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Degradation of Algal Palynomorphs on 34-yr-old Microscope Slides

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Reexamination of 34-yr-old microscope slides of palynological preparations reveals the loss and degradation of algal palynomorphs, in particular the green alga *Pediastrum* and the desmid *Pleurotaenium*. Results from this study suggest that great caution should be used when referring to archived palynological slides.

INDEX DESCRIPTORS: palynology, pollen analysis, degradation of palynomorphs, desmids, *Pediastrum*, *Pleurotaenium*.

Palynology is the study of pollen, spores, algal remains, and an assortment of other forms, collectively referred to as palynomorphs, found in maceration preparations of sediment and sedimentary rocks. The palynological study of ancient rocks yields pollen, spores, acritarchs, chitinozoans, and other forms now generally extinct. The study of Quaternary sediments, sometimes called pollen analysis, deals almost exclusively with extant taxa (Traverse 2007). Traverse (2007) calls the palynological study of any rock or sedimentary deposit paleopalynology, regardless of its age.

Rock and sediment samples are typically treated in consecutive steps with hydrochloric acid (HCl), hydrofluoric acid (HF), acetic anhydride and sulfuric acid, and potassium hydroxide (KOH). This treatment removes mineral grains, pyroclastic particles, diatoms, sponge spicules, and other siliceous forms as well as most of the organic debris making up the sediment. What survives this treatment are only the most resistant forms, pollen and spore walls (exines), and other palynomorphs. The preparation is then often stained and mounted in glycerin jelly (containing glycerol) or silicone oil on glass microscope slides (Faegri and Iverson 1989, Traverse 2007).

Because palynomorphs are made of organic materials, they are subject to oxidation and other forms of degradation. Traverse (2007: table 3.4) discusses the degradation of pollen and spore exines on 10-yr-old microscope slides, which were then mounted in glycerin jelly. Cushing (2011) also reports degradation of pollen mounted on slides using silicone oil. This paper reports on the differential degradation and disappearance of algal forms on 34-yr-old slides mounted in glycerin jelly where the pollen appears little affected.

MATERIALS AND METHODS

Sediment cores from several postglacial pond/marsh systems in the mountains of Montana were obtained in 1978 (Brant 1980). The coring device consisted of three-inch aluminum irrigation pipe using a piston to enhance core recovery. The sediment cores were sealed inside the pipe in the field and stored until the summer of 1979, when the sediment was exposed, examined, and sampled. The samples were then treated with HCl, HF, acetic anhydride and sulfuric acid, and KOH in a typical procedure. The palynomorphs were stained using biological stains, either safranin O or basic fuchsin. Glycerin jelly of commercial manufacture was used to mount the preparations on the microscope slides, then enclosed by a cover slip and ringed with clear nail polish to keep out as much oxygen as possible (Brant 1980).

The prepared microscope slides were examined in early 1980 using a bright field Olympus binocular microscope equipped with a mechanical stage, a FL 40/ 0.75 objective, and widefield 10× oculars for a magnification of 400×. The microscope was also equipped with a FL 100/1.30 used for identification of some palynomorphs. Counting was done using the 40× objective in a number of east-west traverses across each slide, beginning at one corner and proceeding until the desired number of grains was reached. The mechanical stage assured non-overlapping coverage of the slide. All pollen grains and spores, as well as *Botryococcus* and *Pediastrum* colonies, were counted in each sample. Some groups appearing in the preparations, such as desmids, were noted but not counted. The pollen sum consisted of the number of pollen grains and spores, except aquatic types (e.g. *Nuphar*), and usually exceeded 500. *Botryococcus* and *Pediastrum* were not included in the pollen sum (Brant 1980).

In 2013, I selected slides from the original set made in 1979 and reexamined them using a bright field Olympus BH2 binocular microscope equipped with S-Plan objectives. I duplicated the counting procedure using the (40/0.70) objective for a total magnification of 400×, except I counted only *Nuphar* pollen grains, and *Botyococcus* and *Pedisatrum* colonies. I counted the *Nuphar* pollen and *Botryococcus* algal colonies only to assure that an adequate amount of the slide was examined. I also searched many other slides more casually looking for *Pediastrum*.

RESULTS AND DISCUSSION

The walls of pollen grains and embryophyte spores are partly constructed of an organic compound of an extremely resistant material called sporopollenin. This material resists decay and becomes part of the sediment or sedimentary rock. It survives for millions of yrs in the rock and survives the acetolysis (the procedure described above) in the laboratory. It is destroyed, however, by oxidation. But even in sediment I have treated with boiling 30% hydrogen peroxide for diatom preparation, pollen grains are the last organic objects to be destroyed. Traverse

Table 1. Comparison of palynomorph counts from 1980 and 2013.

Sample (depth)	Year of count	*Nuphar* grains	*Botryococcus* colonies	*Pediastrum* colonies
150 cm	1980	8	409	58
	2013	3	229	3[1]
340 cm	1980	7	382	29
	2013	12	359	0

[1]Very degraded and almost unrecognizable.

(2007) talks about spores and pollen surviving the guts of goats and locusts. But sporopollenin does pick up oxygen and will "autoxidize" on microscope slides where the grains degrade by swelling (Traverse 2007).

Some algae also contain sporopollenin, but others seem to acquire resistance to decay and palynological treatments in other ways. Desmids were found in the pollen preparations counted in 1980. In particular, species of *Euastrum*, *Xanthidium* and *Staurastrum* were frequently observed. One sample contained nearly as many *Pleurotaenium nodosum* semicells as there were pollen grains. Interestingly, *Micrasterias* was not found in any of the preparations even though it was observed living in the wetland. What gives these desmids resistance is not clear. *Botryococcus* colonies, on the other hand, are robust palynomorphs found in rocks as old as Ordovician, and owe their resistance to a mixture of organics and hydrocarbons (Traverse 2007).

Pediastrum colonies are green algal coenobia that are often found in pollen preparations found in rocks as old as the Cretaceous. Traverse (2007) says that the resistance to biodegradation of this genus "is one of the puzzles of palynology." It looks delicate under the microscope, and my observations indicate that it is indeed somewhat delicate.

Out of some whimsical desire to look at *Pediastrum* in my old pollen slides, I looked into my old thesis to see which samples contained significant numbers of this palynomorph. To my surprise, when I looked at the slides the *Pediastrum* seemed to be gone, even though the slides were in good condition and the stained pollen was not obviously degraded. I then set about reproducing my counting procedure of two samples that once contained significant numbers of this alga. The number of *Botryococcus* colonies counted in the sample from 150-cm depth in the core would indicate that 32 *Pediastrum* colonies should have been encountered. Similarly, 27 should have been observed in the sample from the 340-cm depth (Table 1). That only three highly degraded colonies of *Pediastrum* were observed is further indication that I did not simply fail to recognize *Pediastrum* when I saw it. *Pediastrum* was simply (at best) highly degraded or was totally gone.

The desmid *Pleurotaenium nodosum* var. *borgei* Gronblad (Prescott et al. 1975, Pl XLIV, Fig. 6) occurs in numbers nearly as great as the number of pollen grains at the 420-cm depth in the core. These forms appear faded, distorted, and swelled in 2013 when compared to photographs taken in 1980 and to the figures in Prescott (1975). The semicells are swollen in diameter relative to their length.

CONCLUSIONS

To establish some fact or principle based upon the absence of evidence, in this case *Pediastrum* colonies, presents a challenge; therefore, I have tried to create the same (or very similar) viewing conditions of 33 yrs ago when establishing that *Pediastrum* colonies have indeed effectively and spontaneously disappeared from these old slides.

Traverse (2007) has shown how pollen and spores mounted in glycerin jelly swell and degrade over a period as short as 10 yrs but show little or no degradation if the oxygen is totally excluded from the material. Here I have demonstrated that the green alga *Pediastrum* and the desmid *Pleurotaenium* also undergo degradation, to the point of destruction for *Pediastrum*, over time. That the pollen grains on these old slides appear to have been degraded little or not at all in my casual observation suggests the relative delicacy of the algal forms. Glycerin jelly-mounted palynological slides are not permanent, and the use of voucher or archived slides mounted with glycerin jelly must be used with great caution to avoid being misled when examining old collections. However, this study sheds no information on palynomorph preservation on slides mounted using different media, or even different laboratory treatments of the original sediment or rock.

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