Ancient DNA in lake sediment records

Marco J.L. Coolen[1] and John A.E. Gibson[2]

[1]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, USA; mcoolen@whoi.edu
[2]Marine Research Laboratories, University of Tasmania, Hobart, Australia

Ancient DNA in lake sediments offers a novel window into past aquatic ecosystems and the environmental changes that triggered past species successions.

The geological record offers our best opportunity for understanding how biological systems function over long timescales and under varying paleoenvironmental conditions. Understanding these ecosystem responses to change is critical for biologists trying to understand how organisms interact and adapt to environmental changes, and for geologists seeking to use these biology-geology relationships in order to reconstruct past climate conditions from sediment records. For example, enumeration of microscopic fossilizing protists, such as diatoms, has become a standard paleoecological approach in the fields of paleoclimatology and paleolimnology. However, the identification of morphological remains is not always straightforward, as many taxa lack diagnostic features preserved upon fossilization. Lipid-based records can be particularly valuable for species that do not leave diagnostic features in the sedimentary record. Nevertheless, the interpretation of these molecular stratigraphic records is often complicated by the limited specificity of many lipid biomarkers.

There is thus a need to find new biomarkers with greater source-specificity that can be used to complement and enhance interpretations based on existing methods. The field of molecular biology offers a most promising approach/technique that is just starting to gain wider utility: The use of ancient DNA preserved in the sedimentary record (i.e., fossil DNA or *f*DNA) to reconstruct past ecosystems. Fossil DNA has been successfully employed to study the succession of species as a result of environmental changes in terrestrial (e.g., Willerslev et al., 2007), marine (Boere et al., 2009; Coolen et al., 2007; 2009; Coolen and Overmann, 2007; Manske et al., 2008), and lacustrine settings (Bissett et al., 2005; Coolen et al., 2004; 2008; Coolen and Overmann, 1998; D'Andrea et al., 2006; Epp et al., 2009). A major advantage of this molecular paleoecological approach is that ancient species can be studied, including those with-

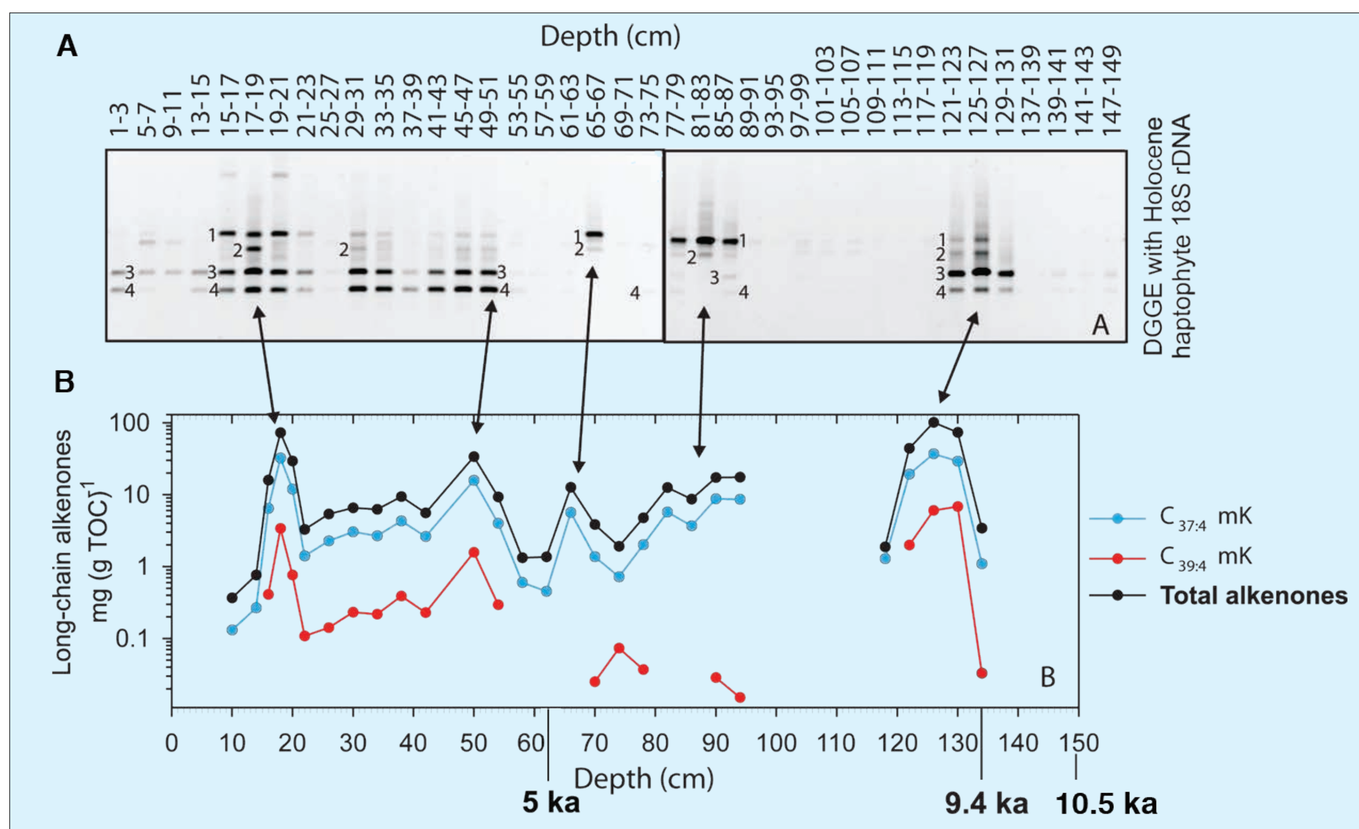

*Figure 1: Stratigraphy of biomarkers of photosynthetic haptophyte algae (18S ribosomal DNA, alkenones [mK, methyl ketone]) recovered from the Holocene sediments of Ace Lake (figure modified from Coolen et al., 2004). (**A**) Denaturing gradient gel electrophoresis (DGGE) gels with PCR-amplified 18S rDNA of Holocene haptophytes. DGGE bands numbered as 1 to 4 were excised from the gel and subsequently sequenced for phylogenetic analysis (Coolen et al., 2004). All sequences were related to non-calcifying* Isochrysis *spp. (data shown in Coolen et al., 2004). (**B**) Quantity of total alkenones produced by* Isochrysis *spp. (black line), the most predominant alkenone (i.e., the 4 times unsaturated C37:4mK; blue line), and C39:4 mK (red line) [mg (g-1 TOC)]. TOC = Total Organic Carbon. Comparable profiles were found for other alkenones that were produced by the* Isochrysis*-related haptophytes (see Coolen et al., 2004). The oldest analyzed sediment layer was deposited at 10.5 ka BP when the lake was freshwater, but the first haptophytes appeared at the onset of meromictic conditions when the lake became a marine basin and salinity reached 10 psu at 9.4 ka BP. The marine basin became an isolated saline lake at ~5 ka BP. Parallel examination of the alkenones and haptophyte sequences, especially from sediments deeper than 65 cm, revealed that the less common C39:4 mK was not biosynthesized by phylotype 1. The species-specific identification of alkenone sources is important for the calibration of alkenone-based SST reconstructions. See Coolen et al. (2004) for further details.*

out diagnostic features retained in the fossil record.

Study sites and analytical approaches for *f*DNA studies

Most *f*DNA surveys have been conducted on Holocene lake sediments that were deposited under cold and/or anoxic conditions. In particular, permanently stratified (meromictic) lakes with anoxic bottom waters and undisturbed laminated sediments provide excellent preservation conditions for *f*DNA. The strong vertical stratification of (microbial) species in meromictic environments makes it easier to identify species that are and were indigenous to the surface waters.

However, despite excellent preservation conditions, the degradation of ancient DNA to shorter fragments occurs within the first several thousands of years after deposition (Coolen and Overmann, 1998). Therefore, only short, less than 500 base pair (bp)-long *f*DNA fragments should be analyzed using molecular methods that involve polymerase chain reaction (PCR; a technique used to amplify DNA across several orders of magnitude to yield billions of copies of a particular DNA sequence of interest). Furthermore, the use of group-specific oligonucleotides (i.e., primers, which serve as a starting point for PCR) instead of universal primers significantly lowers the detection limit of *f*DNA during PCR. Identification of the ancient species composition most commonly involves the separation of PCR-amplified *f*DNA by denaturing gradient gel electrophoresis (DGGE) and subsequent phylogenetic analysis of sequenced DGGE bands (e.g., Boere et al., 2009; Coolen et al., 2004; 2007; 2008; 2009; Manske et al., 2008). DGGE is a fast and inexpensive way to analyze 200 to 500-bp-long PCR-amplified *f*DNA. We recently started to perform parallel phylogenetic surveys of reverse transcribed and PCR-amplified gene transcripts from the environmental samples (Coolen and Shtereva, 2009). Species that express genes in deep anoxic waters and sediment samples at the time of sampling are considered to be metabolically active and indigenous to those samples, and are therefore excluded as proxies for past environmental conditions of the surface waters. In order to further ground-truth *f*DNA data, we always perform a parallel analysis of the more traditional (morphological) proxies (Boere et al., 2009).

*f*DNA in Antarctic lakes: Protists and photosynthetic bacteria

Ace Lake (Vestfold Hills, Antarctica) was originally a freshwater lake. Due to sea level changes resulting from Holocene deglaciation, the lake became a meromictic saline fjord system with sulfur-rich bottom waters at 9.4 ka BP, and then a saline, meromictic lake about 5 ka BP (Cromer et al., 2005; Coolen et al., 2006). We expected these major hydrologic changes to have a strong impact on the ancient pelagic communities of the lake. The onset of photic zone anoxia at 9.4 ka BP was verified from the presence of *f*DNA and carotenoids of anoxygenic photosynthetic green sulfur bacteria, which still inhabit the sulfidic chemocline of Ace Lake today (Coolen et al., 2006). Furthermore, this freshwater/fjord transition was marked by a shift in pelagic cyanobacteria to a predominance of a *Synechococcus* strain (Coolen et al., 2008) that is still abundant in Ace Lake and which requires a minimum salinity of 10 psu for growth. The passing of a critical salinity threshold also resulted in the occurrence of non-calcifying alkenone-biosynthesizing haptophytes related to coastal *Isochrysis* species (Coolen et al., 2004; Fig. 1). Alkenones are of great in-

terest to paleoceanographers because of the strong empirical relationship between the degree of unsaturation in alkenones and growth temperature, which forms the basis for their use as a molecular proxy of past sea surface temperatures (SST) (e.g., Brassell et al., 1986). Based on the phylogenetic information inferred from *f*DNA, it should be possible to calibrate the reconstructed alkenone-based Holocene SST in eastern Antarctica once modern cultivars related to the Holocene haptophytes become available. Thus, the *f*DNA analysis served to identify past (morphologically non-fossilizing) species with clearly defined paleoenvironmental growth requirements, as well as biological sources of fossil lipid biomarkers.

*f*DNA in Antarctic lakes: Ancient copepods

The lack of diagnostic features is not restricted to unicellular aquatic organisms but can also be the case for metazoans. For example, the best preserved copepod remains present in lake sediments are eggs, which often have few if any distinguishing features. Therefore, the *f*DNA approach was developed to track changes in ancient copepod diversity in lakes (Bissett et al., 2005). This approach allowed the characterization of copepod species in sediments from three fresh to brackish Antarctic lakes as old as 10 ka BP. In most cases, the fossil species found matched those of extant lake populations, but analysis of early- to mid-Holocene sediments from one lake revealed a species that is not known to exist today (Gibson and Bayly, 2007). It was furthermore shown that it is possible to recover copepod DNA from lake sediments that underwent long-term refrigeration (4°C) or preservation in polyethyleneglycol (Bissett et al., 2005).

Potential of this new approach

It is obvious that *f*DNA can be used to: (a) study the succession of a large variety of ancient species with defined environmental requirements, including those that lack diagnostic features, (b) identify biological precursors of (lipid) biomarkers, and (c) verify paleoenvironmental information (i.e., alkenone SST) inferred from (lipid) biomarkers. However, the extent to which *f*DNA is preserved and the factors/conditions that control the preservation (survival) of DNA remain largely unknown. These and other issues must be addressed and resolved before *f*DNA techniques can be broadly applied, especially in non-polar lakes. In addition, many group-specific PCR/DGGE runs are required to cover the total diversity of ancient species. We therefore recently explored the use of parallel tag-encoded amplicon pyrosequencing (Sogin et al., 2006) as a molecular paleoecological tool. Price reduction and easier and faster computing of the gigantic sequencing datasets should eventually make pyrosequencing the standard method for *f*DNA studies, thereby replacing currently used molecular methods.

References

Bissett, A., Gibson, J.A.E., Jarman, S.N., Swadling, K.M. and Cromer, L., 2005: Isolation, amplification, and identification of ancient copepod DNA from lake sediments, *Limnology and Oceanography: Methods*, **3**: 533-542.

Coolen, M.J.L., Muyzer, G., Rijpstra, W.I.C., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2004: Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake, *Earth and Planetary Science Letters*, **223**: 225-239.

Coolen, M.J.L., Muyzer, G., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2006: Sulfur and methane cycling during the Holocene in Ace Lake (Antarctica) revealed by lipid and DNA stratigraphy. In: Neretin, L.N. (ed), *Past and Present Marine Water Column Anoxia*, NATO Science Series: IV-Earth and Environmental Sciences, Springer, 41-65.

Coolen, M.J.L. and Overmann, J., 1998: Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment, *Applied and Environmental Microbiology*, **64**: 4513-4521.

Coolen, M.J.L., Talbot, H.M., Abbas, B.A., Ward, C., Schouten, S., Volkman, J.K. and Sinninghe Damsté, J.S., 2008: Sources for sedimentary bacteriohopanepolyols as revealed by 16S rDNA stratigraphy, *Environmental Microbiology*, **10**: 1783-1803.

For full references please consult:

www.pages-igbp.org/products/newsletters/ref2009_3.html