation that has been observed for bacteria.

CONCLUSIONS

The isolation of nucleic acids such as DNA from geologic materials has resulted in a new source of fossil information. If nucleic acids could be isolated from the geologic record in sufficient quantity, and shown to be of land plant origin, their carbon isotope composition could be used to gain insight into the dominant photosynthetic pathway and the paleoecological status of the ancient plant community. The oldest authenticated DNA sequences known come from plant DNA isolated from sediments 400 k.y. to 10 k.y. old (Willerslev et al., 2003). Note that the amount of DNA isolated from each 2 g sample collected by Willerslev et al. (2003) was at most 1/1000 of the mass required for $\delta^{13}C$ analysis. Nevertheless, there is reason to think that specific plant compounds could be incorporated in large amounts into the sedimentary record: Fogel and Tuross (1999) demonstrated that high molecular weight subunits of Rubisco can be found in degraded higher plant material after 12 months of decomposition, and the total amount of remaining Rubisco was more than 50% of the original amount in some species. Because living and dead plant tissues comprise the vast majority of organic carbon input to soils and to many nearshore sediments, nucleic acid extraction from large samples (i.e., kilograms) of Holocene—and perhaps older—sediments could yield sufficient amounts for C isotope analysis. On the basis of the results presented here, we think that the $\delta^{13}C$ value of land plant nucleic acids isolated from geologic sediments and sedimentary rocks would reflect the $\delta^{13}C$ value of the ancient plant community, after the demonstrated isotopic enrichment ($= 1.39‰ \pm 0.48‰$) was subtracted. This technique could be particularly important to validate Holocene shifts between C3 and C4 photosynthesis widely suggested for specific taxonomic groups, based on inference from mixed organic substrates (e.g., Nordt, 2003).

ACKNOWLEDGMENTS

We thank W.M. Hagopian, C. Hansen, L. Knudsen, and J.W. Mundy for help and advice in the laboratory. This project was supported by the Danish-American Fulbright Association. This manuscript was improved thanks to J. Pross and an anonymous reviewer.

REFERENCES CITED

Benner, R., Fogel, M.L., Sprague, E.K., and Hodson, R.E., 1987, Depletion of ^{13}C in lignin and its implication for stable carbon isotope studies: Nature, v. 329, p. 708–710.

Blair, N., Leu, A., Muñoz, E., Olsen, J., and Des Marais, D.J., 1985, Carbon isotopic fractionation in heterotrophic microbial metabolism: Applied Environmental Microbiology, v. 50, p. 996–1001.

Boom, A., Marchant, R., Hooghiemstra, H., and Sinninghe Damsté, J.S., 2002, CO_2- and temperature-controlled altitudinal shifts of C4- and C3-dominated grasslands allow reconstruction of palaeoatmospheric pCO_2: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 177, p. 151–168.

Burr, K., Harper, R., and Linacre, A., 2001, One-step isolation of plant DNA suitable for PCR amplification: Plant Molecular Biology Reporter, v. 19, p. 367–371.

Chase, M.W., and 41 others, 1993, Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*: Missouri Botanical Garden Annals, v. 80, p. 528–280.

Coffin, R.B., Velinsky, D.J., Devereux, R., Price, W.A., and Cifuentes, L.A., 1990, Stable carbon isotope analysis of nucleic acids to trace sources of dissolved substrates used by estuarine bacteria: Applied Environmental Microbiology, v. 56, p. 2012–2020.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., and Tu, K.P., 2002, Stable isotopes in plant ecology: Annual Review of Ecology and Systematics, v. 33, p. 507–559.

Doyle, J.J., and Doyle, J.L., 1987, A rapid DNA isolation procedure from small quantities of fresh leaf tissue: Phytochemical Bulletin, v. 19, p. 11–15.

Ehleringer, J.R., 1993, Carbon and water relations in desert plants: An isotopic perspective, *in* Ehleringer, J.R., et al., eds., Stable isotopes and plant carbon-water relations: San Diego, California, Academic Press, p. 155–172.

Fogel, M.L., and Tuross, N., 1999, Transformation of plant biochemicals to geological macromolecules during early diagenesis: Oecologia, v. 120, p. 336–346.

Hayes, J.M., 2001, Fractionation of the isotopes of carbon and hydrogen in biosynthetic processes: Reviews in Mineralogy and Geochemistry, v. 43, p. 225–277.

Jahren, A.H., 2002, The biogeochemical consequences of the mid-Cretaceous superplume: Journal of Geodynamics, v. 34, p. 177–191.

Jahren, A.H., Porter, S., and Kuglitsch, J.J., 2003, Lichen metabolism identified in Early Devonian terrestrial organisms: Geology, v. 31, p. 99–102.

Lockheart, M.J., Van Bergen, P.F., and Evershed, R.P., 1997, Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms; implications for the study of higher land plant–derived sedimentary organic matter: Organic Geochemistry, v. 26, p. 137–153.

Lodhi, M.A., Ye, G.-N., Weeden, N.F., and Reisch, B.I., 1994, A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*: Plant Molecular Biology Reporter, v. 12, p. 6–13.

Marino, B.D., and McElroy, M.B., 1991, Isotopic composition of atmospheric CO_2 inferred from carbon in C4 plant cellulose: Nature, v. 349, p. 127–131.

Nordt, L.C., 2003, Late Quaternary fluvial landscape evolution in desert grasslands of northern Chihuahua, Mexico: Geological Society of America Bulletin, v. 115, p. 596–606.

Park, R., and Epstein, S., 1961, Metabolic fractionation of ^{13}C and ^{12}C in plants: Plant Physiology, v. 36, p. 133–138.

Pearson, A., Sessions, A.L., DeLong, D.F., and Hayes, J.M., 2002, Carbon isotopic analysis of selected nucleic acids from environmental samples: Geochimica et Cosmochimica Acta, Supplement 1, Abstracts of the 2001 Goldschmidt Meeting, v. 66, p. A585–A585.

Petersen, G., and Seberg, O., 2003, Phylogenetic analyses of the diploid species of *Hordeum* (Poaceae) and a revised classification of the genus: Systematic Botany, v. 28, p. 293–306.

Petit, R.J., Kremer, A., and Wagner, D.B., 1993, Geographic structure of chloroplast DNA polymorphisms in European oaks: Theoretical and Applied Genetics, v. 87, p. 122–128.

Porebski, S., Bailey, L.G., and Baum, B.R., 1997, Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components: Plant Molecular Biology Reporter, v. 15, p. 8–15.

Reichle, D.E., Dinger, B.E., Edwards, N.T., Harris, W.F., and Sollins, P., 1973, Carbon flow and storage in a forest ecosystem, *in* Woodwell, G.M., and Pecan, E.V., eds., Carbon and the biosphere: Washington, D.C., National Technical Information Service, p. 345–365.

Scott, L., 2002, Grassland development under glacial and interglacial conditions in southern Africa: Review of pollen, phytolith and isotope evidence: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 177, p. 47–57.

Sessions, A.L., Hayes, J.M., Pearson, A.M., and DeLong, E., 2002, Carbon isotope ratios of nucleic acids from environmental samples: American Chemical Society, Abstracts of Papers, v. 224, Part 1, p. 002-GEOC.

Smith, B.N., and Epstein, S., 1971, Two categories of $^{13}C/^{12}C$ ratios for higher plants: Plant Physiology, v. 47, p. 380–384.

Sugiura, M., and Takeda, Y., 2000, Nucleic acids, *in* Buchanan, B.B., et al., eds., Biochemistry and molecular biology of plants: Washington, D.C., American Society of Plant Biologists, p. 260–309.

Takigiku, R., 1987, Isotopic and molecular indicators of origins of organic compounds in sediments [Ph.D. thesis]: Bloomington, Indiana University, 248 p.

Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Gilbert, M.T.P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., and Cooper, A., 2003, Diverse plant and animal genetic records from Holocene and Pleistocene sediments: Science, v. 300, p. 791–795.

Zhang, J., and Stewart, J.M., 2000, Economical and rapid method for extracting cotton genomic DNA: Journal of Cotton Science, v. 4, p. 193–201.

Manuscript received 13 June 2003
Revised manuscript received 30 October 2003
Manuscript accepted 7 November 2003

Printed in USA