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A SIMPLE METHOD TO SEPARATE POLLEN FOR AMS RADIOCARBON DATING AND ITS APPLICATION TO LACUSTRINE AND MARINE SEDIMENTS

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ABSTRACT. We present a simple method for manually separating pollen concentrates for radiocarbon accelerator mass spectrometry (AMS) dating using a mouth pipetting system. The required equipment is readily available from scientific equipment supply houses at minimal cost. Pollen samples from lake sediments required about 4 h of hand picking, whereas samples from marine sediments required about 8 h labor. Pollen dates from marine sediments were much older than expected. We are attempting to resolve whether this is due to contamination of the pollen or the presence of significant quantities of old reworked pollen. Pollen dates from lake sediments associated with Mazama Ash were consistent with other published ages; however, replicate dates on pollen samples from above the ash were consistently older than the surrounding sediment. Our results suggest that caution must be used when interpreting pollen dates if the potential for sediment reworking is present.

INTRODUCTION

The ability to obtain reliable radiocarbon dates from pollen using accelerator mass spectrometry (AMS) is well established (Brown et al. 1989, 1992). A number of methods have been developed to concentrate pollen samples for AMS ^{14}C analysis. In the first published work on the topic, Brown et al. (1989) obtained pollen concentrates from lake sediments using a variation of the standard palynological pretreatment of samples (Faegri and Iversen 1975) followed by repeated bleaching and sieving. Brown et al. (1989) dated bulk sediments and pollen concentrates from samples associated with previously dated horizons, including the Mazama Ash. Pollen dates were younger than bulk sediment dates, but were consistent with the range of commonly cited dates for the Mazama Ash. In a later paper, Brown et al. (1992) tested a set of modified preparation procedures on 6 samples from peat deposits associated with the Mazama Ash. The procedures were sufficient to remove nearly all non-pollen materials and the dates again were consistent with other published dates for the Mazama Ash.

Efforts to concentrate pure pollen samples from sites in other regions have generally found that the procedures developed by Brown et al. (1989) did not remove sufficient organic materials to produce a datable sample (Long et al. 1992; Regnell 1992). New methods have been developed to improve the removal of chemically resistant organic matter from peats (Richardson and Hall 1994) and hard-water lakes (Long et al. 1992; Regnell 1992). These methods include the use of strong acids such as H_2SO_4 (Regnell 1992), microbiological degradation (Richardson and Hall 1994), centrifugation (Regnell and Everitt 1996), and manual separation with a micromanipulator (Long et al. 1992). Although each of these new procedures has resulted in some improvements, they typically required purchase of expensive specialized equipment and materials (Long et al. 1992; Regnell and Everitt 1996), or were not able to completely remove contaminants (Regnell 1992; Richardson and Hall 1994).

We have developed a simple, inexpensive method for manually separating pollen from detrital material for AMS dating. We have tested our procedures on lake sediments associated with Mazama Ash from the Sierra Nevada, California, and on varved marine sediments of known age from the Santa Barbara Basin, California.

METHODS

Lake sediment samples used in this study were from a 2-cm Livingstone core recovered from Lake Moran, California, USA (38°23′00″N, 120°07′45″W, elev. 2006 m), by Roger Byrne and Eric Edlund, University of California, Berkeley. The lake lies in a granitic basin dammed by a terminal moraine on the western slope of the Sierra Nevada. Mazama Ash is present as a 3-cm thick layer at a depth of 229–232 cm and has been identified as the Tsoyawata Ash bed (Llao Rock event) that was a precursor to the climactic Mazama eruption (Byrne, personal communication). At Osgood Swamp (38°50′45″N, 120°02′30″W), about 50 km northeast of Lake Moran near the crest of the Sierra Nevada, tephra was also identified as the Tsoyawata Ash (Davis 1978; Bacon 1983; Hallett et al. 1997). In this region, the climactic Mazama Ash bed has been found only at sites east of the Sierra Nevada (Davis 1978). The climactic Mazama Ash and the Tsoyawata Ash have been found together at several localities in the Lahontan Basin (Davis 1978) and in Mono Lake (Davis forthcoming).

One-cm thick samples were obtained from immediately above and below the ash. The annual pollen accumulation rate for Lake Moran exceeds 50,000 grains cm^{-2} yr^{-1}, with pine representing 50% of the pollen sum; consequently only a small sample (3–5 g wet weight) was processed.

Marine sediments were obtained from box cores recovered from the Santa Barbara Basin, California, USA (34°15′N, 119°52′W), by Tim Baumgartner, Scripps Institute for Oceanography. Anoxic conditions in the basin support virtually no benthic life and support the preservation of annually laminated varves. Varve thickness averages 2.0 mm per pair of laminae (Hulsemann and Emery 1961), or 5 yr per centimeter, a rapid sedimentation rate that allows for high-resolution sampling. The annual nature of the Santa Barbara sediments has been confirmed by ^{14}C age determination (Emery 1960), ^{210}Pb measurements (Koide, Soutar and Goldberg 1972; Krishnaswami et al. 1973; Bruland 1974), correlation with precipitation records and tree-ring indices (Soutar and Crill 1977), and through correlation with fluctuations in microfossil assemblages associated with strong El Niño events (Schimmelmann et al. 1990). Average annual pollen accumulation rate in the Santa Barbara Basin is only 3500 grains cm^{-2} yr^{-1}, (Mensing 1993), and pine represents only 10% of the pollen sum; therefore, relatively large samples (45–65 g wet weight) were required for processing. Three consecutive 5-yr sediment samples from 1963–1977 and 4 additional 10–30-yr samples from 1860–1950 were treated for pollen extraction.

Pollen extraction generally followed Brown et al. (1989). Carbonates were removed with 10% HCl. Sediments were then boiled in KOH for 20 min to remove humic acids. Silicates were digested with 49% HF in a hot bath for a minimum of 1 h, followed by 10% HCl in a boiling bath for 10 min. Following treatment with 2%–3% NaOCl, samples were sieved with 38 μm and 74 μm mesh to concentrate pine pollen, the largest common pollen type in our study areas. The >74 μm and <38 μm fractions were decanted into vials for dating. Visual analysis of both size fractions revealed that they contained primarily non-pollen material. The 38–74 μm fraction was then treated with NaOCl twice more, followed by sieving after each treatment. The 2nd and 3rd bleach/sieve treatments eliminated significant amounts of amorphous organic material while preserving most of the pollen. Experiments with more than 3 sieving treatments showed that the additional sieving removed only marginal amounts of detrital material. However, the treated extract still contained a significant quantity of non-pollen material that needed removal prior to dating.

Pollen separation used a simple manually operated mouth pipetting system (Fig. 1). Components include a polystyrene mouthpiece, latex tubing (ca. 5 mm o.d., 3 mm i.d.), a disposable universal pipette tip, and a 9-inch borosilicate glass Pasteur pipette. The materials are very inexpensive and are readily available from any scientific equipment supplier. Tubing was cut to a comfortable work-

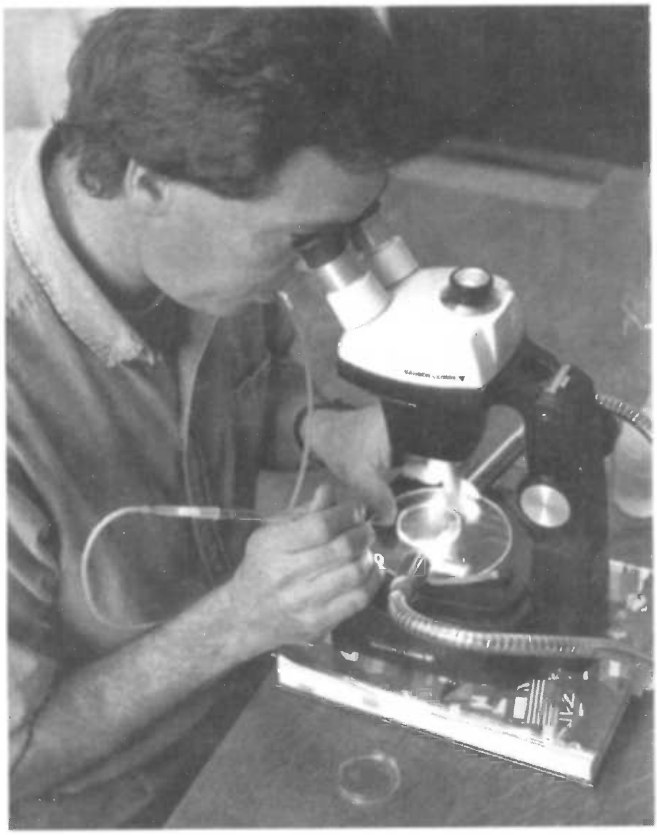

Figure 1 Photograph of the mouth pipetting apparatus in operation

ing length (ca. 60 cm) with one end fitted to the mouthpiece and the other end placed on the taper of the disposable pipette tip. A small swab of cotton was placed inside the disposable pipette tip to absorb moisture. The Pasteur pipette was prepared by heating over a flame and drawing out to an approximately 100-μm orifice. This was then inserted into the large opening of the plastic disposable pipette.

A small (ca. 0.1 mL) sample of pollen concentrate was ejected into a petri dish and flooded with deionized water to disperse the material. Pollen and other organic material quickly settled to the bottom. Pollen in the 38–74 μm fraction was easily identified for picking using a zoom binocular microscope in the range of 30–45× magnification under a fiber optic light source. The bleached pollen shows up clearly against a dark background. Pollen was aspirated into the Pasteur pipette. The small pipette orifice draws in the material through capillary action, and with gentle, periodic suction on the mouth pipette, one can rapidly vacuum pollen grains. This can be controlled to the extent that one can easily pick up a grain of pollen without also drawing in detritus that lies immediately adjacent. At this low magnification, we never found it difficult to hold the apparatus steady and guide the orifice to individual pollen grains. Generally 100–200 grains were collected in the pipette before ejecting the pollen directly into a vial for storage.

Samples were pipetted from the vials directly into quartz combustion tubes, and dried in a vacuum centrifuge. An excess of CuO oxidizer was added together with several mg of Ag powder, the tubes were flame-sealed under vacuum, and samples were combusted at 900 °C. The resulting CO_2 ali-

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quots (typically 25–70 μg of carbon) were cryogenically purified and then converted to graphite by a hydrogen reduction with an Fe or Co catalyst, and ^{14}C was measured by AMS. Ultra-small samples such as these are sensitive not only to contamination of the samples by trace quantities of contemporary carbon (most likely adsorbed CO_2), but also to contamination by "dead" carbon and/or to fractionation effects (Brown and Southon 1997; Kirner et al. 1997; van der Borg et al. 1997; Pearson et al. 1998). Numerous small aliquots of ^{14}C-free coal and a Modern ^{14}C standard (HOxI) were used as process blanks to monitor the combustion and graphitization process, and results from these test samples were used to correct the measured pollen data (Donahue et al. 1990; Brown and Southon 1997). Dates were calculated according to Stuiver and Polach (1977).

RESULTS AND DISCUSSION

Brown et al. (1989) suggested that only 200–500 spruce pollen grains should be sufficient to obtain an AMS date. We found that due to the smaller size of pine pollen compared with spruce, and the difficulty in graphitizing very small samples, a sample of about 10,000 grains (ca. 70 μg of carbon) was necessary for reliable AMS dating. Manual picking of pollen grains averaged 1000–2500 grains per hour depending on the sample material. Separating a 10,000-grain sample from Lake Moran required only 4 h labor, whereas the Santa Barbara Basin material averaged 8 h per sample because pollen was less abundant and chemically resistant organic material in the 38–74 μm fraction was more abundant. However, even for pollen-poor sites, isolation of a sufficient quantity of nearly pure pollen for AMS dating required only one day of additional labor after the chemical extraction procedures. For all samples, manual separation of pollen produced a nearly pure concentrate (Fig. 2).

The Lake Moran pollen sample from below the ash (LM 232-233) produced a date of 6880 ± 175 BP (Table 1). The LM 232-233 38–74 μm fraction received the same preparation treatment, except that pollen was not separated from the non-pollen matrix. This sample produced an identical date. The LM 232-233 >74 μm fraction, which contained no pollen but did include some charcoal, produced a date of 7000 ± 60 BP, which is not inconsistent with the other 2 dates. The 38–74 μm and >74 μm fractions from above the ash (LM 228-229) give nearly identical dates, both younger than published Mazama Ash dates. However, pollen sample LM 228-229, from above the ash, returned a date of 7120 ± 150 BP, our oldest Lake Moran date. Another pollen sample was picked from the same preparation and the date on this sample (7040 ± 80 BP) confirmed the earlier results.

Unlike the climactic Mazama Ash, the Tsoyawata Ash has few published dates and no AMS dates. Bulk sediment from below the ash at Osgood Swamp in California dated 6990 ± 300 BP (Haynes et al. 1967). Carbonized twig fragments from the upper 1 cm of a soil directly below the ash at a site near Crater Lake, Oregon, produced a date of 7015 ± 45 BP. At Wildcat Lake, Washington State, Blinman, Mehringer and Sheppard (1979) found both ashes and obtained a date of 6940 ± 120 BP on bulk sediment below the lower ash and a date of 6750 ± 90 BP on bulk sediment between the 2 ashes. They used a complex process derived from upper core pollen accumulation rates to calculate the time between the 2 ash falls to be approximately 140 yr. Other authors have suggested that a period of <200 yr separates the 2 ash falls (Bacon 1983; Young 1989).

Many efforts have been made to accurately date the climactic Mazama event (see Hallett et al. 1997 for a recent review). New AMS dates on charcoal and twig fragments from Mazama air-fall deposits suggest a weighted mean age of 6730 ± 40 BP (Hallett et al. 1997). Adding the estimated time between the 2 ashes to this date gives an approximate age of 6870–6930 ± 40 BP for the Tsoyawata event. Each of our pollen dates falls within this range of potential dates; however, the pollen dates from above the ash are older than the sedimentary matrix of the same strata. Previous studies have

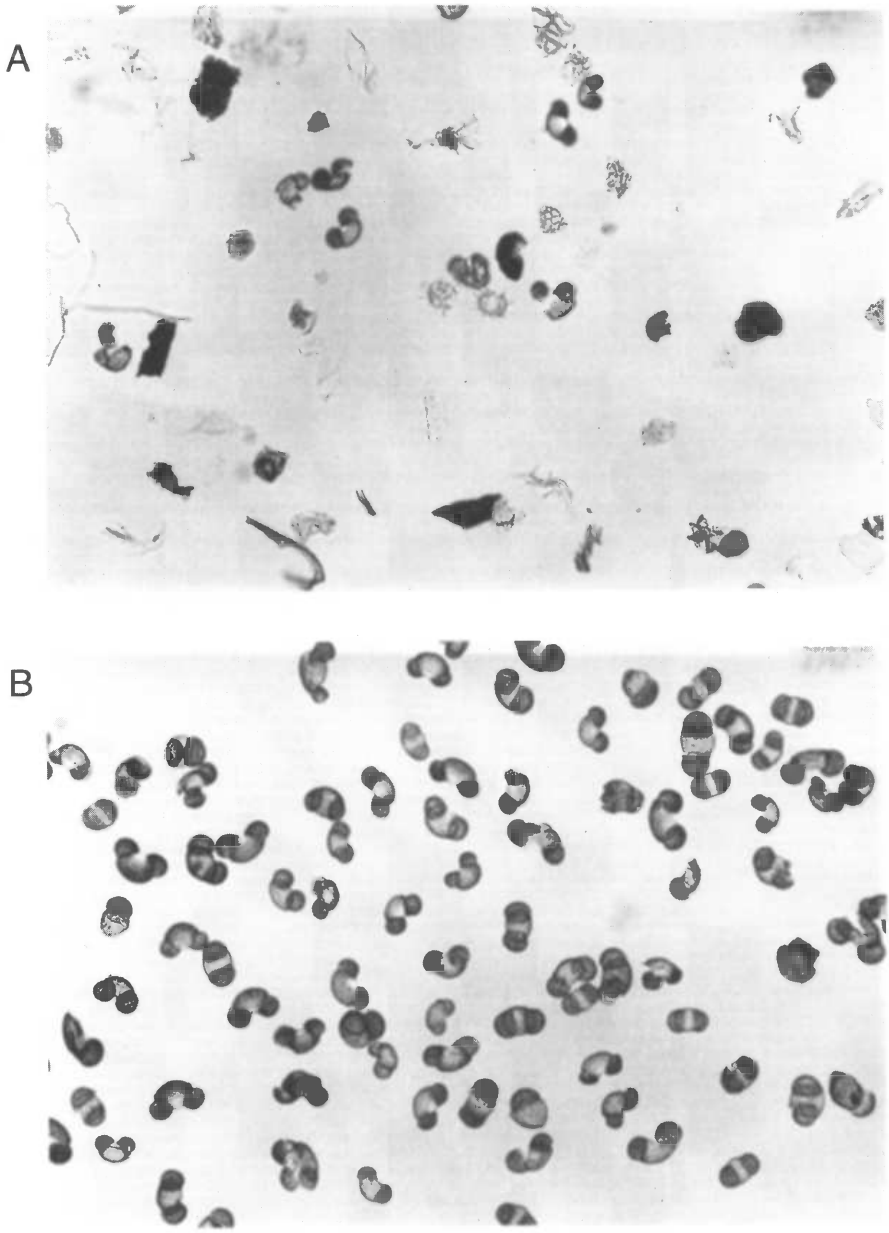

Figure 2 Photomicrographs showing samples before and after manual purification of the 38–74 μm fraction. 2A. Before separating pollen; note the presence of organic matter, unknown algal bodies and charcoal fragments. 2B. Pure concentrate remaining after separation of pine pollen.

consistently found pollen dates to be younger than the surrounding sediment, suggesting that the pollen represented a more accurate chronological indicator than the bulk sediment (Long et al. 1992; Regnell 1992). Although in general we would agree with this reasoning, our results suggest that the presence of reworked pollen in a sample could potentially provide a date older than sediment from the same strata. Mazama Ash may have altered soil infiltration rates, channeling runoff and leading

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to localized downcutting. Palynological evidence for this period shows high percentages of oak pollen, suggesting conditions drier than today, and possibly lowered lake levels. As lake levels fluctuate, pollen eroded from near-shore sediments can be redeposited in the central basin. This is more likely to occur at times of rapid climate change, precisely the periods for which paleoecologists desire accurate dates.

For the Santa Barbara Basin samples, the exact calendar age for each sample was known from varve counts (Tim Baumgartner, personal communication 1993). We dated pollen from strata prior to and associated with the ^{14}C spike caused by nuclear testing in the 1950s and 1960s. The 3 pollen samples between 1963 and 1977 dated above modern as expected (Table 1), whereas the fraction modern averages 67% of the atmospheric value for this period, suggesting either that as much as 33% of the material is intrusive dead carbon, or that a large fraction of pre-bomb pollen is in the sample. Dates for the 4 pollen samples prior to the bomb peak were much older than expected, which suggests that any intrusive component must be quite old. We can suggest 2 possible reasons for these results; the pollen could be contaminated with residual old carbon, or a large contribution of old reworked pollen is in the sample. Similar arguments may also apply to charcoal in the Santa Barbara Basin since our 2 charcoal samples gave comparable or older dates than pollen. We are currently attempting to resolve this question, since it may have important implications for interpretation of pollen diagrams from the Santa Barbara Basin.

The <38 μm fractions produced very old dates. This is not simply due to the presence of marine organics, because the reservoir age for the Santa Barbara Basin is only 825 yr (Ingram and Kennett 1995). These ages could be due to the presence of petroleum seeps or black carbon (Masiello and

Table 1 Dates obtained for samples from Lake Moran (LM) and the Santa Barbara Basin (SBB). All dates were determined at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry (CAMS). Lake Moran samples are depth in cm down the core and Santa Barbara Basin samples represent calendar years AD.

CAMS #	Location	Sample	Material	^{14}C age (BP)	Fraction modern
42474	LM	228–229	Pollen	7120 ± 150	
46338	LM	228–229	Pollen	7040 ± 80	
29051	LM	228–229	38–74 μm bulk fraction	6620 ± 60	
29052	LM	228–229	>74 μm bulk fraction	6650 ± 50	
29053	LM	232–233	Pollen	6880 ± 170	
29054	LM	232–233	38–74 μm bulk fraction	6880 ± 60	
29055	LM	232–233	>74 μm bulk fraction	7000 ± 60	
26573	SBB	1963–1967	Pollen	>Modern	1.1998
26574	SBB	1968–1972	Pollen	>Modern	1.1453
26575	SBB	1973–1977	Pollen	>Modern	1.0725
26580	SBB	1963–1967	<38 μm bulk fraction	4450 ± 60	
26579	SBB	1968–1972	<38 μm bulk fraction	7400 ± 60	
26578	SBB	1973–1977	<38 μm bulk fraction	4760 ± 50	
39879	SBB	1860–1890	Pollen	1080 ± 140	
39880	SBB	1890–1920	Pollen	1210 ± 140	
39881	SBB	1920–1950	Pollen	860 ± 140	
39882	SBB	1933–1942	Pollen	1760 ± 240	
26577	SBB	1963–1967	Charcoal	>Modern	1.0793
26576	SBB	1968–1972	Charcoal	1060 ± 100	

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Druffel 1998). However, neither of these explanations seems reasonable for explaining the old pollen dates given the chemical treatment and appearance of the pollen.

CONCLUSION

We have developed a simple and inexpensive method for separating pure pollen samples for AMS ^{14}C dating. The equipment and method require virtually no investment. The only expensive equipment necessary, a binocular microscope capable of about 35× magnification, is commonly present in most pollen labs. We have demonstrated that samples sufficiently large for AMS dating (50–100 μg) can be efficiently extracted even from pollen-poor sediments. We are continuing to test samples from the Santa Barbara Basin to better understand dating pollen from the marine environment. The pollen dates from Lake Moran are consistent with other published dates for the Tsoyawata/Mazama Ash; however, our results raise the possibility that pollen present in sediments above the ash may include some percentage of old pollen that has been reworked and redeposited. Our results suggest that bulk sediment dates for a particular stratum do not necessarily indicate when the pollen was deposited; direct dating of pollen provides a more accurate date.

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