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TESTING POLLEN SORTED BY FLOW CYTOMETRY AS THE BASIS FOR HIGH-RESOLUTION LACUSTRINE CHRONOLOGIES

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ABSTRACT. Documenting leads and lags in terrestrial records of past climate change is critical to understanding the behavior of Earth's natural climate system and making reliable predictions of future climate conditions. However, uncertainties of several hundred years in age models make it difficult to distinguish synchronicity and feedbacks in paleo archives. In lakes this is often due to the lack of terrestrial macrofossils in climate-sensitive locations, such as high alpine or dryland settings. The potential of radiocarbon (^{14}C) dating of pollen has long been recognized, but the difficulty of cleanly separating pollen from other kinds of organic carbon has limited its usefulness. Here we report ^{14}C ages on pollen separated by flow cytometry, from a set of closely spaced samples from Mono Lake, California. The accuracy of the pollen ages is tested using well-dated bracketing tephras, the South Mono and North Mono-Inyo tephras. In spite of the purity of the sorted samples, the pollen dates are older than the bounding tephras by ~400 yr, similar to some other pollen-dating studies. While improvements in sample preparation protocols are planned, understanding the geological processes involved in the production, preservation, and deposition of pollen at each site will be critical to developing robust high-resolution age models.

KEYWORDS: flow cytometry, lakes, paleoclimate, pollen, radiocarbon.

INTRODUCTION

High-resolution and high-precision age models for proxy records are required to understand the teleconnections and feedbacks in the global climate system, including abrupt forcings such as volcanic eruptions (Lavigne et al. 2013) and changes in sea ice cover (Denton et al. 2005; Li 2005). Without good chronologies, each climate record stands alone, and analyses of regional and global patterns are limited. High-precision correlations are especially important in assessing the realistic nature of climate model predictions, because models are much better at producing patterns in space than time series at individual locations. However, paleoclimatic reconstructions are typically time series measured at a single location, and are relatively isolated when age models are of low resolution. This weakness is only highlighted by the increasing number of very high-resolution proxy reconstructions, such as those from scanning XRF and paleomagnetic U-channel analysis (Grimm et al. 2011; Vigliotti et al. 2014; Noble et al. 2016). Great progress in regional and global reconstructions has been made in the last several decades using highly precise uranium-series age models on speleothems (Wang et al. 2001, 2008; Zhang et al. 2008; Cheng et al. 2016), and tree-ring records provide (sub-) annual-precision records over the last 1000 to 2000 yr (e.g., Cook et al. 2007). However, for regions with few suitable speleothems, such as the western United States (Asmerom et al. 2010; Lachniet et al. 2014), a well-dated network of terrestrial reconstructions may be lacking for periods predating tree-ring records.

High-precision, accurate chronologies are especially elusive in terrestrial climate records from archives such as lakes and meadows. Many lakes of interest in the western United States and other arid regions are climatically sensitive because they are hydrologically closed, but have little terrestrial vegetation to produce macrofossils or charcoal, and scant run-off to carry these materials into the lake (Benson et al. 1996, 2003; Placzek et al. 2006; Kirby et al. 2010). Other

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lakes are excellent recorders of glacial activity, a powerful indicator of seasonal temperature, but sit at elevations or latitudes where vegetation is sparse, and thus also lack macrofossils (Clark and Gillespie 1997; Crann et al. 2015; Xu et al. 2015). Although bulk sediment is sometimes used in the absence of other materials, such sampling homogenizes all the carbon-bearing materials in a lake, including dissolved, particulate, organic, inorganic, recycled, and freshly eroded carbon, and thus the resultant radiocarbon (^{14}C) dates are of unknown accuracy. On the other hand, pollen is produced abundantly by most terrestrial plants and is spread widely by wind transport.

Pollen has long been used as a proxy indicator of vegetation change and, since the development of accelerator mass spectrometry (AMS) for ^{14}C in the late 1980s, a number of attempts have been made to develop methods to isolate pollen for ^{14}C dating (Brown et al. 1989, 1992; Long et al. 1992; Mensing and Southon 1999; Neulieb et al. 2013). None of the methods tried have been widely adopted, because either specialized equipment or extensive time was required. Most pollen dates reported in the literature are therefore still a combination of pollen and micro-charcoal, plant detritus, and amorphous organic material. The pollen is produced by vegetation with carbon directly from the atmosphere and (presumably) quickly deposited in the place where it is preserved, while the other materials all bear carbon of some indeterminate age (Piotrowska et al. 2004; Munroe 2007; Li et al. 2012; Lozano-García et al. 2015).

Recent application of flow cytometry, a technique developed to sort biological cells and organisms, demonstrates the potential for efficient, complete purification of pollen from sediments (Byrne et al. 2003; Tennant et al. 2013). This approach is potentially very powerful for improving terrestrial chronologies, but its application to real sediment sequences raises a number of questions: How short and variable is the time from production of pollen to deposition in the sediment? What is the potential for contributions from pre-aged pollen on the landscape, or erosion of ancient pollen in surface deposits? Can different sources of pollen be discriminated by sieving or by sorting parameters? What is the most efficient protocol for concentrating the pollen before cytometric sorting, and cleaning afterward? What kinds of variations in protocol may be required for different kinds of sediments?

As a preliminary exploration of these and other questions, we have applied cytometric sorting to pollen for AMS dating in a short sequence of sediment from Mono Lake, California. Located on the eastern flank of the Sierra Nevada (38.0°N, 119.0°W), on the western edge of the arid Great Basin region (Figure 1), Mono Lake is a climatically sensitive lake with potential for rich proxy information. A well-dated decadal-resolution absolute lake level curve for the last 2000 yr was developed by Stine (1987, 1990a), taking advantage of the artificial reduction in lake level during the 1980s. Sediment cores from the lake can help to fill gaps in the curve, as well as provide a lake history before 2000 yr BP, if sufficient chronologies can be developed.

One such core, BINGO-MONO10-4A-1N, records the last >10,000 yr in multiple proxies (Zimmerman et al. in revision) but over that period has an average uncertainty of ± 325 yr (95% confidence interval) in the age model (Figure 2). The age model is based on ^{14}C dating of macrofossils and the identification of two tephras, the North Mono-Inyo and South Mono tephras. These tephras are comprehensively described from exposures around the basin (Sieh and Bursik 1986; Bursik et al. 2014) and their ages determined by ^{14}C dating of leaves and twigs from bushes killed by the eruptions (Bursik and Sieh 2013). The end of the North Mono-Inyo sequence is further constrained by a dendro-chronological age on the last phase of the eruption, of late summer AD 1350 (Millar et al. 2006). Pollen separated from the sediment between these

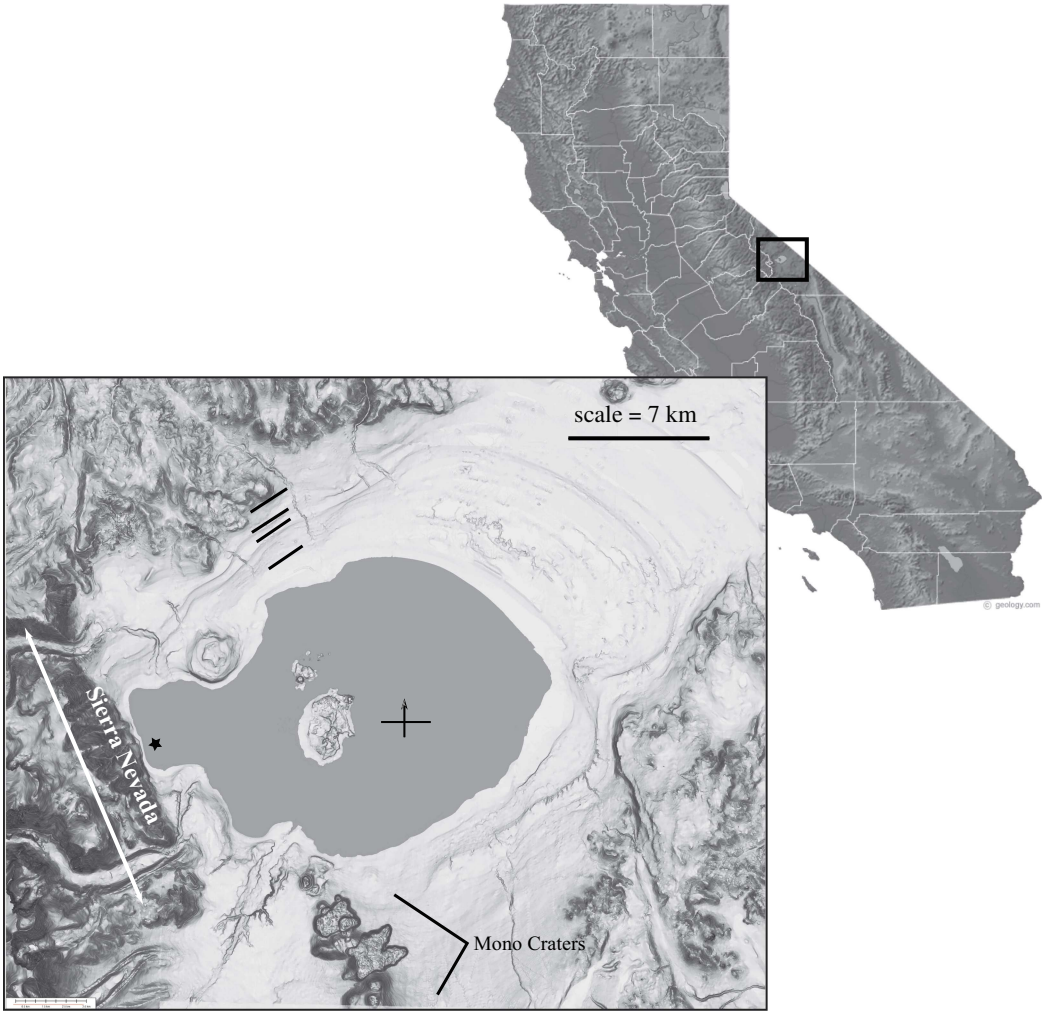

Figure 1 Location and digital elevation map of the Mono Lake basin in the eastern Sierra Nevada, on the western edge of the Great Basin. More than 75% of the modern inflow to the basin comes from the main Sierran streams, fed by rain and snowmelt from the mountains. Eruptions of the Mono-Inyo Craters to the south of the lake are the source of the North Mono-Inyo and South Mono tephras. Deep-lake silts of the Pleistocene cover much of the lake plain and late Pleistocene shorelines appear on the map as bathtub rings around the eastern edges of the basin on the DEM. In the modern climate little to no surface run-off drains the north, east, and south parts of the basin, but run-off from these areas may have been greater in the past (Zimmerman et al. 2011). The BINGO-MONO10-4A-1N core site is marked by the black star, at 2.8 m water depth (1942.3 m above sea level [m asl]); maximum lake depth is ~1897 m asl, off the southern shore of Paoha Island (Scholl et al. 1967).

tephras should therefore date to between 1345 ± 55 and $615\ ^{+30}/_{-15}$ cal BP, unless it has been stored on the landscape or altered by chemical preparation.

METHODS

Twelve samples were collected from between the South Mono and North Mono tephras in core BINGO-MONO10-4A-1N (hereafter: BINGO/10-4A), which is archived at the National Lacustrine Core Repository at the University of Minnesota (LacCore). Previous work

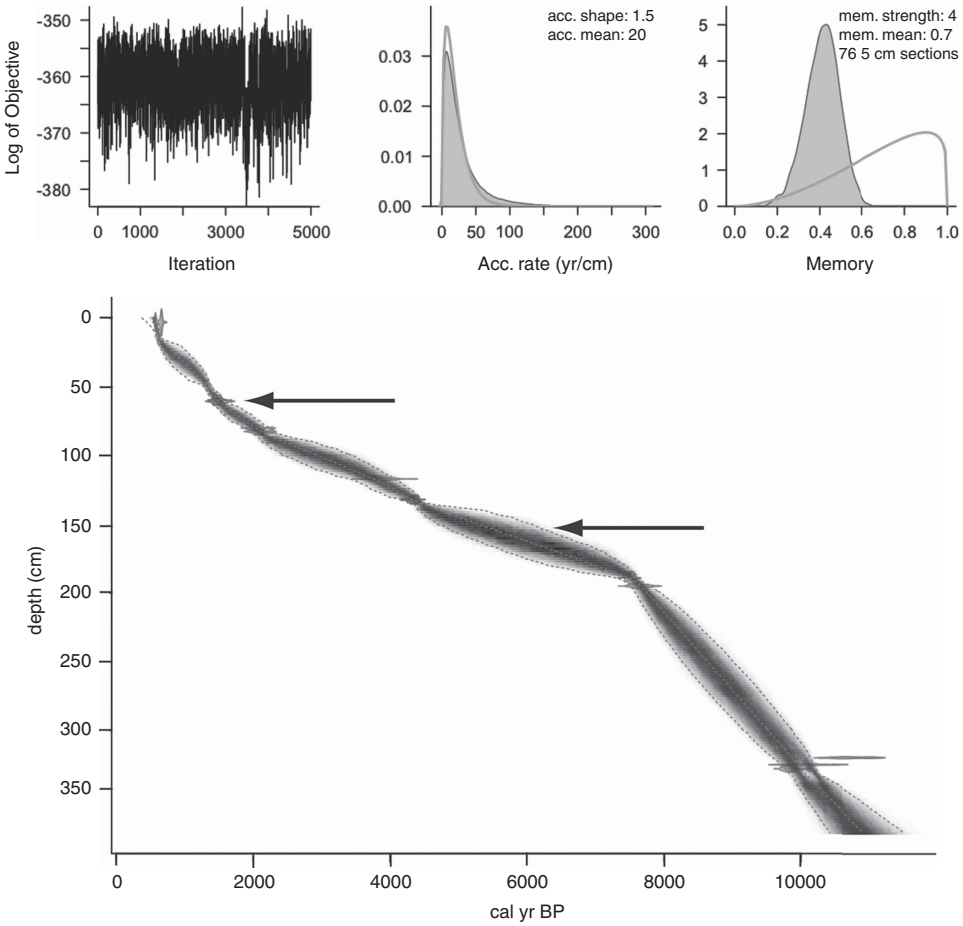

Figure 2 Bayesian age model for full BINGO-MONO10-4A-1N core; 95% confidence envelope is as narrow as 59 yr near dates, but expands to as much as 1480 yr in intervals between dates (arrows). Sub-plots at top are diagnostics of the Bacon age model and show (left) the distribution of the Markov Chain Monte Carlo (MCMC) iterations produced by Bacon, with a lack of structure indicating a stationary distribution; (center) the prior (solid gray line) and posterior (light gray histogram) distributions of the accumulation rates; and (right) prior and posterior distributions of the memory, a measure of the variability of the accumulation rate between neighboring depths in the core.

(Davis 1999) suggests that pollen concentrations in this interval at Mono Lake may be as high as 50,000 grains per cm^3 of sediment. To balance stratigraphic resolution with sufficient pollen for dating, samples were 1 cm in the depth direction, and approximately 2 cm^3 in volume. Pollen was concentrated at LacCore using the standard protocol for pollen intended for ^{14}C dating (Figure 3).

This protocol differs from the standard LacCore pollen prep for palynological studies in the following ways: baked glassware is used in place of organic carbon-based utensils (e.g., wooden stirrers, plastic tubes); samples are sieved to isolate the 20–118 micron fraction, rather than the 7–160 micron fraction; new heavy-liquid solution (LST – lithium heteropolytungstate) is used instead of hydrofluoric acid to remove silicates; resistant organic matter is removed with bleach rather than acetolysis; and no ethanol or tertiary butyl alcohol are used. The goal of this

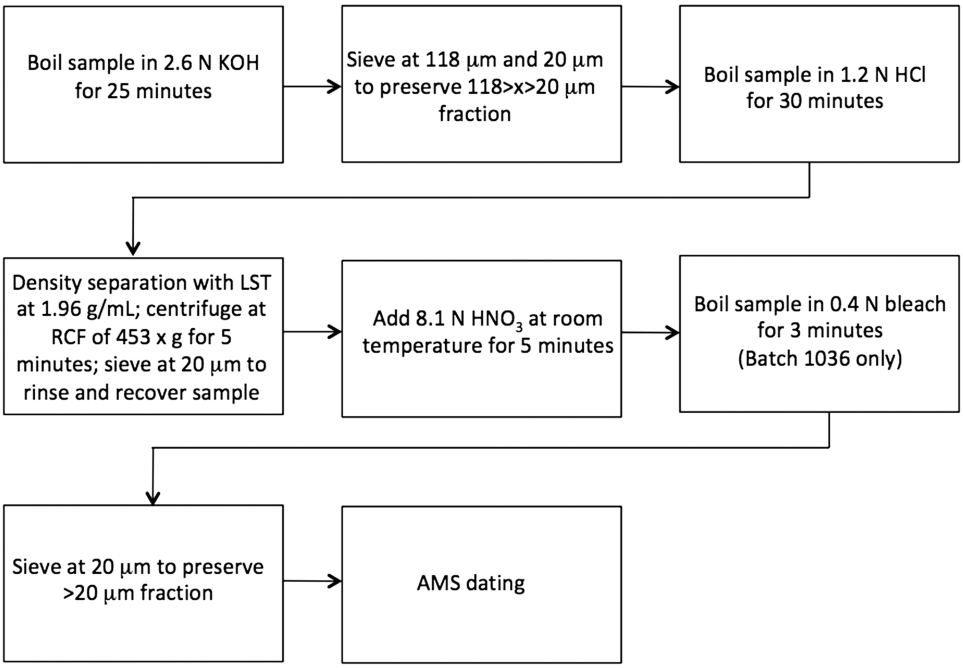

Figure 3 Flow diagram of pollen concentration method used at the National Lacustrine Core Repository (LacCore) for pollen intended for ^{14}C dating.

^{14}C-dating-optimized protocol is to isolate the sample from any other carbon-bearing materials, to prevent contamination of the carbon isotope signature of the pollen. Other ^{14}C pollen preparation methods include use of hydrofluoric acid (Brown et al. 1989, 1992) but this is avoided in the LacCore protocol due to the use of prebaked glassware. The 12 samples were divided into two batches for the pollen concentration procedure, batch 1036 (8 samples) and batch 1037 (4 samples). All preparation steps were the same, except that batch 1036 was boiled in 0.4 N bleach for 3 min after nitric acid treatment, while batch 1037 was not, causing the 1036 samples to be white in color, while the 1037 samples were tan.

After concentration, the samples were sent to the Indiana University-Bloomington Flow Cytometry Core Facility (FCCF) for sorting by flow cytometry. Because the prepared pollen is in the 20–118 micron size fraction, the COPAS (Complex Object Parametric Analyzer and Sorter) Select machine was used. This sorter is specially designed to sort objects between 20 and 400 microns by optical density, size, and fluorescence. All samples were sorted using ultrapure water, and a minimum goal of 10,000 grains per sample was set, with 20,000–30,000 as an ideal, to strike a balance between sorting time and sufficient carbon for AMS analysis. Previous work suggests that this is enough pollen from western U.S. Holocene lake sequences to yield 80–150 micrograms of carbon, a small but feasible amount for a ^{14}C analysis at CAMS.

The FCCF is a central facility and receives many biological samples from a wide variety of projects, potentially including materials exposed to tracer ^{14}C. Tracer solutions contain tens to hundreds of times the natural level of ^{14}C and are extremely powerful tools in biological experiments, but even a small amount presents a severe contamination risk to natural-level samples. To protect the sorted samples and the CAMS lab from potential tracer contamination,

the COPAS sorter was swiped to test for tracer-level ^{14}C just before each sorting session. Swipes were sent ahead to CAMS and measured separately; no tracer-contaminated swipes have been detected thus far.

^{14}C analyses were done at the Center for AMS at Lawrence Livermore National Laboratory. Because of the extensive prior preparation, at CAMS the samples were only rinsed three times with high-purity MQ water, centrifuging between rinse steps, and combusted and graphitized according to standard procedures (Vogel et al. 1984). AMS analysis followed routine protocols, and all dates are reported in conventional ^{14}C yr with 1-sigma uncertainty (Stuiver and Polach 1977). Dates for the tephras follow the weighted-mean ^{14}C ages reported by Bursik and Sieh (2013); 555 ± 40 ^{14}C BP and 1440 ± 40 ^{14}C BP, respectively. Individual ^{14}C dates (for both pollen and tephras) were calibrated using the IntCal13 calibration curve in the Calib 6.0 program (Stuiver and Reimer 1993; Reimer et al. 2013), and are reported as the median probability age with 2-sigma uncertainties (Table 1).

Bayesian age models for the core interval studied were developed in the Bacon program (Blaauw and Christen 2011). The sediment shows undisturbed bedding and clear but minor variation of sediment type, and so we used the prior assumptions of superposition and increasing age with depth. Because we have 8 dates over only 30 cm, we allowed Bacon to use a 1-cm thickness, allowing the maximum flexibility; all other settings were the Bacon defaults. The assumption of superposition allowed Bacon to eliminate the tails of the ^{14}C probability density functions where they result in age reversals, reducing the possible age range for any depth interval.

RESULTS

Although the bulk samples were all ~2 cm^3, they yielded highly variable amounts of pollen (Table 1). Previous pollen work shows that pine dominates the Holocene assemblage at Mono Lake (average 50–70% of total; Davis 1999), and this is reflected in smear slides of the concentrates. The smear slides also showed that the 20–118 micron fraction of the samples contained variable amounts of charcoal, silicate minerals, volcanic glass, and unidentifiable organic matter (Figure 4). In a conventional pollen concentrate, the silicates and glass are unimportant to the ^{14}C date because they do not contain carbon, but the charcoal and organic matter would contribute carbon of an unknown age to the ^{14}C measurement, potentially changing the age significantly. The bleach step did not appear to cause any difference in the behavior of batches 1036 and 1037; no offset in the ^{14}C ages was observed, nor a systematic difference in initial purity or sorting time, indicating that the bleach step did not improve the purity of the sample in this case.

The sorting times varied from 2 to 8 hr because of differences in the size of the samples and the fraction of the material that was pollen (Table 1). Three samples yielded <1500 grains, and were not submitted for ^{14}C dating. Two samples had extremely high pollen concentrations, with pollen grains representing 18–20% of the grains sorted, and yielded 30,000 and 54,000 grains in a few hours of sorting. The other seven samples produced 9000–17,000 grains each, with pollen concentrations of 1–2%. Sample 1037-1 had a particularly high pollen concentration, and was sorted to 54,000 grains, in order to split the sample into two aliquots for replicate ^{14}C dates.

The ^{14}C ages of the pollen samples are generally in stratigraphic order within their calibrated ranges (Figure 5). However, the pollen separates were all relatively small, yielding between 30 and 110 micrograms of carbon. One sample (1037-2) did not graphitize properly, and is not

Table 1 Pollen concentrations, radiocarbon dates, and calibrated ages.

CAMS ID	Sample ID	Core depth (cm)	Pollen grains (#)	Sorting time (hr/min)	Final pollen content	Graphite (mg C)	$F^{14}C$	1-sigma	^{14}C age (yr)	1-sigma (yr)	Cal. age (yr)	2-sigma (yr)
172104	1036-1	23.5–24.5	8,886	3 hr 25 min	1.40%	0.03	0.8875	0.0106	960	100	865	185
172105	1036-2	27–28	17,236	8 hr 40 min	1.50%	0.06	0.8588	0.0066	1220	70	1145	150
t.s.	*1036-3*	*28.5–29.5*	*1,407*	*2 hr 30 min*	*1.90%*							
172106	1036-4	32–33	16,483	7 hr 5 min	0.20%	0.11	0.8472	0.0041	1330	40	1265	65
172107	1036-5	34–35	17,021	4 hr 25 min	2%	0.02	0.7901	0.0159	1890	170	1835	445
t.s.	*1036-6*	*36–37*	*1,102*	*1 hr 15 min*	*2.40%*							
t.s.	*1036-7*	*38–39*	*1,197*	*1 hr 10 min*	*2%*							
172108	1036-8	41–42	17,128	3 hr 55 min	3%	0.03	0.8178	0.0105	1620	110	1525	250
172109	1037-1	43–44	54,040	5 hr 10 min	19.50%	0.09	0.8264	0.0042	1530	45	1420	95
172112	1037-1 dup	43–44				0.07	0.8297	0.0054	1500	60	1395	110
t.s.	*1037-2*	*46–47*	*30,931*	*2 hr 30 min*	*22.50%*							
172110	1037-3	48.5–49.5	19,261	3 hr 25 min	4.10%	0.05	0.8091	0.0064	1700	70	1610	200
172111	1037-4	51.5–52.5	17,604	4 hr 30 min	2%	0.03	0.7878	0.0105	1920	110	1865	280

*median probability as calibrated with Calib 6.1/IntCal09
calibrated ages and 2-sigma uncertainty rounded to the nearest 5-year
t.s. = too small; insufficient CO^2 to make graphite

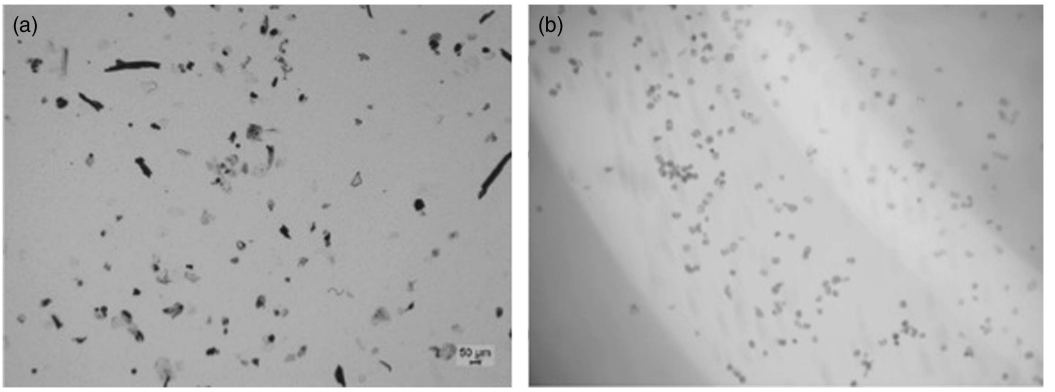

Figure 4 Photomicrographs of sample 1037-1 (43–44 cm): (a) after chemical pretreatment at LacCore, pollen is abundant, but charcoal and other organic material are also abundant; (b) after sorting by flow cytometry, sample is nearly pure pine pollen.

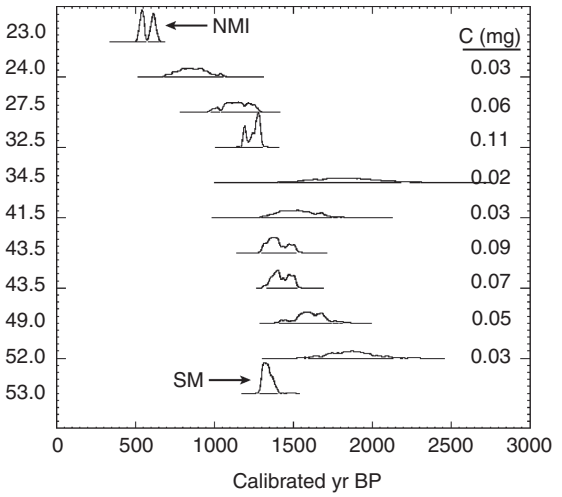

Figure 5 Probability density functions (PDFs) of calibrated ages of pollen dates and North Mono-Inyo (NMI) and South Mono (SM) tephras, from Calib 6.0, using IntCal13 (Stuiver and Reimer 1993; Reimer et al. 2013). Note that the vertical axis is only to indicate the depth of each sample, and is not a linear scale. The amount of carbon in each pollen AMS sample is indicated to the right; samples close to 0.1 mg mostly reflect the complexity of the calibration curve (e.g., 32.5 cm), while PDFs of dates with <0.05 mg are smeared by the large AMS uncertainty (e.g., 52.0 cm).

discussed further. Samples of less than ~300 micrograms of carbon require a background correction scaled to the sample size (Zimmerman et al. 2012) and so the analytical uncertainties were larger than desired, between ± 40 and ± 170 yr, 1-sigma (Table 1). Unfortunately, the combination of large analytical uncertainty and the shape of the calibration curve in this interval led to large uncertainties in the calibrated ages. Most of the calibrated ages had precisions of ± 150–250 yr (2-sigma), but the smallest sample had a calendar-calibrated precision of ± 445 yr.

The ages of the North Mono-Inyo and South Mono eruptions provide a basis for judging the accuracy of the pollen ages. The bottommost sample, just above the South Mono tephra, has a ^{14}C age of 1920 (± 110) ^{14}C yr, 480 yr older than the age of the tephra (1440 ±40 ^{14}C yr BP).

The topmost pollen sample, just below the North Mono tephra, has a ^{14}C age of 960 (± 100) ^{14}C yr BP, 405 yr older than the tephra (555 ±40 ^{14}C yr BP).

A Bacon model for this core interval using only the tephras (without the pollen dates) (Table 2 Model 1, Figure 6A) results in a mean 95% confidence interval of 227 yr, with a minimum close to the younger tephra, and a maximum range near the middle of the sequence. A Bacon age model based only on the pollen (Table 2 Model 2; Figure 6A) is overall more precise, because the larger number of dates constrains the envelope of uncertainty better, but it is less accurate. Subtracting 400 ^{14}C yr from the age of sample 1036-1 stratigraphically below the North Mono-Inyo tephra makes it slightly older than that tephra, as it should be. Adopting that offset to adjust the other pollen dates yields a model with a mean 95% confidence interval of 158 yr, more precise than the tephras alone (Table 2 Model 3, Figure 6B).

DISCUSSION

The value of pollen dating in building lacustrine age models is two-fold: first, to provide dates at a desired depth in a sediment sequence, for example, across a significant change in a proxy record; and second, to provide the most precise dates possible, by adjusting the sample size. Both of these are rarely possible in macrofossil-poor lakes, where age models rely on dating the available macrofossils, wherever they are found, and whatever size they are. We have demonstrated that it is possible to acquire an age at any depth that contains sufficient pollen, but two issues need to be addressed for future application of the technique: first, the small size of the

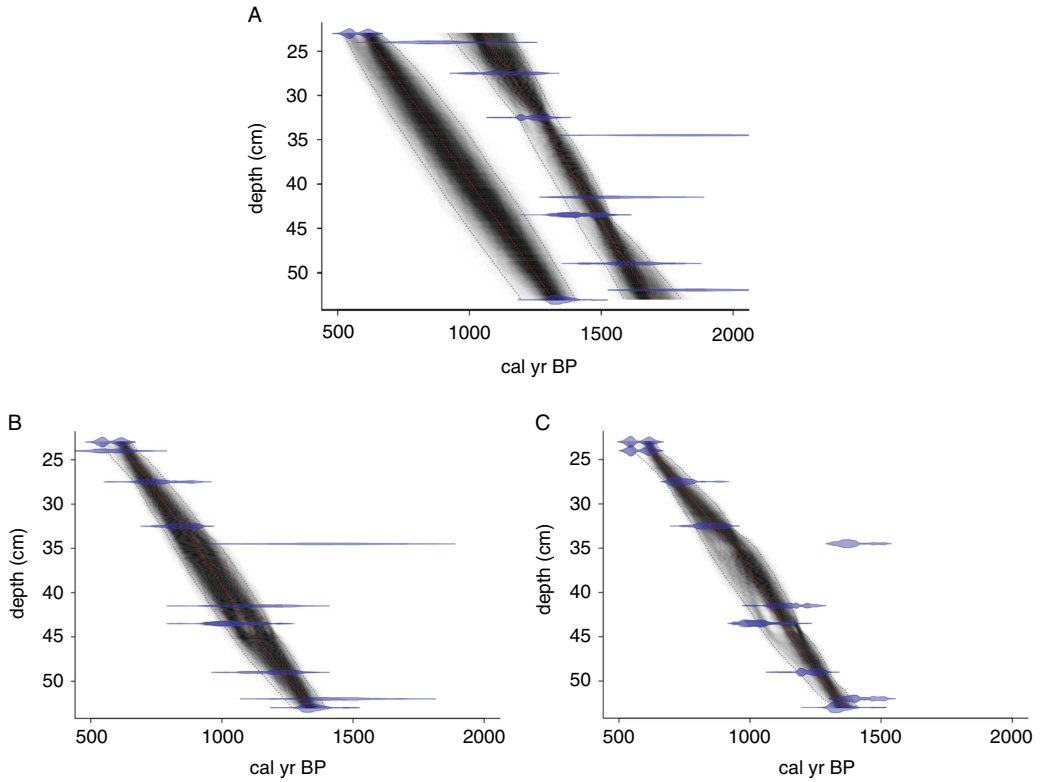

Figure 6 Bacon age models for datasets discussed here: A: tephras only (left curve), and pollen only (right curve); B: tephras + pollen-400 ^{14}C yr; C: tephras + pollen-400 ^{14}C yr, with hypothetical 35-yr uncertainties.

samples, which resulted in large uncertainties on the ^{14}C ages; and second, the causes of the significantly older age of the pollen samples compared to the well-dated tephras. Although several potential improvements to the sample preparation methods may help to overcome these issues, geological processes are likely to be more significant, and more complex to address.

One problem that should be relatively simple to rectify in future pollen-dating studies is the size of the samples. In principle, the amount of carbon in a ^{14}C sample may be increased by combining samples from adjacent depths. Although this reduces the resolution of the age model, ^{14}C measurements with smaller analytical uncertainties can be calibrated with greater precision, leading to a better overall precision. The decision to combine adjacent samples relies on a reasonable ability to predict the size of the AMS sample from the sorted pollen separate, which in turn requires improvement in our handling procedures.

For example, in this study samples 1036-4 and 1036-5 had similar pollen counts (Table 1), but 1036-4 gave 0.11 mg of carbon and had an analytical uncertainty of ± 40 yr, while 1036-5 yielded 0.02 mg of carbon, resulting in an analytical uncertainty of ± 170 yr. Given the difficulty of removing the rinse water from the centrifuge tube while leaving the pollen behind, even after centrifuging, we suspect that the difference in weight between these two samples is because some pollen was lost from 1036-5 during the three rinse steps. Reducing or eliminating post-cytometry, pre-AMS cleaning steps, and development of efficient techniques for any required handling, should address this issue. Further, the color difference between the bleached and unbleached samples made handling of the latter significantly easier, suggesting that elimination of the pre-cytometry bleach step is desirable for samples where it is not required. Ideally, samples would be examined microscopically on arrival at the AMS sample lab, and the decision to combine samples or not made based on expected carbon content, sedimentation rate and depth between samples, required precision of the age model, and other project-specific considerations.

An informative exercise is to consider the precision of the age model if the samples had all contained >0.3 mg C. In that case, the AMS analytical uncertainties would likely have been the canonical 35 yr, and the simple calibrated age ranges would have been much smaller (Figure 7). For example, the worst-case sample in this experiment, 1036-5, had 0.02 mg C and a ^{14}C uncertainty of ± 170 yr (1-sigma), which translates into a calibrated uncertainty of ± 445 yr (2-sigma). If this had been a 0.3 mg sample with the same central age (1890 ^{14}C yr BP) and a 35-yr analytical uncertainty, the calibrated 2-sigma uncertainty would be reduced to ± 95 yr.

The reduction in calibrated range is primarily a function of the actual sample size, where the smallest sample (0.02 mg) had the largest reduction, and the calibrated uncertainty of the three largest samples (0.11–0.07 mg C) was reduced by only 0–20 yr. The irregular shape of the calibration curve plays a secondary effect in this sample set: two samples of 0.05 and 0.03 mg had nearly the same reduction in uncertainty, because the larger sample sits in a valley in the curve, while the smaller is on a little peak. Used in the same Bayesian model described above, this hypothetical "reduced uncertainty" dataset (with the 400-yr offset of the pollen dates as in Model 3) results in Model 4 (Table 2, Figure 6C), which has only slightly better precision than Model 3, using the tephras and the pollen with the real uncertainties (Table 2, Figure 6B). Either would permit correlation of paleoclimatic proxy features on the sub-centennial to multi-decadal scale.

A second effect that may be improved with examination of the chemistry protocols is the "old" age of the pollen relative to the tephras. Although the potential for contamination by various

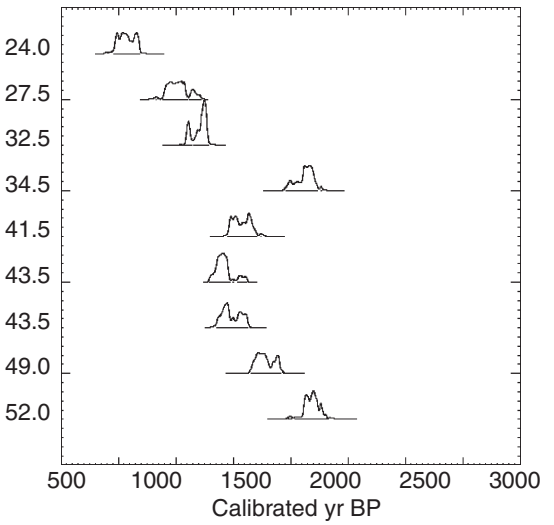

Figure 7 Probability density functions (PDFs) of pollen dates with hypothetical 35-yr uncertainties, from Calib 6.0, using IntCal13 (Stuiver and Reimer 1993; Reimer et al. 2013). Note that vertical axis is only to indicate the depth of each sample, and is not a linear scale.

Table 2 Mean, minimum, and maximum widths in years, of 95% confidence interval for Bacon models.

Bacon model input parameters	Mean (yr)	Minimum depth (cm)	Minimum years	Maximum depth (cm)	Maximum years
1. Tephras only	227	23	144	36	252
2. Pollen only	173	44	141	24	234
3. Tephras + pollen–400 yr	158	23.5	115	38	194
4. Tephras + pollen–400 yr, 35 yr uncert.	143	52.5	95	37	190
Model 1 – Model 3	69		29		58

carbon-bearing materials has been recognized since the work of Brown et al. (1989) and carbon-based reagents were carefully avoided in this study, the addition of petroleum-based (^{14}C dead) carbon from laboratory reagents may not be the only source of alteration during the chemical preparation. Byrne et al. (2003) found that chemical pretreatment with a variety of non-carbon bearing reagents (trisodium phosphate, hydrochloric acid, potassium hydroxide, etc.) caused the $\delta^{13}C$ of pine pollen samples to shift as much as 4 per mil lighter than untreated pollen. Although they could not rule out the possibility that the shift was caused by the physical removal of isotopically heavier components of the pollen, they suggested that the carbon-isotope composition of the sporopollenin phase itself may have been altered by the use of strong acids. Additional systematic testing of these variants in conjunction with flow cytometry purification and ^{14}C dating is in order. Regardless, a shift of a few per mil in the isotopic composition of the pollen cannot account for the 400-yr offset of the pollen ages obtained in this study.

The geological setting and processes of a lake basin influence the accumulation of pollen in the lake's sediments and the resulting pollen ^{14}C age, and are likely more significant and more difficult to control than effects of sample preparation. For example, the shift of 400 yr observed in sample 1036-1 requires a contribution of only 15% of the carbon from pollen 4000 ^{14}C yr old, and only 6% from pollen >30,000 ^{14}C yr old. Both of these are likely ages of old pollen delivered to modern Mono Lake sediments, because higher lake levels during those periods deposited sediment at elevations that are now above lake level and may be actively eroded.

Further, the date at 34.5 cm depth is >250 yr older than the date below it, constituting a serious reversal in age at this scale (Table 1, Figure 6). If the dates were on macrofossil material, it might be assumed that the bit of charcoal or plant material had been stored and re-transported, and it would be ignored as an outlier. It is somewhat more difficult to explain a purified pollen age in this way. There was nothing different about this sample in the smear-slide evaluation of the concentrated separates before sorting, and the sorting time, grains sorted, and final pollen content were not strikingly different than the sample above. The two samples below (36.5 cm and 38.5 cm) did not contain enough pollen for dating, and the 34.5 cm sample occurs at the time of the pluvial between the mega-droughts of the Medieval Climate Anomaly, when Mono Lake (and other western Great Basin lakes) rose briefly and then fell (Stine 1990b, 1994; Adams 2003). It seems likely that the change in lake level may have reactivated older deposits around the lake, which are common in the basin.

This effect is especially likely where old deposits on the surface are common, as is the case in the western Great Basin. Mono Lake, like many lakes in the region, was much larger during glacial periods, and the regression at the beginning of the Holocene exposed Pleistocene lake sediments all around the lake. These sediments are eroded into the lake by the streams, as well as being lifted from the surface by winds, depending on vegetation cover and wind speed/direction. Both fluvial and aeolian transport are likely to be variable in time as well as space, increasing, for example, during a dry period when vegetation dies, or a drop in lake level induces down-cutting by the streams. As suggested by the above example, old pollen may also be introduced by increased run-off and erosion during wet periods. The amount and age of reworked pollen contributing to a lake sequence will depend on basin geomorphology, patterns of surface run-off, the geometry and location of older deposits, and whether streams are aggrading, down-cutting, or in approximate equilibrium at any particular time.

Interestingly, Mensing and Southon (1999) also found an offset of 400 yr between pollen and non-pollen dates in the sediments of Lake Moran, in the western Sierra Nevada. The 38–74 micron and >74 micron fractions of the sediment just above the Mazama tephra were consistent with the best age for that eruption, (~6640 ^{14}C BP) while pollen from the same depth interval was older by 400 yr (~7040 ^{14}C BP). Their pollen samples were purified by mouth pipetting, and so (similarly to our results) the older age cannot be attributed to non-pollen contaminants. Instead, Mensing and Southon (1999) suggest the possibility that soil infiltration rates may have been altered by the tephra, inducing down-cutting and erosion of older pollen.

The potential for older reworked pollen to be included in the sorted pollen fraction makes microscopic examination of the pollen separates critical. If the older pollen is physically or chemically degraded and can be identified visually after sorting, the sample may be discarded as undateable if the fraction is high enough, or flagged as potentially problematic and the date treated with caution. In locations where older periods were characterized by significantly different vegetation (e.g., forest vs. grassland, oak vs. spruce), it may be possible to identify the presence of reworked pollen even where it isn't visibly degraded. The possibility that sorting

parameters used by the flow cytometry system might be able to distinguish pollen of different types and stages of degradation also remains to be explored.

Whatever the cause of the age offset, in this case the tephra dates can be used to do an approximate correction of the ^{14}C dates, similar to the comparison of macrofossil-bulk sediment pairs in lakes where macrofossils are rare. The premise of the approach is to test the similarity of the offset at different depths in the sediment sequence, in hopes of finding a consistent offset through time that can be reliably used to correct bulk-sediment dates that do not have a macrofossil pair. Here we have corrected the pollen ages based on the well-constrained age of the North Mono-Inyo tephra, subtracting 400 yr from each ^{14}C age before calibration. This results in the oldest date, just above the South Mono tephra, being 80 yr older than the mean ^{14}C age of that tephra, well within the 2-sigma uncertainty of both dates.

As with bulk-macrofossil pairs, this correction method is not recommended without careful attention to the specific case at hand, however. If the cause of the offset is the chemical preparation, it should be consistent between samples prepared in the same way, and avoidable with appropriate chemistry. However, in the case of a contribution of old pollen from the environment, the correction will certainly vary between lakes, probably even in similar settings (e.g., western Great Basin lakes), *as well as through time in a particular lake*. Further, the contribution of old pollen may potentially change pollen ^{14}C ages by hundreds or thousands of years, depending on the balance of production and deposition of contemporary pollen with erosion and deposition of old pollen stored on the landscape.

IMPLICATIONS AND FUTURE DIRECTIONS

Sorting by flow cytometry may potentially be a breakthrough in building pollen-based age models for lake records, but additional testing of preparation protocols is required. Several tasks will help to address the problems and uncertainties we observed here:

1. The techniques that have been used to prepare the raw mud for sorting, including sieving, heavy-liquid separation, and chemical concentration, must be compared to determine their value for flow cytometry of pollen for AMS. The protocol using the fewest preparation steps to yield the cleanest final sample is the ideal, but the variety of techniques needed for common kinds of mud (and pollen) must be systematically tested. An additional consideration is the time necessary to sort sufficient pollen grains from a sample for a reliable AMS analysis, as sorting costs are generally charged per hour.
2. The potential range of sorting parameters must be explored, to address the possibilities of separating pollen of different types, as well as the potential to eliminate badly degraded pollen from a sample during sorting.
3. How much pollen is in the sediment and what type (i.e., how big), as well as what other material remains after sieving and concentration, will determine the amount of sediment needed and the time required for sorting (and thus the cost). These set practical limits on the precision of the age models that can be produced by this approach, and suggest that different approaches may be appropriate for initial age models on long sediment sequences, versus high-resolution age control for time periods of particular interest.
4. The possible addition of modern and ^{14}C-dead carbon during sample preparation for AMS measurement is routinely monitored for other sample types (e.g., wood, charcoal, carbonates) by preparing materials of known age and similar matrix alongside samples. To test for addition of old carbon or modern carbon during the preparation of pollen,

modern and ^{14}C-dead pollen standard materials must be identified and characterized. These should then be prepared alongside samples using the same physical and chemical protocols for both.

Once protocols are developed to reliably produce robust ^{14}C dates, a number of geological questions will need to be studied and addressed on a case-by-case basis. What kinds of pollen (size, amount of carbon, plant life cycle, dispersion method) are most desirable for ^{14}C dating? Once the pollen has been released from its plant of origin, how long can it sit on the landscape before being sufficiently degraded to be unrecognizable to the flow cytometer? How does the potential residence time vary with climatic and geomorphic characteristics of a lake basin? In a specific lake or climatic setting, what changes in landscape dynamics or climate conditions produce the most reliable, or most erroneous ^{14}C ages? Are there particular kinds of lakes or climatic settings where pollen dating always, or never, produces reliable age models? What are the limits to the resolution achievable by the technique in different settings? With the proper attention to these dynamics of the specific basin being studied, AMS dating of flow cytometry-sorted pollen may be the key to high-precision, high-accuracy correlation of proxy records of past terrestrial climate.

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