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Analysis of maize (Zea mays subsp. mays) pollen: Normalizing the effects of microscope?slide mounting media on diameter determinations

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ANALYSIS OF MAIZE (*ZEA MAYS* SUBSP. *MAYS*) POLLEN: NORMALIZING THE EFFECTS OF MICROSCOPE-SLIDE MOUNTING MEDIA ON DIAMETER DETERMINATIONS

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Abstract

Maize (*Zea mays* subsp. *mays*) dominates the record of prehistoric agriculture in the Neotropics. Nonetheless, many significant questions of *Zea* systematics and evolution persist. Palynology provides a record central to addressing those questions, but determining pollen grain diameter remains a significant methodological issue: diameter is a key characteristic in identification, and diameter seems to be space-time dependent — the latter phenomenon but little understood. One issue in analyzing diameter is the confounding effect of microscope-slide mounting media. This study provides correction factors to normalize diameter among silicon oil, glycerine jelly, and acrylic resin (du Pont Elvacite), the last coming into increasing use without previous study of its effect on pollen grain size.

INTRODUCTION

Despite the importance of maize (*Zea mays* subsp. *mays*) in the prehistoric agriculture of the Neotropics (Cowan and Watson, 1992) and the perseverance of palynologists, identification of fossil maize pollen persists as a methodological issue and remains far from straightforward. Standard approaches rely on the large size of maize pollen vis-à-vis most other grass (Poaceae) taxa. However, overlaps among the size ranges of maize and closely related taxa as well as the putative time dependency of maize pollen size (Galinat, 1961) demand identification through size-frequency analysis (Buell, 1946; Sluyter, 1995) rather than the vague, but all too common, reliance on "several large Poaceae grains." Accurate size determinations are a corollary to the size-frequency method, but accuracy remains problematic because microscope-slide mounting media differentially affect pollen diameter by as much as 10%, enough to confound differentiation of maize and closely related taxa. In some cases, those methodological issues have resulted in long-running controversies over the identification of fossil maize pollen and the origins, dispersals, systematics, and phylogeny of *Zea* (Barghoorn et al., 1954; Mangelsdorf et al., 1978; Beadle, 1981; Iltis, 1983).

Size-frequency analysis of fossil maize pollen, and therefore this methodological study, pertain more to the Neotropics than to the temperate Americas. The Southwest region, for example, typically has not yielded prehistoric maize pollen in high enough concentrations or with sufficiently good preservation to permit effective use of size-frequency analysis (Martin and Schoenwetter, 1960). In contrast, the high concentrations and good preservation of prehistoric maize pollen in records from Middle America has encouraged use of size-frequency analysis (Byrne and Horn, 1989; Straka and Ohngemach, 1989; Sluyter 1995). Moreover, although some records from the Southwest do have high concentrations and good preservation of prehistoric maize pollen (Sluyter, 1991), the ranges of closely related taxa which produce pollen close in size to maize do not extend into temperate latitudes and the need for size-frequency analysis is not as great as in the Neotropics.

This study contributes correction factors for the normalization of diameter determinations among mounting media in order to facilitate size-frequency analysis, maize pollen identification, and comparisons among records. Previous studies have addressed the characteristics of silicone oil and glycerine jelly; this study is the first to include acrylic resin (du Pont Elvacite), a medium increasingly coming into usage among palynologists (D. W. Engelhardt, written commun., 1993; Wrenn, 1996).

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MAIZE POLLEN ANALYSIS

Maize pollen morphology typifies all grass taxa: subspheroidal, monoporate, annulate, and essentially psilate to scabrate under light microscopy (Kapp, 1969; Bassett et al., 1978). Schemes to conclusively differentiate maize pollen by exine sculpturing (Irwin and Barghoorn, 1965; Tsukada and Rowley, 1964) or the ratio of annulus diameter to grain diameter (Barghoorn et al., 1954) have failed to yield consistently reproducible results and do not discriminate between maize and the teosintes (*Zea perennis*, *Zea mays* subsp. *parviglumis, Zea* spp.) (Kurtz et al., 1960; Whitehead and Sheehan, 1971; Grant, 1972; Doebley and Iltis, 1980; Iltis and Doebley, 1980; Ludlow-Wiechers et al., 1983; Straka and Ohngemach, 1989; Fearn and Liu, 1995).

Therefore, major diameter has emerged as the single credible differentiating parameter, maize pollen attaining a greater maximum size than that of any other grass taxon. Based on acetolyzed pollen from twelve Mexican landraces mounted in silicone oil, maize has a major diameter of 58-99 μm (Whitehead and Langham, 1965). Other studies have yielded similar ranges; although some non-Mexican varieties attain major diameters of ca. 120 μm (Ludlow-Wiechers et al., 1983). Problematically, the other species and subspecies of *Zea* (Doebley and Iltis, 1980; Iltis and Doebley, 1980) have a major diameter range of 46-87 μm, based on acetolyzed pollen from eleven teosinte taxa mounted in silicone oil (Whitehead and Langham, 1965), which overlaps that of maize. Moreover, Galinat (1961) provides an empirical and theoretical argument that early maize might have produced teosinte-sized pollen — which consequently would overlap with the upper range of yet another Neotropical grass: *Tripsacum* spp. (33-57 μm, based on acetolyzed pollen from gama grass [*T. dactyloides*] mounted in silicone oil [Whitehead and Langham, 1965]).

The resulting opportunity for confounding maize, teosinte, and *Tripsacum* fossil pollen when employing maximum-size criteria alone, demands analyses of the frequency distributions of the major diameters of large grass-pollen grains occurring in stratigraphic sequence — both to definitively distinguish between taxa and to test Galinat's (1961) hypothesis that maize pollen diameter increases with time. Buell (1946) provides the classic application of the size-frequency comparison, applied to pine (*Pinus* spp.); Byrne and Horn (1989), Straka and Ohngemach (1989), and Sluyter (1995) apply the method to maize.

Size-frequency analysis, however, and comparisons among records and with the characterizing diameter ranges of extant taxa (Whitehead and Langham, 1965), relies on accurate measurement — a goal complicated by the effects of taphonomic context, pollen preparation, and microscope-slide mounting media. Taphonomic context influences pollen size — apparently (Andersen, 1960; Praglowski, 1966); however, the paucity of data and theory precludes evaluation or further discussion. Preparation technique also affects size, but the "acetolysis method" (Faegri and Iversen, 1975) does not introduce bias if applied for four to eight minutes (Christensen, 1946; Reitsma, 1969), and its near ubiquity among Quaternary palynologists facilitates comparison among records. Mounting media, in contrast, remain varied and can significantly affect size. Silicone oil is a potential standard medium because it preserves the true dimensions of pollen grains (Andersen, 1960; Faegri and Iversen, 1975) and has served to characterize the major diameters of maize, teosinte, and *Tripsacum* (Whitehead and Langham, 1965). However, since silicone-oil slides require horizontal storage and careful transport, palynologists regularly employ several other media. Among them, glycerine jelly enjoys a long tradition and continuing popularity even though slides can deteriorate within decades (Andersen, 1960; Faegri and Iversen, 1975). Glycerine jelly also distends pollen grains progressively over time — probably due to exine softening and pressure of the cover-slip as the medium desiccates and shrinks (Cushing, 1961; Whitehead and Sheehan, 1971). The time dependency of this "Cushing effect" might explain why correction factors to normalize measurements made on glycerin-jelly slides to silicone oil vary so widely among studies: 0.8 (Faegri and Iversen, 1975) to 0.94 (Whitehead, 1965). Acrylic resin is becoming increasingly popular due to its permanence and transportability but its effects on pollen size have not previously received study.

METHODS

The following analysis, therefore, employed silicone oil (Dow Corning 200 Fluid, 2,000 centistokes viscosity), acrylic resin (du Pont Elvacite 2044), and glycerine jelly (Anderson Laboratories, lot GO-20, melting point 33°C) in order to establish correction factors. Acetolysis preparation of modern maize pollen (Greer Laboratories, lot 53W149-8) for four minutes and subsequent staining with Safranin-O (Kodak Cl 50240) provided the material to make one microscope slide with each medium. The acetolysis, silicone oil, and glycerine jelly methods followed Faegri and Iversen (1975). The acrylic resin method derives from D. W. Engelhardt (written commun., 1993), as follows.

Acrylic resin is nontoxic, "permanent," places all of the pollen in one focal plane, minimizes sorting by pollen size, and has an appropriate index of refraction. Prepare the acrylic resin by adding 80 g to 165-170 ml of xylene; turn the bottle to dissolve and allow to settle for two days;

transfer the solution to an amber dropper bottle. Prepare the hydroxyethyl cellulose carrier (Union Carbide Cellosize WP3H) by adding 1 g to 100 ml of distilled water; stir over low heat until dissolved; add several drops of phenol; filter the solution into a dropper bottle. To prepare slides, place one drop of carrier on a cover-slip; add one drop of washed pollen residue; thoroughly mix the two and distribute the pollen over the cover-slip to within several millimeters of its edge; completely evaporate the carrier on a slide warmer set at ca. 30°C; place a large drop of acrylic resin on a slide; slowly (to minimize air bubbles) lower the cover-slip into place; leave the slide on the slide warmer overnight in order to cure the acrylic resin and disperse any air bubbles; remove any excess acrylic resin with a razor blade.

Measurement of the major diameters of fifty relatively uncrumpled grains on each slide employed an ocular graticule for an initial measurement one day after slide preparation and a subsequent measurement thirty days after. The relatively normal distributions (e.g., the mean, median, and mode of silicone oil at one day after measurement are all equal to 91 μm, with 62% of the measurements falling within $\pm 1\sigma$ and 94% within $\pm 2\sigma$) and small sample sizes relative to population dictated t-tests to estimate the statistical significance of inter-sample variation and the veracity of the correction factors.

RESULTS

Table 1 presents the results of those measurements and the derived correction factors. Silicone oil is stable over time. Acrylic resin distends grains within one day by ca. 10%, with subsequent stability. Glycerine jelly progressively distends grains over time, within one day by ca. 10% and after thirty days by ca. 15%. Andersen (1960) had similar results with glycerine jelly: immediate distension by ca. 10%, and after thirty days by ca. 20%. Multiplication by a correction factor (the ratio of the means) normalizes the frequency distribution of each sample to that of silicone oil. For example, a transformation of sample 3 to sample 5 (all 50 measurements normalized to silicone oil through multiplication by 0.91) yields a probability value (*P*) of

TABLE 1. Effects of mounting media on major diameter parameters of modern maize pollen and correction factors for normalization to the silicone oil standard.

Sample number	Mounting medium and correction factor	Time to measurement (days)	Major diameter parameters			t-Test pair sample numbers	t-Test *2-tailed* (*P*)
			Count (each)	Mean (mm)	1 s (mm)		
1	silicone	1	50	91.0	±5.2	—	—
2	silicone	30	50	91.2	±4.6	1 and 2	0.8598
3	acrylic	1	50	99.6	±7.0	1 and 3	0.0001
4	acrylic	30	50	99.1	±7.1	3 and 4	0.7663
5	acrylic (^{X} 0.91)	1	50	90.6	±6.4	1 and 5	0.7171
6	acrylic (^{X} 0.91)	30	50	90.2	±6.4	1 and 6	0.5123
7	glycerine	1	50	98.7	±4.5	1 and 7	0.0001
8	glycerine	30	50	102.8	±3.5	7 and 8	0.0001
9	glycerine (^{X} 0.92)	1	50	90.9	±4.1	1 and 9	0.8579
10	glycerine (^{X} 0.89)	30	50	91.0	±3.1	1 and 10	0.5317

72% between samples 1 and 5, thus indicating no significant differences vis-à-vis silicone oil. The other correction factors achieved similar probability values: acrylic resin, time independent = 0.91; glycerine jelly, 1 day = 0.92, 30 days = 0.89 (Table 1).

DISCUSSION AND CONCLUSIONS

Therefore, if practicable, silicone oil is the preferable medium, yielding data directly comparable to the silicone oil standard (Whitehead and Langham, 1965) and offering stability over time. Acrylic resin offers stability as well, and a consistent correction factor of 0.91, at least for as many as 30 days after slide preparation. However, both silicone oil and acrylic resin somewhat tend to crumple maize pollen, perhaps due to their high viscosities, as well as the single aperture, large size, and thin exine of the pollen (Praglowski, 1966). Yet silicone oil advantageously permits manipulation of pollen during microscopy, ameliorating the constraints of grain crumpling and non-equatorial orientation on measurement (Andersen, 1960; Whitehead, 1961). Glycerine jelly, which has a low viscosity while molten during slide preparation, minimizes crumpling but over time progressively distends pollen grains. Note, however, that while glycerine jelly minimized crumpling in the current study, Praglowski (1970) found that glycerine jelly with a 64°C melting point "badly collapsed" 61% of larch (*Larix decidua*) grains and that glycerine jelly with a 42°C melting point had the same effect on only 32% of the same species, one similar in size and morphology to maize but inapeturate. Given the 33°C melting point of the glycerine jelly in the current study, Reitsma (1969) inspires a possible explanation by noting that "hot" glycerine causes a significant increase in pollen size, an effect possibly related to exine softening and thinning that would also promote crumpling, particularly for inapeturate pollen but also for the monoporate grains of Poaceae.

In sum, for palynological studies concerning maize and which employ acrylic resin, silicone oil, or glycerine jelly, the correction factors in Table 1 facilitate identification and comparison with other records. Nonetheless, the instability of glycerine jelly demands measurement immediately after slide mounting. Alternatively, simultaneous preparation of a control slide with modern maize pollen of known size parameters can subsequently serve to normalize measurements taken from the glycerine jelly slides.

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