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Research paper

Experimental investigations into the preservation of pollen grains: A pilot study of four pollen types

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ABSTRACT

Preservation condition of pollen grains from different sedimentary environments can be a useful tool for interpreting pollen assemblages, but current understanding of the controls on pollen preservation is limited. One constraint is the complexity of studying these processes in the natural system. This paper presents the results of a pilot study of laboratory assessments of damage processes. A standard mixture of dried pollen grains (4 taxa: *Alnus, Secale, Dactylis* and *Ambrosia*) was rehydrated and suspended in glycerine, then subjected to two types of treatment, chemical oxidation or agitation in water with added sand or pebbles. The treatments generated distinctive damage patterns, and the pattern of damage observed varies with duration of treatment, combinations of treatments and pollen taxon. The results suggest that laboratory methods, which can generate damaged pollen assemblages rapidly and under controlled conditions, are a useful route for investigating the processes controlling pollen degradation and therefore interpreting the damage spectra recorded from past assemblages in terms of environmental processes.

1. Introduction

Pollen analysis is a widely applied method of reconstructing variations within vegetation patterns through time (Lowe and Walker, 1997). A key assumption in pollen analysis is that the composition of the pollen assemblage recorded from a sediment sample (e.g. ratios of different grain types) is the same as that originally deposited, and therefore that an understanding of the taphonomy and pollen–vegetation relationships observed today can be used to interpret past assemblages and reconstruct past vegetation (e.g. Sugita et al., 1999; Nielsen, 2004; Bunting and Middleton 2005; Anderson et al., 2006; Sugita 2007a,b). The outer wall of pollen grains and spores is rich in sporopollenin, a robust substance which enables preservation in a range of conditions, but not immune to damage. Ideal preserving conditions occur in permanently waterlogged (and thus anoxic) low-energy environments which inhibit sporopollenin degradation.

The preservation state of pollen grains is sometimes recorded and interpreted in terms of the history of the sediment system and its formation (Cushing, 1967; Lowe, 1982; Wilmshurst and McGlone, 2005b; Tweddle and Edwards, 2010), used as a measure of how reliably the recovered assemblage reflects the original deposit (Bunting and Tipping, 2000; Tipping, 2000; Tipping et al., 2009) or used as a marker of the risk of losing the palaeoecological archive within the sediments in the near future (e.g. Jones et al., 2007). Sedimentary processes tend to be relatively slow-acting, which limits direct empirical investigation of the phenomena of pollen assemblage degradation. In this paper, we briefly review the literature on this topic, and present the results of preliminary attempts to cause degradation and pollen assemblage alteration under controlled laboratory conditions over relatively short time frames.

In many cases, preservation problems are dealt with by ignoring them (e.g. review in Tipping 2000), unless preservation is so poor that it is problematic to count the assemblage at all, in which case the sample is usually set aside. However, almost all pollen assemblages show some degree of imperfect preservation. Havinga's (1984) twenty-year field experiment showed clearly that different palynomorphs have different susceptibilities to degradation processes and that poor preservation conditions alter the composition of the assemblage. For example, in his leaf mould experiment, 98% of *Corylus avellana* grains were lost over the course of 20 years, but only 50% of *Taraxacum* grains and 60% of *Quercus* grains were lost. These shifts in relative abundance could lead to pronounced changes in interpretation of the pollen record. Pollen degradation also increases the difficulty of identifying pollen grains. Grains which remain relatively recognisable when damaged or which are resistant to damage will be recorded more readily (Hall, 1981), and lower levels of taxonomic discrimination will be possible in damaged samples (e.g. identification of Rosaceae sub-types).

Post-depositional damage to pollen grains is usually divided into two types, oxidation and mechanical damage. In samples from contexts with good oxygen availability, the most susceptible taxa

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tend to have thinner wall structures (Havinga, 1964, 1967), but decay susceptibility is not directly correlated with the percentage of sporopollenin in the cell wall (Birks and Birks, 1980), suggesting that simple chemical reactions are not the only processes involved; biological activity also affects grain preservation. At present little is known of the biology of pollenivores, but bacteria are known to damage both the exine and protoplast of modern pollen grains (Elsik, 1971; Birks and Birks, 1980).

Mechanical damage can be caused by a number of agents, including transport in water, compaction and repeated wetting and drying. In a fluvial environment pollen grains are generally transported with silts and clays in suspension, but collisions with larger grains can cause mechanical damage (Holmes, 1994; Brush and Brush, 1972). Compaction is only an effective agent of damage at very high pressures and where the protoplast has been removed before compression; this rarely occurs on timescales relevant to the Quaternary palynologist (Sangaster and Dale, 1961). Repeated wetting and drying can cause swelling and shrinking of grains, leading to loss of structural integrity, splitting and crumpling (Holloway, 1989; Campbell, 1991; Campbell and Campbell, 1994).

There is no single method for quantifying pollen preservation. Cushing (1967) proposed classification of grains into 6 groups; corroded, degraded, crumpled and exine thinned, crumpled but exine normal, broken and well preserved. A five-category system which amalgamates the two crumpling classes is the most commonly used method in the literature (see Birks, 1970; Lowe, 1982, Moore et al 1991), sometimes with further modification (Long et al., 2000). However, these groupings assume that a grain is affected mostly or entirely by one type of damage, which is not always the case (e.g. Holloway 1989). Jones et al. (2007) record mechanical and oxidative damage separately; using a gridded record sheet with mechanical damage classes on one axis and oxidative classes on the other axis also allows recording of the relationship between the two types of damage (Delcourt and Delcourt (1980) on individual grains.

Another method of assessing preservation state is to examine the fluorescence of pollen grains under UV lighting (Van Gijzel (1971). Intensity of fluorescence varies with age (van Gijzel, 1971; Philips, 1972) and location along the colour spectrum relates to preservation (Van Gijzel, 1967, 1971; Yeloff and Hunt, 2005).

Published interpretation of preservation data generally focuses on detection of erosional input into a sedimentary system, during the late glacial (Cushing, 1967; Lowe, 1982; Tipping, 1987; Crowder and Cuddy, 1973), in fluvial contexts (Birks, 1970; Fall, 1987) or in response to human disturbance of vegetation (Wilmshurst and McGlone, 2005b; Tweddle and Edwards, 2010). Preservation data has been used to screen samples from archaeological contexts to exclude those most likely to be affected by post-depositional biasing (e.g. Bryant and Hall, 1993; Tipping, 1987; Tipping et al., 1994; Bunting and Tipping, 2000; Jones et al., 2007). Archaeologically-related samples often come from contexts that do not preserve pollen well, such as palaeosoils or floor surfaces, which makes interpretation complex (Bryant and Hall, 1993; Holloway, 1989). Bunting and Tipping (2000) propose a check list of 'tests' which might indicate altered assemblages, and Bunting and Tipping (2001) apply these tests to archaeological samples from a Bronze Age barrow cemetery.

Investigation of the processes leading to the development of a degraded assemblage is essential to better interpret the preservation spectra from past pollen assemblages. Three experimental approaches can be characterised: comparison of surface samples, field experiments and laboratory experiments. Wilmshurst and McGlone (2005a) compare pollen assemblages from moss, soil and lake sediments, and attribute observed differences to the relatively wet, acidic environment provided by moss polsters and the influence of inwashed soil on the lake assemblages. Havinga (1964, 1967, 1971, 1984) carried out field experiments by mixing pollen grains and spores with inert sediment, sealing sub samples into nylon mesh bags ('teabags') and placing these in environments with different levels of biological activity, then recovering them at intervals over a 20 year period. He reports that higher levels of biological activity lead to a greater overall intensity of degradation, and shows that if pollen types are ranked according to susceptibility to degradation, the rank for a given type varies with preserving environment. Laboratory experiments have shown that repeated cycles of wetting and drying can cause mechanical damage through repeated shrinking and swelling of the cell wall (Holloway, 1989; Campbell and Campbell, 1994), and that varying the particle size of bedload in flume experiments can also affect the amount of mechanical damage observed (Holmes 1994).

1.1. Summary of current knowledge

The damage spectrum relates to the taphonomy of the assemblage; for example corrosive processes occur in environments with high biological activity and mechanical processes affect grains that are transported and deposited within minerogenic environments. Different grains are variably susceptible to damage. Reported rankings vary with the authors, the type of degradation emphasised and the basis of the ranking (Faegri, 1971; Birks and Birks, 1980; Havinga, 1984), but similar groupings are generally agreed upon. This is useful for interpretation when one degradation process is strongly dominant. However, if a sample at first experiences oxidative damage and is then exposed to mechanical damage it is not clear which ranking of taxa will be most appropriate, and how the response to the second process is altered by the first. There is also no clear basis on which to decide whether the damage spectrum indicates that the composition of the sample has been significantly changed, or to help extract information from biased assemblages.

2. Aim of our experiments

Preservation of grains is an important aspect of palynology which is currently poorly understood. Empirical data and inferences from the sedimentary record provide a framework, but controlled experiments clearly have the potential to improve our understanding. If damage spectra can be produced within a laboratory setting, it will be possible to explore the interaction of factors, to test and extend the susceptibility ranking systems to a wider range of taxa, and to assess the effectiveness of proposed screening or assessment protocols (e.g. Bunting and Tipping, 2000; Jones et al., 2007) in detecting situations where an assemblage is substantially altered from the original. Simple techniques are therefore applied to investigate the impact of energetic interaction with mineral material, chemical oxidation, and a combination of these factors has upon the condition of pollen grains.

3. Methods

3.1. Polleniferous medium

The basic pollen assemblage used in all experiments was a mixture of known quantities of commercially sourced *Alnus, Ambrosia, Secale* and *Dactylis* pollen grains (Sigma-Aldrich), rehydrated in a humid environment and suspended in glycerol. These taxa were chosen both because of ready availability and because differential susceptibility to damage is expected (with *Dactylis* and *Secale* being more susceptible and *Ambrosia* least susceptible). The suspension was then mixed using a magnetic mixer for 7 days prior to the commencement of the experiments and remixed for at least 24 h before the start of each experimental run to ensure a homogenised solution.