

## A methodical and taxonomic study of dispersed oak pollen (Fagaceae) from the Holocene of Rolla, Missouri, USA

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### Abstract

Oak pollen has great potential to be used in reconstructing paleovegetation and paleoclimate. Pollen grains from Holocene sediments of Rolla, Missouri, USA were studied. The goal of this study was to evaluate the morphology of dispersed oak pollen using light and electron microscopy. Three types of oak pollen were recognized: Group 1, Group 2, and Group 3. They occurred in the sample in the following approximate percentage: 52, 32 and 16% respectively. Pollen of Group 1 corresponds to that of white oaks, pollen of Group 2 to that of red oaks. Pollen of Group 3 differs from oak pollen known for the studied region. While electron microscopy shows a good prospect for a detailed interpretation of dispersed oak pollen, many factors should be taken into account when assessing past vegetation: the paleo-forest density, location of sampling, apparent wind direction during pollination, and the “resolving ability” of pollen morphology for different oak species. The intensity of flowering and direction of the pollen transport can significantly influence the quantity of pollen of a particular species in the spectrum. The possibility of medium or long-distance transport of a small quantity of oak pollen from other regions should also be considered in the interpretations of fossil spectra.

**Key words:** *Quercus*; spectrum interpretation; pollen transportation; sculpture; exine ultrastructure

### Introduction

Oaks are a dominant component of many Quaternary pollen spectra and are often included in paleovegetation and paleoclimate reconstructions (e.g., Naryshkina & Evstigneeva 2009, Denk *et al.* 2010, Hayashi *et al.* 2012). The interpretation of pollen spectra for anemophilous types should consider the possibility of pollen transfer from other regions. This depends on the ease of transporting the pollen, direction of prevailing wind, vegetation composition etc. Experiments on the pollen “behaviour” of different plant species in relation to their transport help to improve the reliability of interpreting fossil pollen assemblages (e.g., Fedorova 1948, 1950, Kabailene 1976, Brush & DeFries 1981).

Categorizing oaks at the species level using light microscopy (LM) is complicated (e.g., Van Benthem *et al.* 1984). The pollen size and shape (sometimes, other characters) are used to distinguish between deciduous and evergreen species in Eurasia, or to define oak species, but the reliability of such determinations at the LM level can be questionable. The exine sculpturing was shown to be useful in recognizing different oak morphotypes, which generally correspond to groups of species or sometimes even to species (see review in Liu *et al.* 2007, Denk & Grimm 2009, Tekleva *et al.* 2014). This in turn will help to clarify the systematic composition of dispersed pollen spectra, ecology and possible phytogeographic links of the plants producing such pollen.

During the last decade, studies using scanning electron microscopy (SEM) to examine oak pollen from different regions have been accumulating and the data helps create more detailed reconstructions (see review in Tekleva *et al.* 2014). TEM data on the exine ultrastructure of modern oaks has shown that there are differences between oak species in the sporoderm as well (Denk & Tekleva 2014). Therefore, information from the exine ultrastructure can be used as additional characteristics to define more precisely dispersed fossil pollen. This is especially important in the case of oak species which show variability in their exine sculpturing (e.g. most species with verrucate surface). Unfortunately, transmission electron microscopy (TEM) studies of fossil oak pollen are scarce and it is necessary to fill this gap.

In this paper we studied dispersed oak pollen from the Holocene deposits, Bray Area Lake, Rolla, Missouri with

LM, SEM and TEM. Bray Area Lake is a man-made impoundment located next to a single-family residence and originally created in the 1940s (Haselwander & Oboh-Ikuenobe 2017). Thus, in this case correlations can be drawn between the oak species known for this region and the dispersed pollen in the lake sediment sample. The aim of this paper was to distinguish groups of dispersed *Quercus* Linnaeus (1753: 994) pollen in the studied sample with LM, SEM, and TEM, and to compare them with oak species growing in the region for a more detailed interpretation of the pollen spectra.

## Material and methods

The material examined for this study comes from the Holocene deposits of Rolla in Bray Area Lake, N37°55'11.5", W091°49'41.0" (Bray Conservation Area, Rolla, Phelps County, Missouri, Fig. 1). Bray Area Lake is an education and recreation site that was donated to the Missouri Department of Conservation in 1995, and includes an upland forest, an early successional field, a spring fed stream, pond, and a seasonal stream (*mdonline, Missouri department of conservation, Marguerite Bray Conservation area*). The percent composition of trees, including *Quercus* by species, is given in Table 1 and based on US Forest Service data.

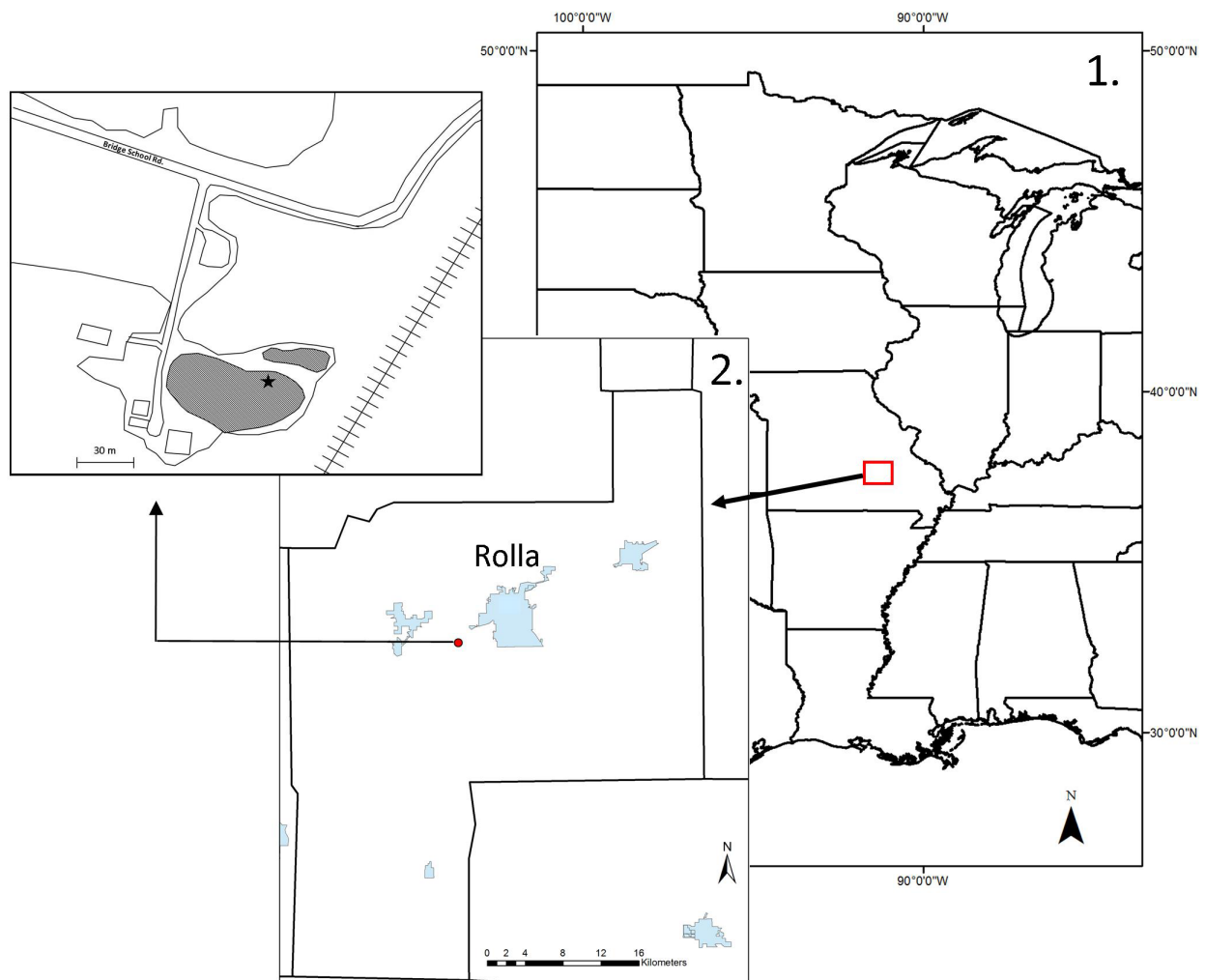
**TABLE 1.** Percent composition of trees and *Quercus* species in regional forests

Percent composition of trees in regional forests	
Taxon	%
<i>Quercus</i>	65.79
<i>Carya</i>	5.27
<i>Juglans</i>	5.27
<i>Acer</i>	2.63
Cupressaceae ( <i>Juniperus</i> )	7.89
<i>Sideroxylon lanuginosum</i>	2.63
<i>Diospyros virginiana</i>	2.63
<i>Fraxinus</i>	2.63
<i>Cornus</i>	2.63
<i>Gleditsia triacanthos</i>	2.63
Percent composition of <i>Quercus</i> species:	
<i>Q. velutina</i>	32
<i>Q. stellata</i>	24
<i>Q. alba</i>	24
<i>Q. marilandica</i>	12
<i>Q. shumardii</i>	4
<i>Q. muehlenbergii</i>	4

Three cores were obtained using 2 inch (5.08 cm) diameter PVC pipe, which was pushed into the lake-bottom sediments. The longest core from Bray Area Lake was used for study. Cores were sampled in 3 cm intervals into 16 g samples, and these samples were processed into palynological slides using standard techniques (Traverse 2007) at Global Geolabs, including the addition of a *Lycopodium* Linnaeus (1753: 1100) spike. Strew mounts prepared from palynological residuum were counted to at least 500 total palynomorphs on a Nikon E100 microscope.

The sample is from core #2, 3–6 cm depth, which occurs in an organic rich mud containing plant fragments, few pebbles, and less than 5% medium grained sand (quartz). In the pollen assemblage *Quercus* is the dominant pollen type (35%); other common pollen types include Cupressaceae, *Pinus* Linnaeus (1753: 1000), *Fraxinus* Linnaeus (1753: 1057), Asteraceae, Poaceae, Juglandaceae, *Carya* Nuttall (1818: 220), and *Populus* Linnaeus (1753: 1034). Uncommon

pollen types include *Larix* Miller (1754), *Ephedra* Linnaeus (1753: 1040), *Alnus* Miller (1754), *Ulmus* Linnaeus (1753: 225), *Corylus* Linnaeus (1753: 998), *Plantago* Linnaeus (1753: 112), Chenopodiaceae, and Cyperaceae (Haselwander 2016).



**FIGURE 1.** Map of locality: Bray Conservation Area, Rolla, Phelps County, Missouri, USA. Star indicates core location.

Sixty oak pollen grains were picked up from the residue and subsequently studied with light (LM), scanning (SEM) and transmission (TEM) electron microscopy. Each studied pollen grain was photographed with a Carl Zeiss Axioplan-2 light microscope equipped with a 100x oil immersion objective and a Leica DFC-420 digital camera. Then the cover glass with the drop of immersion oil was removed and the same pollen grain was transferred with an eyelash mounted on a needle from the LM slide to a SEM stub, covered with gold and observed under a Tescan Vega-II XMU SEM (accelerating voltage 30 kV).

In total, four whole slides were examined, with LM and SEM photos used to estimate the ratio of the three oak types encountered. Running ratios were calculated each time a slide was completed. Ratios were similar across the entire study, with a variance of 2–4%. This consistently small variance is interpreted to indicate that the sample size in this study is adequate.

Some of the studied pollen grains were then removed from the stubs and prepared for TEM according to standard methods (Meyer-Melikian *et al.* 2004). Some of them were first stained with 1% OsO<sub>4</sub>; others were directly embedded in epoxy resin unstained. Pollen grains were sectioned with an ultramicrotome Leica UC6. Some of the ultrathin sections were stained with lead citrate and uranyl acetate (others were studied unstained), and examined under Jeol 100 B and Jeol 1011 TEMs (Jeol, Japan) at accelerating voltage 80 kV and with a CCD GATAN ES500W under control Digital Micrograph GATAN at the Laboratory of Electron Microscopy, Biological Faculty, Lomonosov Moscow State University.

## Results

The studied pollen grains can be divided into three groups according to their exine sculpturing and sporoderm ultrastructure. We wanted to trace the relative proportion of each type. Several pollen grains were covered with some substance that prevented the identification of the grain. The approximate ratio between the occurrence of Group 1 (28 grains), Group 2 (17 grains) and Group 3 (9 grains) pollen is 52%, 32% and 16% accordingly.

There are no evident differences in pollen size or aperture structure between the three groups. Pollen grains are ellipsoidal, they are rounded trilobated or rounded from polar view (Fig. 2A, B; Fig. 3A, B; Fig. 4A, B), and oval from equatorial view (Fig. 2C, D; Fig. 3C, D; Fig. 4C, D). Pollen grains are tricolpate or tricolporoidate and medium-sized, falling into 21.9–28.2  $\mu\text{m}$  for the polar axis and 14.5–25.5  $\mu\text{m}$  for the equatorial diameter (as measured in SEM; in LM the figures for the same pollen tend to be 1–3  $\mu\text{m}$  larger). Differences in sculpture pattern observed at the SEM level can sometimes be seen at the LM level, but it is commonly uncertain. In other words you can see in LM that there are different oak species but there are no distinct and accurate characters to distinguish the pollen grains. The oak exine is tectate, and columellate (Fig. 2H–K; Fig. 3H–L; Fig. 4H–K). The foot layer is discontinuous and of an uneven thickness throughout the pollen grain (Fig. 2J, K; Fig. 3J–L; Fig. 4H, I). The endexine is less electron dense and appears to be homogeneous (Fig. 2J; Fig. 3J, K; Fig. 4H, I). The endexine thickness is not uniform and sometimes “drops” (considerably thicker small regions) of the endexine can be seen (Fig. 2J; Fig. 3K; Fig. 4I). The foot layer is the first to disappear toward aperture regions; then it is followed by the infratectum and tectum. The endexine thickens toward aperture regions (Fig. 2I; Fig. 3I; Fig. 4J).

### Group 1 (Fig. 2)

The sculpture in SEM is verrucate to microverrucate. Perforations are often observed between (micro)verrucae. The sculptural elements are of different sizes, mostly less than 1  $\mu\text{m}$  in diameter, some are about 0.5  $\mu\text{m}$  and others are less than 0.5  $\mu\text{m}$ . The verrucae are not flattened, of a regular rounded outline, can be situated densely, but usually with each microverruca standing alone. The exine surface between the microverrucae appears to be formed by vertically oriented rod-like elements, covered with more sporopollenin (Fig. 2E–G).

In TEM the ectexine is of uneven thickness (corresponding to verrucae and the surface between them). In verruca regions the ectexine is 0.6–1  $\mu\text{m}$  and the tectum is 0.32–0.6  $\mu\text{m}$ , between them the ectexine is 0.3–0.56  $\mu\text{m}$  and the tectum is 0.15–0.25  $\mu\text{m}$ . Almost no channels or perforations in the tectum were observed. The columella height is 0.13–0.23  $\mu\text{m}$ , the width is 0.13–0.5  $\mu\text{m}$ . The thickness of the foot layer is from 0 (discontinuous) up to 0.2  $\mu\text{m}$ . The endexine is from 0.02 up to 0.12  $\mu\text{m}$  at endexine “drop” regions (Fig. 2H–K).

### Group 2 (Fig. 3)

The sculpture in SEM is verrucate or microverrucate, with microechinate suprasculpture. Sometimes rare perforations were observed. The sculptural elements are up to 1  $\mu\text{m}$ , flattened, with protruding tips of rod-like elements. This makes the verrucae seem microechinate. The verrucae or groups of verrucae occur as scattered features. The exine surface between the microverrucae appears to be formed by vertically oriented rod-like elements, covered with sporopollenin (Fig. 3E–G).

In TEM the ectexine is of uneven thickness (corresponding to verrucae and the surface between them). In verrucate regions the ectexine is 0.72–1.06  $\mu\text{m}$  and the tectum is 0.32–0.58  $\mu\text{m}$ , between them the ectexine is 0.42–0.69  $\mu\text{m}$  and the tectum is 0.13–0.32  $\mu\text{m}$ . Sometimes channels in the tectum were observed. The columella height is 0.16–0.31  $\mu\text{m}$ , the width is 0.13–0.44  $\mu\text{m}$ . The thickness of the foot layer is from 0 (discontinuous) up to 0.25  $\mu\text{m}$ . The endexine is from 0.05 up to 0.29  $\mu\text{m}$  at endexine “drop” regions (Fig. 3H–L).

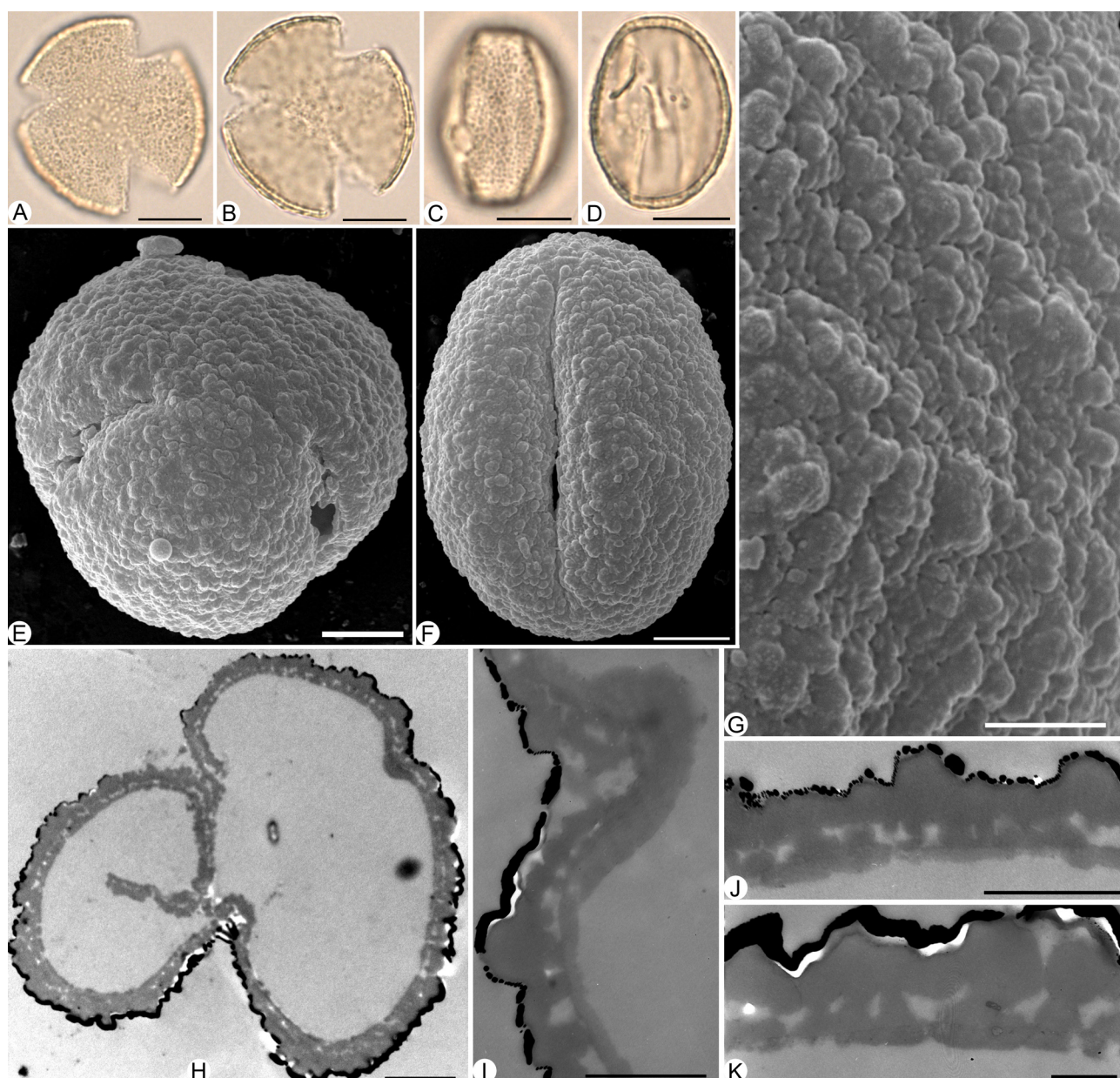
### Group 3 (Fig. 4)

The sculpture in SEM is verrucate, with granular suprasculpture. No perforations were observed. The sculptural elements are of different sizes, mostly less than 1  $\mu\text{m}$  in diameter, some are about 0.5  $\mu\text{m}$  and others are less than 0.5  $\mu\text{m}$ . The verrucae are not flattened, of a regular rounded outline, can be situated densely, but usually with each microverruca standing alone. The exine surface between the microverrucae appears to be formed by vertically oriented rod-like elements, covered with sporopollenin (Fig. 4E–G).

In TEM the ectexine is of uneven thickness (corresponding to verrucae and the surface between them). In verrucate regions the ectexine is 1–2  $\mu\text{m}$  and the tectum is 0.4–1.3  $\mu\text{m}$ , between them the ectexine is 0.5–1  $\mu\text{m}$  and the tectum is 0.2–0.4  $\mu\text{m}$ . Rare channels in the tectum were observed. The columella height is 0.2–0.5  $\mu\text{m}$ ; the width is 0.1–0.33



$\mu\text{m}$ . The thickness of the foot layer is from 0 (discontinuous) up to  $0.5\ \mu\text{m}$ . The endexine is from  $0.11\ \mu\text{m}$  up to  $0.7\ \mu\text{m}$  at endexine “drop” regions (Fig. 4H–K).



**FIGURE 2.** Pollen morphology and ultrastructure of Group 1, A–D. LM, E–G. SEM, H–K. TEM. A, B. Polar view, two different foci. C, D. Equatorial view, two different foci. E. Polar view. F. Equatorial view. G. Exine sculpturing. H. Section through whole pollen. I. Aperture region. J, K. Non-aperture region. Scale bar  $0.4\ \mu\text{m}$  for K,  $1\ \mu\text{m}$  for I, J,  $2\ \mu\text{m}$  for G, H,  $5\ \mu\text{m}$  for F,  $10\ \mu\text{m}$  for A–E.

## Discussion

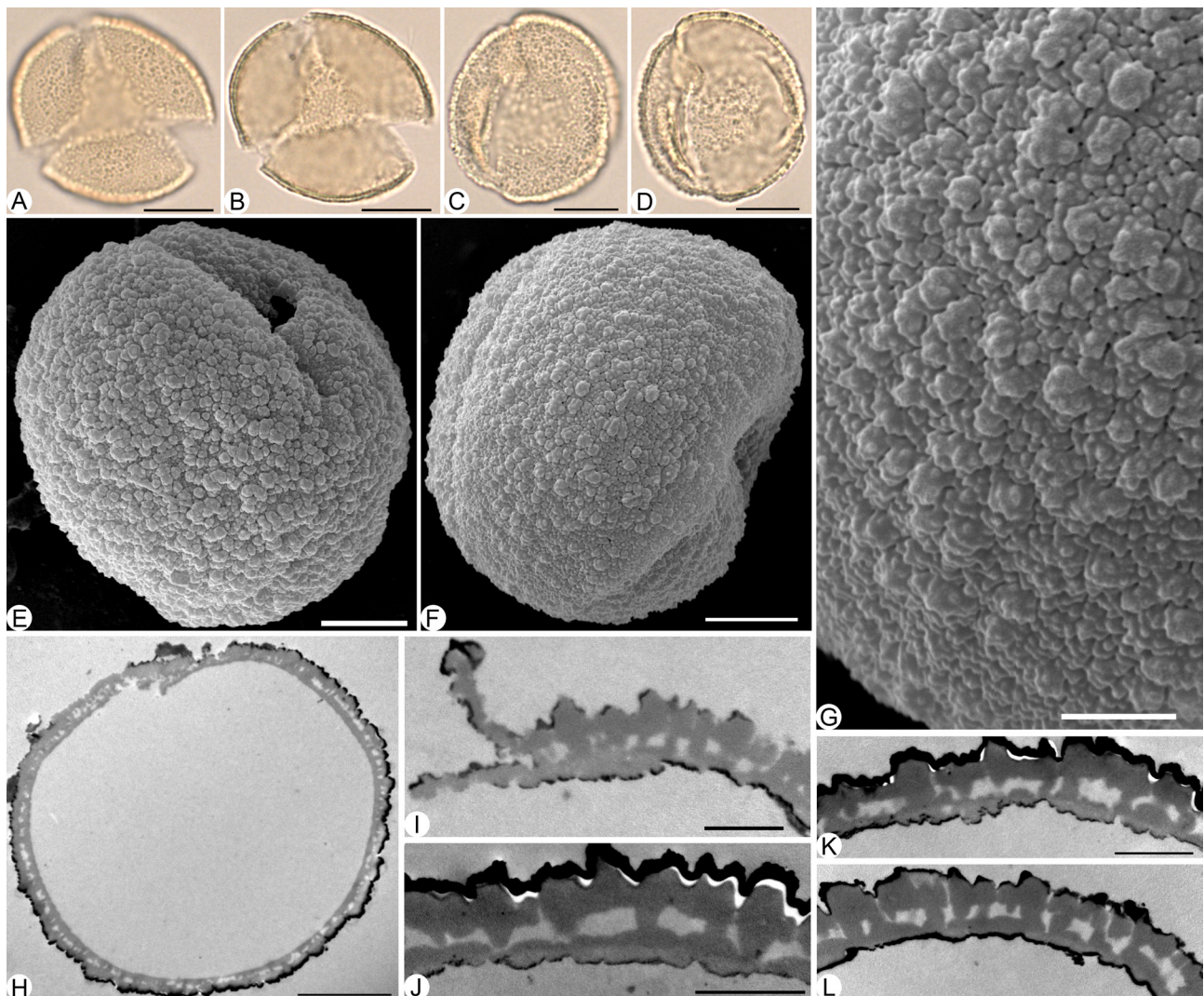
### Utility of different methods applied in studying dispersed oak pollen

Light microscopy is a routine palynological method widely used for determining pollen grain identity up to a likely taxonomic level according to general pollen morphology. Oak pollen can be reliably distinguished to the generic level. Pollen size and shape are sometimes used to distinguish deciduous and evergreen oaks in the spectrum and some researchers even define oak pollen to the species level in LM (e.g., Jarvis *et al.* 1992, Gil-Garcia *et al.* 2013) but such identifications are often debatable.

With the accumulation of works on the exine morphology of modern species SEM has proved to be a useful tool in recognizing oak pollen below the generic level (see a detailed analysis in Liu *et al.* 2007, and a review of later works in Tekleva *et al.* 2014). Molecular studies recognize six infrageneric groups within the genus *Quercus* and



they are consistent with data of pollen morphology (Denk & Grimm 2009, 2010, Hubert *et al.* 2014). The number of works studying oak pollen in SEM from fossil dispersed spectra is increasing (Nakagawa *et al.* 1996, Kataoka 2006, Naryshkina & Evstigneeva 2009, Grimsson *et al.* 2015 and others). With SEM, dispersed oak pollen can be distinguished to the level of an infrageneric group (e.g., section), groups of species, or even to the specific level. There are two methods used in such works. The first is to drop a pollen sample directly on a SEM stub and observe the exine sculpturing only (e.g., Nakagawa *et al.* 1996, Kataoka 2006, Naryshkina & Evstigneeva 2009, Hayashi *et al.* 2012); the second one is to study the same pollen grains first in LM, then in SEM and obtain combined data on general morphology and exine sculpturing (e.g., Ferguson *et al.* 1998, Denk *et al.* 2010). The latter is more detailed and useful for comparison with other data. With more information, there is a greater possibility of accurately identifying the grain.



**FIGURE 3.** Pollen morphology and ultrastructure of Group 2, A–D. LM, E–G. SEM, H–L. TEM. A, B. Polar view, two different foci. C, D. Equatorial view, two different foci. E. Polar view. F. Equatorial view. G. Exine sculpturing. H. Section through whole pollen. I. Exine section towards aperture region. J–L. Non-aperture region. Scale bar 1 µm for I–L, 2 µm for G, H, 5 µm for F, 10 µm for A–E.

We used an even more detailed method of studying the same oak dispersed pollen in LM, SEM, and TEM (Tekleva *et al.* 2014, this study). The data on the exine ultrastructure (TEM) can differentiate between different oak species better when given a larger pool of TEM data on different oak species. So far, few TEM studies have been made on modern and fossil oak pollen (see review in Tekleva *et al.* 2014).

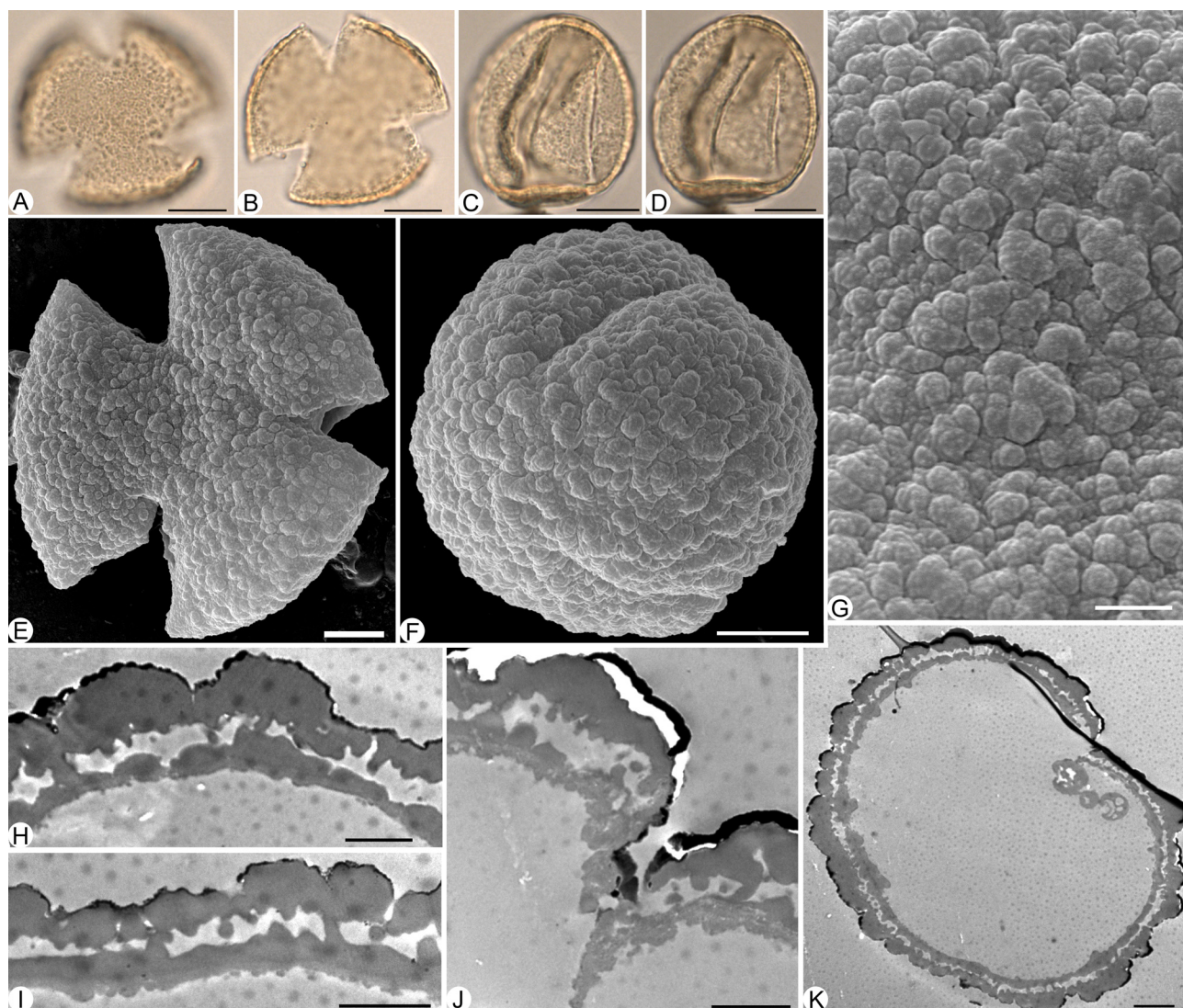
### Comparison of the studied oak pollen with pollen of oak species known for the area (Rolla) and for the state of Missouri

Among the studied pollen, three main morphotypes were distinguished according to their exine sculpturing. This does



not mean that the three morphotypes necessarily correspond to three oak species; a single morphotype can include more than one species.

Liu *et al.* (2007) in their paper made a comprehensive analysis of exine sculpturing in modern oak species and recognized four general pollen types among them: scabrate-verrucate (Type 1), rugulate (Type 2), of rod-like elements (Type 3), and uniformly fine granular (Type 4).



**FIGURE 4.** Pollen morphology and ultrastructure of Group 3, A–D. LM, E–G. SEM, H–K. TEM. A, B. Polar view, two different foci. C, D. Equatorial view, two different foci. E. Polar view. F. Equatorial view. G. Exine sculpturing. H, I. Non-aperture region. J. Aperture region. K. Section through whole pollen. Scale bar 1  $\mu$ m for H–J, 2  $\mu$ m for K, 5  $\mu$ m for G, 10  $\mu$ m for A–F.

Our first (Group 1) and second (Group 2) morphotypes were dominant and can be attributed to the Type 1 (scabrate-verrucate) sensu Liu *et al.* (2007), which is usually characteristic of deciduous oaks. The third morphotype (Group 3) corresponds with Type 2 (rugulate) sensu Liu *et al.* (2007), which occurs both in deciduous and evergreen oaks and is rather similar to, e.g., *Q. myrtifolia* Willdenow (1805: 424). The studied exine ultrastructure also supports the separation of these three morphotypes.

The pollen investigated in this study comes from sediments which are about 70 years old. This allows direct correlation of oak pollen with the modern oak species which grow in the area. There are six oak species with the white oak *Q. alba* Linnaeus (1753: 996) dominant in the region (Rolla city) and the black oak *Q. velutina* Lamarck (1785: 721) being rather common. These two species were expected to be dominant among oak pollen in the spectrum too. Some rarer species in the study area include *Q. marilandica* Münchhausen (1770: 253), *Q. coccinea* Münchhausen (1770: 253), *Q. stellata* Wangenheim (1787: 44), and *Q. rubra* Linnaeus (1753: 996). These are not thought to be common in the pollen samples. Also, as this is an inhabited area, another possibility might be that pollen encountered in our pollen spectrum represents an exotic species.

Comparing dispersed oak pollen of our sediment sample with illustrations in Solomon's works (1983a, b) who studied pollen morphology of North American white and red oaks in SEM, we can correlate our Group 1 pollen with white oaks (*Q. alba*, *Q. stellata*, *Q. bicolor* Willdenow (1801: 396), *Q. macrocarpa* Michaux (1801: 4), *Q. lyrata* Walter (1788: 235), *Q. prinoides* Willdenow (1805: 440) and Group 2 with red oaks (*Q. velutina*, *Q. marilandica*, *Q. rubra*, *Q. muehlenbergii* Engelm (1877: 391). While the first and second morphotypes show similarity to several deciduous oak species known for this region, the third morphotype (Group 3) ornamentation is more similar to that of some evergreen oaks (e.g. *Q. myrtifolia* or *Q. virginiana* Miller (1768) as shown in Solomon 1983a, fig. 18, 19, Solomon 1983b, fig. 7d), however no evergreen species has been identified in the region according to the literature (<http://plants.usda.gov/core/profile?symbol=QUMY>, <http://plants.usda.gov/core/profile?symbol=quvi>).

There are about 20 species and 16 hybrids of oaks in Missouri (e.g., Missouri department of conservation, Knotts & Main 2005), and all of them are deciduous. They belong to the so-called red (or black) and white oaks: *Q. alba*, *Q. velutina*, *Q. marilandica*, *Q. coccinea*, *Q. rubra*, *Q. stellata*, *Q. macrocarpa*, *Q. pagoda* Raf., *Q. prinoides*, *Q. texana* Buckley (1860: 444) (or alternatively as *Q. nuttallii* E.J.Palmer (1927: 52), *Q. lyrata*, *Q. palustris* Münchhausen (1770: 253), *Q. imbricaria* Michaux (1801: 15), *Q. shumardii* Buckley (1860: 444), *Q. michauxii* Nuttall (1818: 215), *Q. bicolor*, *Q. falcata* Michaux (1801: 28), *Q. nigra* Linnaeus (1753: 995) (deciduous or tardily deciduous), *Q. phellos* Linnaeus (1753: 994), *Q. muehlenbergii*. Among them, pollen grains of Group 3 can be compared to willow oak *Q. phellos* with similar rugulate sculpture and granular suprasculpture. However, this species does not grow in Phelps County, and in Missouri the willow oak is only found natively in the southeastern counties. Also, the nearest two small "spots" about 30–50 km in diameter of this species are in the northeast of Missouri, at the border with Illinois (near St. Louis) and in Illinois around Breese and Carlyle cities (USDA Forest service, Northeastern area, State and Private forestry). If we make an approximate estimation this is about 125–130 km from Rolla to the closest border of *Q. phellos* occurring on the southeast regions and about 140 km—with the northeastern "spot". The question is whether oak pollen could have been transported for such a distance?

### Possibility of long-distance transportation of oak pollen

Different opinions exist in the literature on how far oak pollen can transport with many authors indicating that oak pollen are mostly present within their area (e.g. Erdtman 1943, Malgina 1950, Mandrioli *et al.* 1984, Sladkov 1967). Experimental studies confirmed that pollen grains of broad-leaved species do not transport for long distances by wind, and within 100 m of the forest border the concentration of oak pollen falls down to 3% (e.g., Fedorova 1948, 1950). Studies of pollen transportation by water streams also suggest that oak pollen do not remain in the water very long before being deposited and therefore they mostly do not transport for long distances (Fedorova 1950, Brush & DeFries 1981). At the same time, more oak pollen grains are found in prevailing wind directions (up to 13 %) and a small amount to individual pollen grains can be found far from the forest (see discussion in Kabailene 1976). This has been repeatedly observed in different parts of the world (Erdtman 1938, Hernandez-Ceballos *et al.* 2011, Jato *et al.* 2007, Recio *et al.* 1999, Rousseau *et al.* 2008). An interpretation of a small percentage of oak pollen as long-distance pollen dispersal has sometimes been discussed for fossil pollen spectra (e.g., Denk *et al.* 2010, Evstigneeva & Naryshkina 2012, Tekleva *et al.* 2014).

Kabailene (1976) referred oak pollen to the group of "medium transporting" (75% of the pollen can transport for 55–100 km). She pointed out that the distance of pollen transport also depends on how far the forest border was located from the place of sampling. According to her work, with the increasing of this distance to 50 km from the forest border oak pollen can be found more than 900 km away from the nearest oak source. This provides a possible explanation for the presence of oak pollen untypical for the region in our material.

### Pollen morphology for identification of oak species

This study indicates that several factors should be considered when interpreting oak pollen from fossil spectra.

Dispersed oak pollen grains are sometimes identified down to species level based on LM. However, such identifications are difficult to make with confidence. At the LM level, the main pollen characteristics available are pollen size, shape, aperture structure, exine sculpturing and exine thickness. Pollen size and shape are important to consider but they cannot be used separately to determine oak pollen species because they overlap in many oak species and thus the characteristics can fail to identify individual dispersed pollen grains. Though based on these characters, it is sometimes possible to approximately evaluate the ratio of pollen grains produced by evergreen and deciduous oak species for some regions in the assemblage (e.g., where the vegetation is represented by few oak species and the pollen size of evergreen and deciduous species differs).

Exine sculpturing at the LM level cannot be distinguished with enough detail to identify different oak species.



Nevertheless it is interesting to note that after studying a particular sample and distinguishing several sculpture patterns in SEM, the patterns seen in SEM can sometimes also be recognized at the LM level. The aperture structure in different *Quercus* species is thought to differ concerning the absence/presence of the geniculum but when you deal with an individual dispersed fossil pollen grain it is difficult to use this character. The exine thickness also shows low potential at the LM level due to the low magnification.

The exine ornamentation in SEM was shown to be the most significant in distinguishing different oak species/group of species among dispersed pollen (Grimsson *et al.* 2015, Liu *et al.* 2007, Tekleva *et al.* 2014). However, exine ornamentation as a distinguishing feature has its own risks.

First it is important to sort out the terminology used by different authors for describing the sculpture. This is necessary to be able to make a reliable comparison as not all the described characteristics might be clearly illustrated. Illustrations are usually insufficient to document variability that often occurs in oak species. For example, some oak species show a high variability and include several sculpture patterns known from different oak species (e.g., Makino *et al.* 2009, Solomon 1983a, b). There are some exceptions to this trend, as some species can be distinguished rather well by their exine sculpturing (e.g., *Q. phillyreoides* A.Gray (1858: 406), *Q. dentata* Thunberg (1784: 858) in Makino *et al.* 2009, Naryshkina 2015). It is important to study several specimens preferably from different regions per one modern species to document variability of pollen morphology within the same species. Unfortunately, this is rarely done.

Reasons for the variability of exine ornamentation in oak pollen are thought to include hybridization of oak species, and wider variability within some sculpture types (see discussion on this in Tekleva *et al.* 2014). This means that pollen morphology has different resolving abilities for different oak species. For example, it is often more difficult to distinguish different oak species/groups among oak pollen with (micro)verrucate sculpture, where an overlaying variability of the verruca pattern can be observed (Tekleva *et al.* 2014). Solomon (1983a, b) believed that unlike the pollen morphology of white oaks, the pollen morphology of red oaks is rather inconsistent which impedes possible distinction of red oaks in a dispersed pollen assemblage. All these aspects of oak pollen morphology obscure species-level determination which might contribute to the analysis.

## Conclusion

The exine sculpturing of oak pollen as seen in SEM is a good prospect for a more detailed determination of fossil dispersed oak pollen, and studies on the topic are accumulating. However, this method has some limitations. Many factors should be taken into account to make a reliable interpretation of pollen spectra: the paleo-forest density, place of sampling (whether it had been located within the then forest, at its margin or in the forestless area), wind direction during flowering, and the “resolving ability” of pollen morphology for different oak species. The intensity of flowering as well as direction of the pollen transport (“to” or “from” the forest) can significantly influence the quantity of pollen of a particular species in the spectrum. Considering the possibility of medium or long distance transport of a small quantity of oak pollen one should be cautious in drawing conclusions about the presence of a particular species (represented by small percentages in the spectrum) in the vegetation.

In the case of oak pollen, we can see that exine sculpturing plays an important role in species recognition but exact identification is not always possible. Some sculpture patterns (e.g., verrucate) show a higher variability within the same species than others. In regions with a high diversity of *Quercus* species the distinction between the species on the level of pollen morphology can also be more complicated than in those with few *Quercus* species. The exine ultrastructure gives additional information that can at least partly help in distinguishing between oak pollen with similar sculpture patterns.

Oak pollen grains from the study were attributed to three types according to their exine sculpturing and ultrastructure. A comparison with the present vegetation shows that two types correspond well with two dominant *Quercus* species from the region from white and red oaks, while the third one is rather unusual. The young age of the sample allows one to make a direct correspondence with the oak species growing in the region today, and thus we can see that this third type was most probably transported from another region or is represented by an exotic species planted by someone in the region. This confirms that the interpretation of fossil spectra should be very careful when it involves pollen occurring in smaller percentage.

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