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Phylogenetic diversity and evolutionary relatedness of alkenone-producing haptophyte algae in lakes: Implications for continental paleotemperature reconstructions

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ABSTRACT

Alkenones have been found in an increasing number of lakes around the world, making them a promising new tool for continental paleoclimate reconstruction. However, individual lakes may harbor different species of haptophyte algae with different sensitivities to temperature variations, thus presenting a significant challenge to the use of lacustrine alkenones for paleotemperature reconstructions. To explore the extent of lacustrine haptophyte diversity, we conducted the first comprehensive phylogenetic and geochemical survey of lacustrine alkenone producers. We sampled 15 alkenone-containing lake surface sediments from a variety of geographic locales and inferred identities of environmental sequences using 18S ribosomal RNA (rRNA) gene-based phylogenies. For two lakes, BrayaSø in southwest Greenland and Tso Ur on the Tibetan Plateau, we also analyzed both surface and downcore sediments to characterize haptophyte populations through time. In parallel with phylogenetic analyses, we determined the alkenone distributions (including C_{37}/C_{38} ratios, and the presence/absence of C_{38} methyl ketones and C_{40} compounds) in all the samples. The resulting alkenone profiles from this study do not all align with traditional "marine" versus "coastal/lacustrine" alkenone profiles. Additionally, our genetic data indicate the presence of multiple haptophyte species from a single lake sediment sample; these distinct haptophyte populations could not be discerned from the alkenone profiles alone. These results show that alkenone profiles are not a reliable way to assess the haptophyte algae in lakes and that DNA fingerprinting is a preferred approach for species identification. Although closely related haptophyte species or subspecies may not warrant different temperature calibrations, our results emphasize the importance of genetic data for inferring haptophyte identities and eventually selecting alkenone–temperature calibrations for paleoclimate reconstructions.

1. Introduction

Marine haptophyte algae produce unsaturated long-chain alkenones (LCAs) that serve as a well-established paleotemperature proxy (U^K_{37} or $U^{K'}_{37}$) in marine sediments (Brassell et al., 1986; Conte et al., 2006; Marlowe et al., 1984; Prahl and Wakeham, 1987; Volkman et al., 1980). Alkenones are produced by a limited number of haptophyte algal species within the order Isochrysidales (Medlin et al., 2008; Müller et al., 1998), notably *Emiliania huxleyi* and *Gephryocapsa oceanica* in the open ocean (Marlowe et al., 1984; Volkman et al., 1980), and *Isochrysis galbana* (Marlowe et al., 1984; Versteegh et al., 2001) and *Chrysotila lamellosa* in coastal regions (Conte et al., 1998; Marlowe et al., 1984; Rontani et al., 2004; Volkman et al., 1980, 1995). *E. huxleyi* and *G. oceanica* (Conte et al., 2006; Müller et al., 1998) are the dominant alkenone producers in marine surface waters, enabling the application of a global $U^{K'}_{37}$ calibration to marine sea surface temperature (SST) reconstructions.

Long-chain alkenones (LCAs) in lake sediments have great potential for continental climate reconstructions. LCAs have been increasingly reported in lakes around the world, including Antarctica, Europe, Greenland, South America, China and the western United States (Coolen et al., 2004; D'Andrea and Huang, 2005; Liu et al., 2008; Pearson et al., 2008; Toney et al., 2010; Zink et al., 2001). Lacustrine alkenone unsaturation ratios correspond to lake seasonal surface water temperatures and mean annual air temperature (Chu et al., 2005; D'Andrea and Huang, 2005; Sun et al., 2007; Zink et al., 2001). However, individual alkenone-containing lakes may require different calibrations, depending on the haptophyte species present (Chu et al., 2005; D'Andrea, 2008; Liu et al., 2008; Prahl and Wakeham, 1987; Prahl et al., 1988; Rontani et al., 2004; Sun et al., 2007; Versteegh et al., 2001; Volkman et al., 1995; Zink et al., 2001). Previous studies have

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generated a variety of LCA profiles from different lakes (Liu et al., 2008; Pearson et al., 2008), with varying ratios of C_{37}/C_{38} alkenone, percent concentrations of tetraunsaturated C_{37} ketones (% $C_{37:4}$), and the presence/absence of C_{38} methyl ketones and C_{40} alkenones. These variable distributions suggest that alkenones from different lakes could be produced by a diversity of haptophyte species, and therefore a single global temperature calibration may not be viable.

Although alkenone signatures provide a simple way to compare alkenones from different lakes, they do not provide definitive taxonomic information at the species level. Recent studies identified haptophyte species using genetic methods and enabled the identification of novel haptophytes species without the need for cultivation. Work by Coolen et al. (2004) targeted the 18S rRNA molecule to identify the haptophyte responsible for alkenone production in ancient Ace Lake, Antarctic sediments. These authors identified preserved DNA of a haptophyte closely related to *I. galbana*. D'Andrea and Huang (2005) reported the occurrence of a novel bloom-forming haptophyte species in lakes of Southwestern Greenland with extremely high alkenone concentrations, characterized by an abundance of tetraunsaturated alkenones and the presence of C_{38} methyl and ethyl ketones. Genetic work determined this Greenland haptophyte forms a distinct phylogenetic group from the marine and lacustrine phylotypes within the Isochrysidales (D'Andrea et al., 2006).

This paper aims to expand the phylogenetic dataset of lacustrine alkenone-producing haptophytes and compare 18S rRNA gene-based phylogenetic relationships with the observed alkenone lipid signatures. We studied lake sediments from Greenland, China, Northern Canada, and the continental United States. We hypothesized that different alkenone lipid signatures in lake sediments can be attributed to the genetic diversity of the haptophyte species, and that multiple alkenone-producing haptophyte species could be present in individual lakes. With this comprehensive survey we were able to identify a) presence/absence of biogeographical trends in lacustrine haptophyte distribution, b) relationships between alkenone lipid profiles and genetic rRNA-inferred phylogenies, and c) the accuracy and fidelity of the alkenone signature as a haptophyte identification tool. This dataset of haptophyte alkenone signatures and corresponding species classifications assess the reliability of haptophyte species identification through alkenone signature alone.

2. Methods

2.1. Sampling

Previous studies have demonstrated that DNA of haptophyte algae can be readily extracted from lake sediments (Coolen et al., 2004; D'Andrea et al., 2006). We analyzed sediments from 15 lakes (Fig. 1, Table 1) from the continental United States, Canada, Greenland, and Tibet. Lake surface sediment samples were collected by gravity core, split, and the upper 1 cm was used for analysis. Lakes Clear, Medicine, George, and Skoal sediments were freeze-dried before DNA extraction. All other sediment samples were kept at 4 °C and in the dark until processing. Individual sediment samples were split for DNA and alkenone analysis.

For Lake BrayaSø water column DNA, we collected water on June 20, 2007 at 10 m depth, corresponding to the chlorophyll maximum that day. One liter was filtered using a 0.22 μm pore size Sterivex filter (Millipore, Bedford, MA, USA), treated with Puregene cell lysis buffer (Qiagen, Carlsbad, CA, USA) and kept cool and in the dark until freezing at −20 °C.

2.2. DNA extraction

We extracted DNA from 1 g of lake surface sediment for each lake we examined using MoBio UltraPure DNA Extraction Kit for sediments (Qiagen, Carlsbad, CA, USA) according to the manufacturer's instructions. For lakes BrayaSø and Tso Ur, downcore sediment samples were also extracted for DNA analysis. For water samples, Sterivex filters were extracted using a Puregene Cell Kit (Qiagen) according to the manufacturer's instructions. Genomic DNA was polyethylene glycol (PEG) purified to remove proteins and other contaminants that inhibit PCR reactions: DNA was suspended in PEG at 4 °C overnight, centrifuged, and the pellet rinsed with ethanol (LaMontagne et al., 2002). We quantified total extracted genomic DNA yields using a NanoDrop nucleic acid spectrophotometer (Thermo Scientific, Wilmington, DE).

2.3. DNA amplification and sequencing

We amplified genomic DNA using haptophyte-specific oligonucleotide (Coolen et al., 2004; Simon et al., 2000) primers targeting 18S

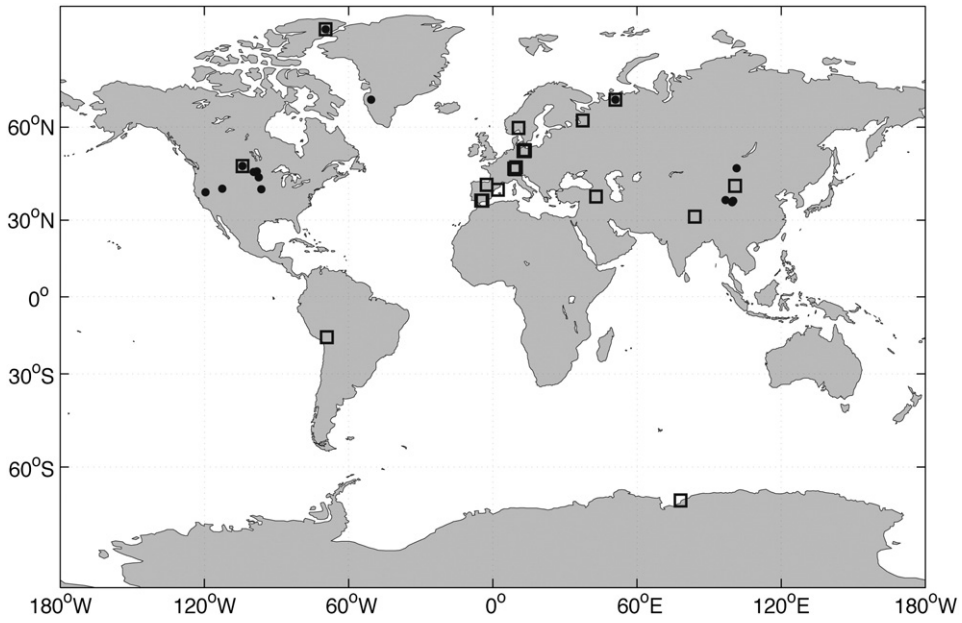

Fig. 1. Map of alkenone-containing lakes. Squares denote previously reported lakes, circles denote lakes analyzed in this study.