

DNA analysis of a single *Pinus* pollen grain used for pollen analysis at species level

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Pollen can be found from snow and ice of glaciers in mid- and low-latitude since most of them are located within a few tens km from vegetation. Although some studies reconstructed past climate by pollen analysis using ice cores obtained in the region, only few attempts have been made so far. This is because pollen concentrations are generally low (around 100-1000 grains/kg). On the other hand, protoplasm of most pollen grains in the glaciers can be observed through a microscope. Such well-preserved pollen grains are difficult to collect from sediment cores of lakes, peat, and so on. Modern pollen analysis is used to identify pollen taxon from pollen morphology. Thus, the identification of related species based on the morphology is difficult and limited primarily to plant genus or family. Obtaining gene information by analyzing DNA of pollen grains from ice cores should allow new pollen analysis that can identify down to species level. The plant species belonging to the same genus are often distributed in different vegetation zones, identification down to the species therefore is extremely useful for reconstructions of past vegetation, climate and environment. This study attempted to analyze DNA of a single *Pinus* pollen grain extracted from snow samples (the section of 0.4-0.5 m depth) taken in Belukha Glacier of Russia's Altai Mountains in July 2003. The pollen grains used for DNA analysis seemed to have reached on the glacier in May to July of 2003. Snow samples were transported in frozen state to Japan and stored in a cold room (-20 degree Celsius) until the analyses were conducted. In this study, the chloroplast genome in *Pinus* pollen were amplified by the multiplex polymerase chain reaction (PCR) and sequenced to identify the species. So far, sequence data have been obtained at success rate of around 20%. A higher success rate could be achieved by improving the multiplex PCR technique. Since an ice core was drilled to the bottom (171 m depth) in July 2003 from the glacier, the present study suggests that the core may allow pollen analysis at species level