Dating Pollen

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Introduction

Pollen grains, the male microgametophytes of seed plants (angiosperms and gymnosperms), are widely distributed in the environment and accumulate in a range of sedimentary environments including lakes, bogs, caves, floodplains, and the deep oceans. While some components of pollen grains such as the generative nucleus degrade quickly in the environment, the resilient pollen wall can be preserved for thousands to millions of years. The widespread distribution and high preservation potential of pollen grains underline the value of ancient or "subfossil" pollen not only as a tool for reconstructing past vegetation history and environmental change (see PALYNOLOGY), but also as a sample material for radiocarbon dating. The wide variety of deposits that have been successfully dated from pollen include peats, lake sediments, aeolian deposits, packrat middens, and Pleistocene ice-wedge casts. Key advantages of pollen as a dating material are that it may be present in deposits where plant macrofossils are scarce or lacking, and it can be identified in terms of the source vegetation, unlike bulk material where the provenance is unknown.

Basis of the approach

Pollen grains, along with the spores of terrestrial lower plants (ferns and mosses), possess several characteristics that make them highly suitable for radiocarbon dating. The pollen wall is composed of the organic biopolymer sporopollenin, which is rich in carbon in the form of both long-chain macromolecules and aromatic compounds. Carbon in sporopollenin is derived from atmospheric CO_2 through photosynthesis, incorporating photosynthates that are either newly acquired during the pollen development stage or derived from stored reserves of at most a few years' age (e.g., Davis and Sparks 1971). Pollen grains therefore provide a reliable and effectively instantaneous "snapshot" of carbon isotopic ratios in the atmosphere during the time of pollen development. Fractionation effects (differential uptake of heavy and light carbon isotopes) occur during photosynthesis, but these are assessed by the parallel measurement of radioactive (^{14}C) and stable (^{13}C) carbon isotopes and are corrected for in the reporting of radiocarbon dating results. Sporopollenin is furthermore remarkably resistant to chemical attack, meaning that pollen grains are inert in the environment and can be considered closed systems with respect to the pool of carbon within them. Finally, the morphological characteristics of pollen grains mean that they can be identified to different taxonomic groups (typically families, genera or species), providing valuable contextual information for the dated sample.

Preparation procedures and practical considerations

Dating of pollen requires the preparation, in a suitably equipped laboratory, of a pollen-rich organic residue or pollen concentrate. This process shares many stages with the preparation of pollen residues for microscopic analysis, while avoiding carbon-containing chemicals (e.g., acetic anhydride, used in acetolysis, and ethanol). Typically, a combination of digestion stages in acidic and alkaline reagents is used to remove carbonates and humic acids from the peat or sediment sample, followed by deionized water washes to remove clay particles. Finally, the concentration of pollen grains is achieved through separation of the organic fraction using dense media (heavy liquids) and microsieving. Dense media separation may either be undertaken at a single target density (e.g., 1.5 times the density of water, or 1.5 *SG*) in which pollen grains, which have a typical density of slightly less than 1.5 *SG*

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(Régnell and Everitt 1992), float, or at a series of decreasing densities (e.g., from 1.6–1.15 *SG*), allowing selection of the purest residue(s) for dating (Vandergoes and Prior 2003). Alternatives to density separations include manual selection of individual grains using a micromanipulator (e.g., Long, Davis, and De Lanois 1992), and isolation of pollen grains using flow cytometry (Tennant et al. 2013). However, the former technique is generally too time-consuming to be practical unless very large pollen grains such as *Picea* are abundant, and the latter has yet to be routinely applied for radiocarbon dating. Sieving using nylon mesh can be used to further concentrate organic particles within the size range of pollen (typically ~20–80 μm, but this may be tailored to suit the predominant pollen types in a specific sample) (Brown et al. 1992).

At each stage of the preparation, it is helpful to inspect the content of the residue under a high-powered transmitted light microscope, using a fine Pasteur pipette to extract a minute droplet of residue and placing it on a microscope slide under a coverslip. This allows the efficacy of the preparation stages to be assessed and modifications to be implemented as necessary, for example in selecting suitable sieve mesh apertures with respect to the size classes of both target pollen types to be retained and any undesirable organic components to be removed. Visual inspection and quantitative estimation (e.g., tally count of objects) of the purity of the pollen concentrate is essential, since the efficacy of the pollen concentration preparation may be strongly influenced by site- and sample-specific characteristics including total pollen concentration in the deposit, as well as the composition and proportions of different organic matter types (pollen, terrestrial plant spores, algal spores, fungal spores, microcharcoal, wood fragments, leaf tissues, etc.).

Particular attention must be paid to maintaining a clean working environment and avoiding contamination with modern carbon in the form of ambient dust, pollen, powders, oils, and so on. This does not require the use of a certified clean-room facility, but simply special attention to minimizing exposure to contaminants by common-sense means, including wearing powder-free gloves at all stages, working with capped centrifuge tubes, pre-cleaning glassware and foil by heating to 500°C in a furnace, and thoroughly rinsing plastics (tubes, mesh, etc.) in deionized water. In light of the small sample size of pollen concentrates and the fairly intense pre-treatments required, it is advisable to undertake the parallel processing and dating of one or more laboratory standards to rule out contamination with modern carbon and to allow for correction factors to be applied if necessary. In any case, it is advisable to discuss the strategy for pollen concentrate dating with the radiocarbon facility prior to undertaking the preparation procedure.

Pollen concentrates should be dried or freeze-dried prior to measurement of the dry weight of sample submitted for dating. Advances in the measurement of small samples and widespread availability of the AMS technique mean that samples containing as little as 200 μg of carbon may be dated at many globally distributed radiocarbon facilities, and even smaller samples may be dated at some facilities. When assessing the size of samples for analysis, it should be taken into account that the pollen concentrates may not be composed entirely of pollen, and that pollen itself is only composed of around 50% carbon. In practice, dry sample weights of around 5–15 mg are recommended. While these amounts are small, quite large quantities of starting material may be required to produce pollen concentrates of sufficient size. At the outset, it is helpful to know the absolute concentration of pollen in the sediment sample, as commonly estimated through the addition of an exotic marker such as *Lycopodium* spores. This approach, of course, cannot be used *directly* in the exact sample to be dated. In a recent case study, approximately 15 g of dry lake sediments with a typical pollen concentration of ca. 200,000 grains per cm^3 were used to generate pollen concentrates ranging from 1–9 mg dry weight and 48% carbon content (Fletcher et al. 2017). Higher, or lower, pollen concentrations in the material would allow for smaller, or demand larger, starting samples, respectively.

Interpretation of pollen dates

In common with all dating approaches, attention should be paid to the characteristics of the sample material when interpreting the results of the radiocarbon dating. In principle, a sample of pollen grains should not be subject to uncertainty associated with dating samples from long-lived organisms, such as wood or charcoal, in which carbon accumulates sequentially over considerably longer timescales (decades to centuries). In practice, however, a sample of ancient pollen extracted from a sedimentary deposit will typically integrate pollen released over multiple seasons of pollen production, depending on the sedimentation rate of the deposit. Nevertheless, the resulting age of the pollen concentrate should provide an accurate estimate of the time of incorporation of pollen into the deposit. In the context of a continuously accumulating deposit, the age of the pollen concentrate will therefore indicate the time of deposition and provide a suitable basis for the development of depositional models and estimation of sedimentation rates. Two potential sources of uncertainty should be considered, namely reworking of old pollen from soils or sediments (e.g., Howarth et al. 2013), and incorporation of organic matter derived from aquatic production in deep lakes and hardwater settings (e.g., Fletcher et al. 2017). Both problems may result in ages that are older than the time of deposition, due either to the incorporation of non-contemporaneous (old) pollen grains, or contemporaneous organic matter containing old carbon associated with aquatic reservoir effects (MacDonald, Beukens, and Kieser 1991). The best guard against misinterpretation is good knowledge of the composition of the dated sample derived from visual inspection of the pollen concentrate prior to dating, accompanied by good contextual (geological, geomorphological, hydrological) information about the site. Comparison of the pollen concentrate ages and other dating results, such as radiocarbon dates on terrestrial plant macrofossils where available, can be highly informative regarding the detection of reworking or reservoir effects.

SEE ALSO: Radiocarbon Calibration and Age Estimation

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