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Leaf wax composition and carbon isotopes vary among major conifer groups

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Abstract

Leaf waxes (e.g. *n*-alkanes, *n*-alkanoic acids) and their carbon isotopes ($\delta^{13}C$) are commonly used to track past changes in the carbon cycle, water availability, and plant ecophysiology. Previous studies indicated that conifers have lower *n*-alkane concentrations than angiosperms and that ^{13}C fractionation during *n*-alkane synthesis ($\varepsilon_{n\text{-alkane}}$) is smaller than in angiosperms. These prior studies, however, sampled a limited phylogenetic and geographic subset of conifers, leaving out many important subtropical and Southern Hemisphere groups that were once widespread and common components of fossil assemblages. To expand on previous work, we collected 43 conifer species (and *Ginkgo biloba*) from the University of California Botanical Garden at Berkeley, sampling all extant conifer families and almost two-thirds of extant genera. We find that Pinaceae, including many North American species used in previous studies, have very low or no *n*-alkanes. However, other conifer groups have significant concentrations of *n*-alkanes, especially Southern Hemisphere Araucariaceae and Podocarpaceae (monkey puzzles, Norfolk Island pines, and yellowwoods), and many species of Cupressaceae (junipers and relatives). Within the Cupressaceae, we find total *n*-alkane concentrations are high in subfamilies Cupressoideae and Callitroideae, but significantly lower in the early diverging taxodioid lineages (including bald cypress and redwood). Individual *n*-alkane chain lengths have a weak phylogenetic signal, except for *n*-C_{29} alkane, but when combined using average chain length (ACL), a strong phylogenetic signal emerges. The strong phylogenetic signal in ACL, observed in the context of a common growth environment for all plants we sampled, suggests that ACL is strongly influenced by factors other than climate. An analysis of $\varepsilon_{n\text{-alkane}}$ indicates a strong phylogenetic signal in which the smallest biosynthetic fractionation occurs in Pinaceae and the largest in Taxaceae (yews and relatives). The relationship between phylogeny and $\varepsilon_{n\text{-alkane}}$ may be related to differences in carbon metabolism among conifer clades. These results have important implications for interpreting *n*-alkane $\delta^{13}C$ values in sedimentary archives, especially outside of North America.

1. INTRODUCTION

The leaf waxes of vascular plants are primarily composed of long-chain *n*-alkyl compounds including the *n*-alkanes, *n*-alkanoic acids, and *n*-alkanols (Eglinton et al., 1962; Eglinton and Hamilton, 1967; Kolattukudy et al., 1976). These leaf waxes are widely used to reconstruct environmental change because they are commonly transported and preserved in sediments from which they are easily extracted and analyzed (Cranwell, 1981; Rieley et al., 1991; Collister et al., 1994a; Freeman and Colarusso, 2001). Of the leaf wax components, *n*-alkanes have been the focus of most geologic studies, although other waxes, such as *n*-alkanoic acids and *n*-alkanols, can

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be informative when preserved (Feakins et al., 2007; Douglas et al., 2012; Sachse et al., 2012). Leaf waxes, especially *n*-alkanes, have been applied to answer various paleoecological questions, such as distinguishing relative changes in paleotemperature or paleohydrology (Castañeda and Schouten, 2011; Freeman and Pancost, 2014; Bush and McInerney, 2015). More recently, analyses of the carbon and hydrogen isotope composition of leaf waxes has greatly expanded their utility for paleoclimate studies, and they have been used extensively to characterize past changes in the carbon cycle, water availability, hydrology, and vegetation (e.g., Pagani et al., 2006; Schouten et al., 2007; Smith et al., 2007; Tierney et al., 2008; Castañeda et al., 2009; Tipple et al., 2011; Niedermeyer et al., 2014).

Despite the widespread use of leaf waxes, many unanswered questions about them remain. For example, the variation in wax concentration and composition among and within many major plant groups has not been quantified and is largely unknown (Diefendorf et al., 2011; Bush and McInerney, 2013). Plant traits such as leaf lifespan, cuticle thickness, and specific leaf area may influence composition of short-chain *n*-alkanoic acids (Mueller et al., 2012), but their influence on the composition of other waxes is largely unstudied. Climate may also influence leaf wax composition, as observed in studies of *n*-alkane average chain length, but relationships with climate tend to be weak and to vary by species (e.g., Dodd and Poveda, 2003; Sachse et al., 2006; Shepherd and Wynne Griffiths, 2006; Castañeda et al., 2009; Vogts et al., 2012; Hoffmann et al., 2013; Tipple and Pagani, 2013; Bush and McInerney, 2015). In addition, the timing of leaf wax synthesis may vary among species, which has important consequences for interpreting isotope signals preserved in these waxes (Sachse et al., 2006, 2009; Tipple et al., 2013). Taken together, these unanswered questions complicate interpretations of leaf waxes found in sediments, especially if plant community composition has changed in combination with climate.

In this study, we focus on understanding variation in *n*-alkane composition among conifers, a major non-angiosperm seed plant group with a long and abundant fossil record. Conifers dominate many high elevation and high latitude ecosystems in the modern world, especially in the Northern Hemisphere, but the group has a global distribution and also includes many tropical members. Prior studies have suggested that conifers differ from angiosperms in their *n*-alkane chemistry, for example, having *n*-alkane concentrations 200 times lower when grown under the same climatic conditions (Diefendorf et al., 2011; Bush and McInerney, 2013). Also, ^{13}C fractionation during *n*-alkane biosynthesis (ε_{lipid}) is ~2‰ less than in angiosperms, suggesting underlying differences in the allocation of carbon to different materials (e.g., carbohydrates, amino acids, lipids) among major plant groups (e.g. Diefendorf et al., 2011). However, Diefendorf et al. (2011) measured only a few Northern Hemisphere conifer species. Many important subtropical, tropical, and Southern Hemisphere conifers groups have been ignored, even though they were more widespread in the past and are common components of fossil assemblages.

To expand on previous studies, we collected 43 conifer species (and *Ginkgo biloba*) from the University of California Botanical Garden at Berkeley. The sample includes species from all extant conifer families and more than 60% of extant genera. By collecting all specimens at a common site we attempted to minimize the confounding effects of climate, allowing potential phylogenetic patterns in the $\delta^{13}C$ of leaf waxes to be expressed more strongly. We find that species belonging to different major conifer lineages have different *n*-alkane compositions and concentrations even when grown in the same climate. A strong phylogenetic signal is also apparent in *n*-alkane isotope fractionation values. These results have important consequences for interpreting sedimentary leaf waxes where conifers are or were part of the plant community.

2. MATERIALS AND METHODS

2.1. Sampling location and leaf collection

Fresh leaf tissue was collected from 43 conifer species and from *G. biloba* at the University of California Botanical Garden at Berkeley (UCBGB; 37.8752°N, 122.2386°W, 210 m) in December of 2011. Evergreen leaf tissue was sampled from the 2011 leaf flush. The total rainfall for 2011 was 602 mm and the average minimum and maximum temperatures are 8.8 and 18.3 °C, respectively (PRISM, 2014). For each species, leaf tissue was collected from multiple branches on the sun-exposed side of a single mature tree per species using scissors, pruners, or pole-pruners. Sampling height ranged from 2 to 3 m except for *Sequoiadendron giganteum* and *Sequoia sempervirens*, which were sampled at ~25 m. The canopy structure at the UCBGB is very open and therefore it is unlikely that soil respiration generates a ^{13}C-depleted CO_2 layer at this sampling height. Sample collection information is reported in electronic annex Table EA-1. Samples (~10 g) were gently rinsed with deionized water, frozen (−20 °C), freeze-dried, and homogenized by powdering with a ball mill.

2.2. Lipid extraction and separation

Powdered leaves were extracted using an accelerated solvent extractor (Dionex ASE 350) with 2:1 (v/v) DCM/MeOH with three extraction cycles at 10.34 MPa (1400 psi) and 100 °C. The total lipid extract (TLE) was base saponified to cleave ester groups with 2.5 ml 0.5 N KOH in 3:1 (v/v) MeOH/water) for 2 h at 75 °C. After cooling, 2 ml of NaCl in water (5%, w/w) was added and then the solution was acidified with 6 N HCl to a pH of 1. The acidic solution was extracted with hexanes/DCM (4:1, v/v), neutralized with $NaHCO_3/H_2O$ (5%, w/w), followed by water removal over Na_2SO_4.

The saponified lipid extract was subsequently separated into four polarity fractions following a modified version of Sessions (2006). Briefly, 0.5 g of aminopropyl-bonded silica gel (Sigma Aldrich, St. Louis, USA) was loaded into 6 ml SPE glass tubes with PTFE frits on a Visiprep SPE vacuum manifold with disposable PTFE liners (Sigma Aldrich). Hydrocarbons were eluted with 4 ml of hexanes, ketones