

## Search for ancient DNA in sediments collected off Shimokita during the CK05-04 Leg.2 and CK06-06 cruises

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The purpose of this study was to establish a method for extracting ancient DNA from eukaryotic unicellular microorganisms (so-called protists), including planktonic microalgae, from sediment cores, and to clarify the relationship between paleoceanographic changes and population genetics. Only a few examples of ancient eukaryotic unicellular microorganisms have been found so far, and they date back to only 15 ka. Fossil DNA has the potential to clarify the relationship between paleoceanographic changes in water-mass properties and differentiation of a genetic population. Off Shimokita, Japan, undegraded organic materials are well preserved in the sediments because the dissolved oxygen concentration in the bottom water is low. DNA may also be preserved under such conditions. We assumed that photosynthetic planktonic organisms are not able to survive in the burial depth of the sediment, although living organisms in surface sediments and the water column are possible sources of contamination. Therefore, we used only planktonic eukaryotic microorganisms in the analyses to exclude possible contamination from organisms living in the sediment surface or in the water column.

The sediment cores used in this study were collected off Shimokita during the CK05-04 Leg 2 (cores C9001A and C9002A and B) and CK06-06 (core C9001C) Shimokita shakedown cruises of the D/V Chikyu. We extracted DNA from 59 layers, two from core C9001A, 13 from cores C9002A and B, and 44 from core C9001C. We also compared methods of extracting DNA from the sediments. PCR amplification and clone analyses of these DNA extracts were carried out with both universal eukaryotic primer pairs and specific primer pairs for diatoms and haptophytes. We obtained PCR amplicons of both nuclear 18S ribosomal DNA and chloroplast 16S ribosomal DNA. We then cloned the PCR amplicons into plasmid vectors for further clone analysis. At least 10 clones were retrieved from each sample and sequenced to confirm the presence of ancient DNA derived from planktonic eukaryotic microalgae.

The extraction results suggested that DNA was preserved in resting spores in the sediment. We compared extraction results at various Bead Beater bead mill homogenization speeds, and found that DNA was best obtained by using a moderate speed (12 000 rpm) or by retrieving the supernatant before extraction to extract DNA. Intensive homogenization not only caused cell walls to break but also broke the DNA into fragments. However, at lower speeds, cell walls were not broken, so the efficiency of DNA extraction was significantly lowered.

Our phylogenetic analyses showed that we retrieved DNA of eukaryotic planktonic microalgae (mainly diatoms) from the sediment surface down to 45 m (core 5, section 4). The lowest layer from which we retrieved diatom DNA corresponded to 5 ka. In the surface sediment (core 1), we identified DNA of various protists, whereas DNA clones were mainly from diatoms below core 2. On the other hand, we identified fungal DNAs from core 29 down to core 40. We expected diatom-derived DNA and fungal-derived DNA to have different existence properties.

The obtained nucleotide sequences were similar to the partial diatom sequence of 18S ribosomal RNA gene of *Chaetoceros socialis* (AY485446) (97% similarity). This species is known to form resting spores, which is consistent with our inference from the extraction results. At least four