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Considerations for the preparation of peat samples for palynology, and for the counting of pollen and non-pollen palynomorphs

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SUMMARY

Peat deposits are valuable archives for studying palaeoclimate, the history of local and regional vegetation, and human impact. The most widely applied laboratory analytical technique has been palynology (pollen analysis), which is often limited to the study of pollen and a few easily recognisable spores; however, a variety of other microfossils can be studied in peat deposits and can provide information on past environmental conditions. Among the so-called non-pollen palynomorphs (NPPs) are fungal and algal spores that can be used as indicators for local hydrological changes and trophic conditions. This article provides an overview of aspects to consider and sample preparation methods for pollen, spores and other non-pollen palynomorph microfossils in peat deposits; advice on aids to pollen identification and counting; and a brief guide to the range of NPPs that can be counted from prepared subfossil-pollen microslides.

KEY WORDS: algal spores, fungal spores, laboratory preparation technique, microfossils, pollen counting.

1. INTRODUCTION

Since its introduction by von Post (1916) in Scandinavia and its mid-20th Century applications in Britain, Denmark and Ireland by Godwin (1940), Iversen (1941) and Jessen (1949) respectively, pollen analysis (palynology) has been regarded as the most widely applied technique in Quaternary palaeoecology (Birks & Birks 1980, Birks 1986 repr. 2003). For Holocene mire sites it has often been either the single or the most important proxy technique for palaeoenvironmental reconstruction used by scientists, although many others are now available or under development, especially for climate reconstruction (Chambers *et al*. in press).

In the later decades of the last century, pollen analysis in north-west Europe was well-served by specialist textbooks; but one (Erdtman 1969), since revised (Nilsson & Praglowski 1992), is out of print, and the most recent editions of others are two decades old (e.g. Faegri & Iversen 1989, Moore *et al*. 1991). Unfortunately, also, a specialist journal—*Pollen et Spores*—has ceased publication. In the 21st century, a new text by Beug (2004) is available (in German). None of the textbooks detail the wide range of other microfossils of various origins that are also preserved in pollen preparations.

Pollen analysis has a long history and multiple purposes, including reconstructing vegetation history, assessing climate response, human impact, and informing biodiversity conservation. Owing to these diverse purposes, and because numerous laboratories have adopted different methods and adapted their own local variants, it is difficult (and would potentially be both contentious and divisive) to propose a single prescriptive protocol for use in peats as has been done for some of the other techniques in this volume. Therefore, this short article aims to alert scientists intending to work on peats to some of the important aspects to consider when preparing to conduct microfossil analysis; to provide a guide to sample preparation from peats; to give advice on aids to pollen identification and counting, including some cautionary remarks; and to provide an overview of the range of non-pollen palynomorphs (NPPs) that can be counted from prepared microslides, alongside pollen.

2. SAMPLE PREPARATION

Before embarking on peat-sample preparation, consideration should be given to the purpose of the analysis. This may well dictate or strongly influence the proposed resolution of the sampling interval; the counting methods to be used; and the likely range of microfossils to be recorded. Traditionally, relative (i.e. percentage) pollen counting has been conducted on microscope slides prepared from peat samples, but 'pollen concentration' counting methods, previously mainly used for lake sediments, has become the normal procedure in some laboratories.

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In north-west Europe this was facilitated by the production and sale (*via* Lund University: http://www.geol.lu.se/kvg/eng/lycopodium.pdf) of 'spore tablets' (Stockmarr 1971, 1973) containing a known amount of 'exotic' pollen (e.g. *Eucalyptus*; probably not appropriate to use for Australian mires) or spores (e.g. *Lycopodium*). Although the latter may also be found native in some habitats close to mires, the spores in the tablets will have been acetylated twice—once in tablet preparation and again during peat sample preparation—so should appear darker than native spores. Spore tablets are not always readily available, and it is possible instead to make up a suspension of exotic pollen, and add an aliquot to each measured volume of peat. Note that the concentration of exotic pollen or spores in the aliquot then needs to be ascertained (Maher 1981); whereas, with tablets, that has already been determined and the data are supplied with the tablets.

Choices for the peat pollen analyst include whether to conduct wide-interval, or evenly spaced close-interval, contiguous and/or fine-resolution sampling; whether to sample volumetrically; if so, whether to use spore tablets (or aliquot of exotic pollen or spores); which alkali to use (KOH or NaOH); what sieve size (e.g. 180 µm or 215 µm); which (if any) method(s) to remove mineral material; whether to use a dehydrated mixture for acetylation (frequently referred to in pollen analytical literature as 'acetolysis'); whether to stain the pollen; which extractant to use; which mountant to use; what slide thickness and which coverslip dimension and thickness; whether to fix coverslip corners or completely seal the slides; and if so, with which sealant. Many of these topics are covered in Faegri & Iversen (1989) and Moore *et al*. (1991).

In considering which preparation method to use, the following should be borne in mind: the intention of percentage-based or pollen concentration methods of pollen counting; desirability of gentle or more rigorous chemical preparation (and possible consequences for susceptible palynomorphs); the presence or otherwise of potentially intractable mineral content (and so any need for removal); the size of mineral content (for example, sand grains can be removed without chemical treatment by swirling beaker contents after the sieving stage and waiting a few seconds before decanting; sand grains will adhere to the beaker and so be left behind; in contrast, silt and clay particles may require chemical treatment for removal); whether charcoal particles will be counted on the pollen slides or from a separate preparation or using other methods (see Mooney & Tinner 2011); whether testate amoebae will be counted, and if so, from pollen microslides or separately (see Booth *et al*. 2010); whether other NPPs will also be counted.

Although an esoteric method using density gradient centrifugation has been proposed for pollen purification (e.g. Forster & Flenley 1993), analysts employ conventional pollen preparation procedures such as the one detailed in Table 1, which is used routinely for peat samples in the Palaeoecology Laboratory at the University of Southampton (UK). This follows Erdtman's (1969) acetylation (using a 9:1 mix of acetic anhydride and concentrated sulphuric acid) to remove cellulose.

Table 1. Pollen preparation schedule, updated by Professor Keith Barber from the preparation protocol detailed by Barber (1976), with minor amendments and footnotes by FMC.

A: SOLUTION OF CARBONATES AND HUMIC COMPOUNDS

1. Using a clean spatula, place a small quantity of peat (*ca.*½ cm^3) of measured volume in a 15 ml or larger centrifuge tube. Add 2–3 exotic pollen tablets. If peats are suspected or known to be calcareous, add a few ml of 10% HCl; if normal acid peats, go to Stage 4 below.

2. When the reaction, if any, is complete, cap the tubes and centrifuge at 3,000 r.p.m. for 3–4 minutes, ensuring first that tubes are balanced by being filled to the same level. This applies throughout the schedule.

3. Carefully decant, i.e. pour away the liquid from the tube, retaining the residue. Do it in one smooth movement. Unless noted to the contrary, all chemicals may be decanted into the fume cupboard sink with water running continuously.

4. Add a few ml of 10% KOH to centrifuge tube, mix on vortex mixer, top up KOH to within 3 cm of top of tube and place in boiling water bath in fume cupboard for 10 minutes.

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5. Using a little distilled water, wash residue through a fine (180 micron) sieve sitting over a 400 ml beaker. Clean centrifuge tubes with distilled water and refill with contents of beaker[1]. N.B. Be especially careful to keep sieves, beakers and centrifuge tubes in correct number order.

6. Centrifuge and decant as often as necessary.

7. Add distilled water, centrifuge and decant. Repeat procedure until brown stain removed from supernatant liquid. **(Two treatments with KOH and two sievings are much more effective than increasing the time at stage 4.)**

B: HYDROFLUORIC ACID TREATMENT [if required, owing to clay or silt content]

N.B. HF burns are painful and slow to heal: read the safety notices before using! HF-resistant rubber gauntlets, face protection (visor) and an apron must be worn over your lab coat. This procedure is unnecessary for highly organic samples.

a. Add a small amount of distilled water to residue and mix thoroughly.

b. Add approximately 10 ml of 40 % HF to centrifuge tube and place in beaker of boiling water or water bath for about 30 minutes. Time required will vary greatly with quantity of silicates present; for very siliceous samples repeat steps a & b. If much silicate is present, samples can be left for 1–2 days in cold HF, and then treated to another dose of hot HF.

c. Centrifuge and carefully decant **into the labelled collector vessel, not down the sink**.

d. Add dilute HCl, mix and put in simmering water bath[2] for 3–5 minutes (This removes colloidal silica etc.); do not boil the HCl.

e. Centrifuge and decant.

f. Add distilled water, mix, centrifuge and decant. Repeat.

C: ACETYLATION

8. Add glacial acetic acid, mix, and centrifuge. Decant into fume cupboard sink with water running during and after[3].

9. Repeat.

10. Make up acetylation mixture, freshly, just before it is required. Using a measuring cylinder, mix acetic anhydride and concentrated sulphuric acid in proportions of 9:1 by volume. Measure out acetic anhydride first, using automatic pipette, then add conc. H_2SO_4 carefully, stirring to prevent heat build-up. Stir again just before use.

11. Add a few ml of the mixture to sample, mix and fill centrifuge tube about $^1/_2$ full.

12. Put in boiling water bath for no more than 1–3 minutes (stirring is unnecessary since acetylation should not be carried on for longer than 3 minutes as it destroys some pollen types. Never leave glass rods in tubes as steam condenses on them and runs down into the mixture, reacting violently).

13. Centrifuge and decant supernatant **either** into a **very large (3 litre) beaker of water** in fume cupboard; if local jurisdictions permit, the beaker may be emptied down the sink, diluting further

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with plenty of running water; **or,** if it is not permitted to flush acids down drains, then instead decant into collector vessel containing powdered calcium carbonate, to neutralize the acid, before disposal.

14. Add glacial acetic acid, mix, centrifuge and decant[3].

15. Add distilled water and 1 ml 10% KOH (and 1 drop of detergent (e.g. Teepol) if material is clumping together), mix, centrifuge and decant.

D: MOUNTING IN SILICONE FLUID

This is a longer, more refined technique than using glycerol or glycerine jelly (see Table 2) and has the important advantage of not altering pollen grain size overmuch. Also, as silicone fluid is practically non-volatile, the slides never dry out and do not need sealing.

16. Add distilled water, mix, centrifuge and decant.

17. Add 1 ml distilled water, 5 ml 100 % ethanol and 1–2 drops of safranin or other stain. Do not over-stain as the grains will be difficult to identify and the staining cannot be reversed! Mix, top up with ethanol, centrifuge and decant.

18. Add 100 % ethanol, mix, centrifuge and decant.

19. Add approximately 1 ml toluene[4] and[5] pour from centrifuge tubes into labelled glass vials. Repeat to wash all pollen residue from centrifuge tubes and balance vials carefully. Seal with polythene tops.

20. Carefully lower vials into centrifuge using forceps, and spin at no more than 750 r.p.m. for 10 minutes.

21. Carefully decant into beaker containing paper tissues in fume cupboard and leave this to evaporate for 24 hours with the fan running; post an Unattended Experiment notice in the holder provided on the outside of the laboratory door.

22. Add silicone fluid (viscosity MS 200/2,000 cs), 2–6 drops, and mix well with a small disposable rod; pollen grains may otherwise clump together. Do not add too much silicone fluid!

23. Allow excess toluene to evaporate from the vials; leave for 24 hours in fume cupboard with fan running (post Unattended Experiment running notice, as before).

24. Make up slides[6]. Fix cover slip[7] with a drop of nail varnish at each corner; no need to seal.

Notes to preparation schedule

[1]*If coarse mineral material is present in beaker, swirl contents and allow sand particles to settle out, before decanting beaker (so as to retain coarse mineral particles within beaker).*

[2]*Some practitioners instead use HCl at room temperature.*

[3]*As with stage C13, if restrictions apply on disposal of diluted acids to drains, then decant into collector vessel containing powdered calcium carbonate, to neutralise the acid, before disposal.*

[4]*Note that toluene is regarded by some as a carcinogen, and so its use may not be permitted in some laboratories. (Its carcinogenic potential is not universally accepted.) An alternative is tertiary butyl alcohol.*

[5]*If contents turn 'milky' or 'cloudy', or the material 'clumps', this means the sample was not fully dehydrated. Add a drop of 100% ethanol to clear.*

[6] *Slides usually 1.2 mm thick.*

[7] *Cover slip: size 0, 22 x 22 mm or 22 x 32 or 22 x 40 mm.*

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Faegri & Iversen (1989) detailed a somewhat different procedure. Another method, used at the University of Amsterdam for sample preparation of peat from raised bogs, and which facilitates counting of NPPs, is given in Table 2.

Practice varies between laboratories, with the 'standard' methods being modified according to local custom, the availability of specific kit and/or materials, Health and Safety considerations and national regulations, the microscopy to be used (e.g. use of phase contrast will require a stain for best results), and the mountant (e.g. silicone fluid or glycerol) chosen. There is robust debate among practitioners as to the most suitable mountant, with adherents strongly advocating their preferences; each has its merits, which are well-rehearsed in Faegri & Iversen (1989), but also its deficiencies. Note, for example, that the mountant used in the laboratory providing Table 1 is different from that specified in Table 2.

Whichever preparation method is adopted, it is desirable for pollen grain-size comparisons that the mountant should match that used for the laboratory's pollen reference collection of type slides; although, in the case of silicone fluid, laboratories have sometimes used different viscosities between typeslides and subfossil samples, perhaps 12500 cs for typeslides but usually 2000 cs for samples, to permit the subfossil pollen grains and NPPs to be turned more easily.

3. POLLEN IDENTIFICATION

3.1 Microscopy

Although Langford *et al*. (1990) held out the prospect of computerised procedures for pollen recognition, and Li & Flenley (1999) investigated automated ways of recognising surface textures, the identification of pollen from peat is invariably done using a binocular light microscope. Counting is usually at ×400 (e.g. ×40 apochromat objective and ×10 eyepieces) or, for pollen-rich slides, at ×600 (same objective, but ×15 wide-field eyepieces). Critical identifications are done using phase contrast (×40 objective) and/or using ×100 objective.

3.2 Pollen images

Published atlases of pollen grains provide photographic images (e.g. the eight volumes of the north-west European pollen flora, commencing with Punt & Clarke (1976); *Atlas of Pollen for Europe and North Africa* by Reille (1992); 120 plates in Beug (2004)) and there are web-based collections of photomicrographs, such as the Australasian pollen and spore atlas (APSA 2011) and the collated images from several laboratories around the world hosted at the University of Arizona (2011). Hooghiemstra & van Geel (1998) provide a world list of Quaternary pollen and spore atlases.

3.3 Pollen keys

For north-west Europe, keys for pollen identification are contained in successive editions of Faegri & Iversen (first edition in 1964, latest in 1989; a key included in the fourth edition was also published separately, see Faegri *et al*. 1989), in Moore *et al*. (1991; also contains photos) and in the Quaternary Research Association's guide by Andrew (1984; contains line drawings). The nomenclature adopted in these differs: Faegri & Iversen's terms for pollen structure differ from those used by Moore *et al*. (1991), who followed Erdtman's (1969) terminology; Andrew (1984) used idiosyncratic and sometimes colloquial descriptions for sculpturing patterns. Scientists working on bogs outside Europe initially had no such recourse to specific regional pollen guides, and so had to apply or adapt the methods pioneered in north-west Europe and devise their own pollen keys (e.g. McAndrews *et al*. 1973, Markgraf & D'Antoni 1978). Some of the publications listed in Hooghiemstra & van Geel (1998) also contain keys.

A pollen key is merely an aid to identification. Those parts of keys that (in effect) require successive binary (yes/no) decisions, especially over features that are difficult to distinguish (requiring a decision: observed/not observed), are prone to producing operator errors, because one 'wrong' answer is likely to lead to incorrect identification.

3.4 Pollen typeslide reference collection

For precise and accurate pollen identification there is no adequate substitute for a pollen reference collection. A reference collection can be made from fresh material collected in the field or from herbarium specimens; pollen can also be bought from suppliers of allergens, but price varies enormously between pollen types. Although some ready-made typeslides can be purchased, material for microscope slides should be prepared, stained (or not), extracted and mounted according to the preparation technique for subfossil pollen used in the laboratory, but usually with the omission of chemical stages for removing mineral material, as this may alter grain size. If microscope typeslides need to be sealed to prevent escape of mountant onto microscope stages and lenses, then 'knotting' (used to seal wood knots) has been found suitable for semi-permanent slides. Three Reference Collections of typeslides produced at Queen's University Belfast, sealed in this fashion and with

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Table 2. Pollen and NPP preparation method, based on a procedure used at the University of Amsterdam.

1. First, cut peat slices of 1 cm thickness, and then, using a sharp 'cork corer' (see right), cut the required cylindrical sample (volume: $\pi r^2 \times 1$). Boil the peat sample in 10 % KOH.

2. Sieve (using mesh size of 215 µm); pour into centrifuge tubes in which *Lycopodium* tablet(s) is/are already dissolved in 10 % HCl. Add distilled water and centrifuge up to a speed of at least 4500 r.p.m. Do not decant the tubes, but take most of the water out of them using a water pump, so that no microfossils will be lost. N.B: sieve very carefully, so that no microfossils are left on the sieve. Burn the (metal) sieves before sieving, so that any microfossils from earlier samples will be destroyed.

3. Wash the material with water and centrifuge until the supernatant is clear (washing two times, but sometimes more than twice is necessary).

Example of a cork corer, which can be used to sample volumetrically from peat (photo courtesy of Carl Roth GmbH + Co. KG.

N.B. The following steps (4–7) are undertaken in the fume cupboard.

4. Dehydrate with 96% acetic acid and centrifuge.

5. Prepare an acetylation mixture by slowly adding 1 part H_2SO_4 to 9 parts acetic anhydride (when making up, stirring the mixture and cooling in a water bath is essential; ensure no contact between acetylation mixture and water; safety glasses and gloves required). Add a small quantity of the mixture to each sample tube.

6. Acetylate the material in the acetylation mixture by heating the tubes to 100 °C for approximately 10 minutes in a water bath.

7. Cool the sample tubes in a water bath and then centrifuge and decant supernatant into collector vessel.

8. Wash residue with distilled water and centrifuge until the supernatant is clear (wash at least twice).

9. Wash residue with 96 % alcohol and centrifuge twice.

10. Decant and wash the sample into a residue tube, using 96 % alcohol. Centrifuge (up to 4500 r.p.m.) the residue tubes. Decant and add drop/drops of glycerine to the residue (dependent upon the residue size).

11. Put the residue tube in an oven at 40 °C for a night.

12. Prepare microscope slides.

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silicone fluid as mountant, were free of blemishes for some 25 years, but have since been found to contain crystalline structures within the mounts that make the typeslides unusable. However, typeslides sealed with varnish deteriorated much earlier.

As bogs tend to be species-poor, it does not require many pollen typeslides to make a rudimentary pollen reference collection of bog taxa. Fens may be species-rich, and so require a more extensive typeslide collection. However, the pollen found as subfossils in peat will not be restricted to mire species; a comprehensive pollen reference collection requires at least several hundred or, in some species-rich regions, potentially thousands of typeslides.

3.5 Precision and accuracy in identification of pollen

While it is desirable to aim for taxonomic precision (i.e. exactness: to the lowest possible taxonomic level) in pollen identification, it is particularly important to be accurate (i.e. correct), because incorrect pollen identifications are potentially far more misleading than those that are imprecise (see Birks 1993). For example, it is incumbent on the analyst in north-west Europe to be honest about the confidence that can be placed in separating *Myrica* (bog myrtle, which grows on some types of bog) from *Corylus* (hazel); and to be aware not just how much taxonomic precision *might* be possible within the Ericales, but how accurate *are* the resulting separate identifications.

4. IDENTIFICATION OF NON-POLLEN PALYNOMORPHS

Traditionally, alongside pollen, spores of a limited range of lower plants have also been recorded by pollen analysts: typically, these include a range of identifiable fern spores (plus those unidentifiable to genus or species, which are often labelled 'Filicales' in pollen diagrams), spores of bog moss (*Sphagnum*) and of various clubmosses (e.g. *Selaginella*, *Lycopodium*, *Huperzia*). From the mid-1970s, some peat-bog pollen analysts also recorded a few taxa within rhizopoda (testate amoebae); use of these has since burgeoned in helping to reconstruct past bog surface wetness (see Booth *et al*. 2010 for details and references). However, there are many other non-pollen microfossils (NPMs; also called non-pollen palynomorphs: NPPs) present on microscope slides prepared for pollen analysis from peats. Among the 'additional' microfossils in raised bog peat are spores of fungi, together with remains of algae and invertebrates.

Descriptions and illustrations of many non-pollen palynomorphs (NPPs, or 'Types') have been published in a series of journal papers in which their indicator value was discussed (van Geel 1978, 1986, 2001; van Geel *et al*. 1995, 2003; van Geel & Aptroot 2006, van Geel & Grenfell 1996, Yeloff *et al*. 2007). Morphological descriptions and pictures were combined with stratigraphic information, often in the form of pollen and macrofossil diagrams. Several hundred Types have now been distinguished, each one with a Type-number. In most cases there was initially no, or hardly any taxonomic/ecological knowledge about the distinguished Types. The identification of the fossils was attempted with the aid of literature and by consulting colleagues in invertebrate zoology, phycology and mycology. Among the NPPs there still are many taxa that are not properly identified (see Wiltshire & Hawksworth, in press, for commentary), but some of them nevertheless can be used as palaeoenvironmental indicators. In such cases, the ecological information was inferred from the co-occurrence (curve matching) with identified taxa (usually of pollen).

For NPPs in raised bog peat the lengthy publication by van Geel (1978) is relevant. For bog studies, the analysis of a selection of NPPs with a clear indicator value is recommended (see below). A selection of NPPs is described and illustrated here. The combination of microfossil analysis with the study of macroremains (Mauquoy & van Geel 2007, Mauquoy *et al*. 2010) and testate amoebae (Booth *et al*. 2010) is recommended. For the application of NPPs in palaeoecological studies, see van Geel (2006 and references therein) and a special issue of *Vegetation History and Archaeobotany* (2010, edited by J.N. Haas). Ideally, analysis of pollen, NPPs, testate amoebae and plant macrofossils are all combined (see van der Linden & van Geel 2006, van der Linden *et al*. 2008a, b, c).

5. POLLEN COUNTING OF PREPARED PEAT SAMPLES

Owing to the non-random distribution of pollen and spores on prepared microscope slides (see Brooke & Thomas 1967), pollen counting should be based on vertical traverses spread across the slide, but avoiding the edges where larger macrofossils tend to be significantly under-represented. Counting of microfossils should normally be continued until at least 400 pollen grains of taxa included in the pollen sum are recorded. Pollen-sum taxa include trees, shrubs and many non-arboreal taxa. Aquatic plants, local mire plants (like Cyperaceae and Ericales) and

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spores are often excluded from the pollen sum, but their percentages will be expressed in relation to the pollen sum. The problem lies in deciding which mire taxa to exclude and which may be considered obligate mire (or exclusively bog) species. For example, Cyperaceae may grow beyond the mire and contribute to the pollen rain; in contrast, some species of Poaceae (a taxon that is not usually excluded) grow on bogs. Practice varies between laboratories and analysts; some analysts prefer to use a total land pollen (TLP) sum, even though this may be dominated in some horizons by local bog heathers, grasses or sedges, in which case a pollen sum of at least 500 is recommended.

Some spores (like Types 10 and 12, and *Sphagnum*) can be so frequent that counting them until the pollen sum is reached is too time-consuming. Therefore, counting of these highly frequently occurring spores can be stopped when, say, 100 pollen-sum taxa (or a certain number of the added exotic spores/pollen) have been recorded. Before entering these data in a spreadsheet, the number of spores should be 'extrapolated', so that (rough) percentages can be calculated.

6. PLOTTING OF POLLEN DIAGRAMS

Pollen and NPP data are usually presented in pollen diagrams, rather than in Tables. Pollen diagrams take many formats. The practice in several leading continental European laboratories was to display the major tree taxa using different symbols in an overlay plot; this helped to save space. Other laboratories separated out the 'curves', which might be plotted as continuous lines or as silhouettes with a solid, hollow, pattern or depth-bar fill style, or as histograms. The diagram was usually hand-drawn once only, in the laboratory house style. In recent decades, graph-plotting computer software has permitted diagrams to be produced from electronic spreadsheets. Although this might have resulted in many different types of plots, paradoxically pollen diagrams now are more similar in format, largely as a result of widespread adoption of what was originally bespoke pollen-plotting software: indeed, the spreadsheet software TILIA and plotting software TILIA*GRAPH (Grimm 1990, note that new software editions are available from 2011) have become standard. Other dedicated software includes PSIMPOLL (Bennett, 1995–2007).

The purpose of the pollen diagram is to present the data in an interpretable form. It helps if the reader can easily make sense of the data presented, and that task is made easier if the diagram follows established conventions. There are some common, but by no means universal, practices. For example, in British and some European Holocene pollen diagrams, major tree taxa follow a conventional order, thought to correspond with their spread following the Last Glacial Maximum (pleniglacial): usually *Betula, Pinus, Ulmus, Quercus, Tilia, Alnus*, etc. Other regions of the world have their own conventions. Although it is not mandatory to follow the usual sequence, analysts should explain for the benefit of the viewer, either in the methods or figure caption, the use of any markedly different schema. In species-rich regions, or for diagrams from valley mires, it may be desirable or even necessary to split the pollen diagram into two plots, with one that contains the non-mire non-arboreal pollen, and another that contains the arboreal (tree and large shrub) pollen and the mire taxa and spores (see Figure 1 for an example).

If exotic pollen or spores have been added to a volumetrically determined sample, then a pollen concentration diagram can also be prepared. Note, however, that the step from a pollen concentration diagram (number of pollen per cm^3 of peat) to a pollen influx diagram (with pollen accumulation rates [PAR] determined as the number of deposited pollen grains per unit surface area per year) requires a large number of radiocarbon dates, and sufficient confidence in reliable determination of the peat accumulation rates.

7. INTERPRETATION OF POLLEN DATA FROM PEATS

7.1 Over-representation of mire taxa

Pollen analysts must confront the question of what exactly is being recorded in the peat pollen and spore record (cf. Räsänen *et al*. 2004). Interpretation of pollen diagrams from peats is potentially problematic, owing to the likely dominance of local mire taxa in pollen and spore spectra. This is not a major problem in wholly *Sphagnum*-dominated mires, because *Sphagnum* spores will be outside the pollen sum; however, problems can arise with non-bryophyte mire taxa. To counter this, many pollen analysts working on raised bogs use a pollen sum of non-mire taxa, but this practice is not universally applied nor is it easily applicable elsewhere. It raises the question in other environments as to which pollen taxa represent plants that grew on the mire surface. For example, in drier episodes in north-west Europe, ling (*Calluna vulgaris*) may have been common or locally dominant on bogs and so, if not excluded from the pollen sum, *Calluna* pollen may periodically be over-represented in the pollen spectra; whereas, in the lower parts of valley mires,

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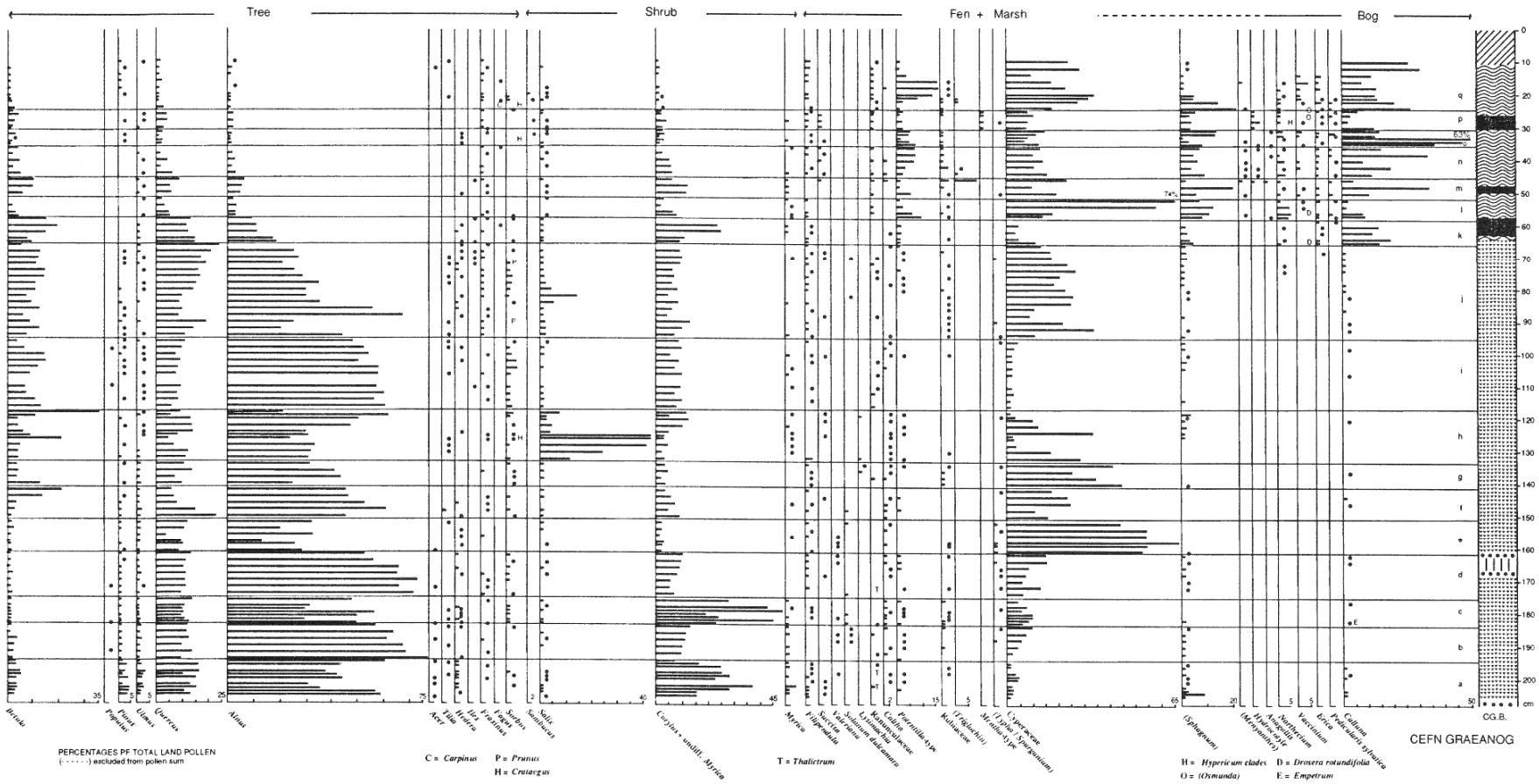

Figure 1. A hand-drawn plot of half of a taxon-rich pollen diagram from a valley mire, North Wales. The contrast between the lower, wood peat and upper, valley mire peat is accentuated by plotting pollen and spore taxa assumed to be from wetland species towards the right-hand side. In this example a total land pollen (TLP) sum was used. Note the traditional, postglacial-spread sequence in the plotting of tree taxa, but with additional tree taxa inserted. The other non-arboreal taxa were plotted in a separate diagram (from Chambers 1998).

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alder (*Alnus glutinosa*) may contribute to wood peat, and so *Alnus* pollen (from alder carr) may be particularly abundant. Neither *Calluna* nor *Alnus* can be considered exclusive to mires; indeed, apart from its presence in alder carr, *Alnus* would normally be considered a forest or riparian tree; as would *Pinus*, which grows on bogs in north-west continental Europe, but would not be excluded from the pollen sum. Poaceae will grow in fens and may be particularly abundant on some degraded bogs (see Chambers *et al*. 2007), but their pollen is not usually excluded; in contrast, Cyperaceae—and in Southern Hemisphere non-*Sphagnum* mires, Restionaceae and Epacridaceae—may well be. The decision rests with the analyst, but the pollen sum must be made clear for the viewer and reader.

7.2 Spurious rises in arboreal pollen

Different mire taxa do not produce the same quantity of pollen; consequently, if they are *not* excluded from the pollen sum, then in relative pollen diagrams, owing to the periodic dominance of particular mire taxa (e.g. *Calluna*, Cyperaceae; but at other times *Sphagnum*, which is already excluded from the pollen sum), spurious rises and falls in arboreal pollen may result. It is likely that pollen concentration diagrams will display synchronous fluctuations of taxa in the same direction as a result of changes in peat accumulation rate. The problem might be overcome if a reliable pollen influx diagram could be constructed; however, it is likely that this would require a large number of wiggle-matched radiocarbon dates.

In surface horizons of heavily grazed degraded mires in the UK, an 'increase' in native arboreal pollen (AP) taxa may occur towards the surface, probably owing to suppression of the flowering of local graminaceous (including Cyperaceae) species; the data imply afforestation, but that may well be spurious. A record of grazing-related NPPs, such as spores of coprophilous fungi (van Geel & Aptroot 2006), can help interrogate and check this apparent rise in AP.

7.3 Simulation methods for quantitative landscape-scale vegetation reconstruction

In recent decades, simulation methods have been developed and applied to assist in interpreting pollen data, although some of the models developed may be better suited to lakes in relatively open flat terrain. Nevertheless, Bunting (2008) shows how simulations of pollen dispersal and deposition can better interpret the pollen signal in wetlands. For an overview, further details and references on simulation methods, see Gaillard *et al*. (2008).

8. INDICATOR POTENTIAL OF SOME NPPs FOUND IN PEAT

Many different fungal 'Types' occur in peat deposits, especially in peat layers that were formed under relatively dry conditions. In peat formed under very wet conditions, fungal remains occur rarely. From the various studies of fossil fungal spores, it became clear that the recorded spores in most cases are of strictly local occurrence. They were fossilised at or near the place where they had been produced, or the spores were deposited only a short distance from the place where sporulation took place. A relevant selection is given here of fungi and algae commonly found in peat from raised bogs (Figure 2).

- *Gelasinospora* spores (Types 1 and 2) indicate relatively dry conditions. Spores are ellipsoidal, 22–30(–37) × 14–20(–24) µm. Spore surface almost black, evenly ornamented with approximately 1 µm wide round or ellipsoidal hyaline pits.
- Type 10 is an indicator for relatively dry conditions. It occurs on the roots of *Calluna vulgaris*. Spores transversely (1–)2–3(–6) septate, (10–)20–30(–50) µm long and (7–)9–10(–12) µm wide, formed at the ends of septate, pigmented, (1–)1.5(–2) µm wide hyphae; basal cells paler (thinner-walled) than the others; not constricted at the septa or hardly so. In each septum a pore approximately 0.3 µm wide.
- Type 12 is also a dryness indicator. It grows on a variety of bog plants. Spores curved, (7–)10–13(–14) µm long, with two transverse septa, constricted at the septa. Apical cell (4–)6–7(–8) µm wide, thick-walled, dark brown, with a sub-apical germ pore about 0.7 µm wide. Central cell brown; basal cell hyaline and thin-walled.
- Spores of *Meliola ellisii* (Type 14). The fungus is a parasite on *Calluna vulgaris*. Spores 41–55 × 14–18 µm, three-septate, inequilateral (one side almost straight), slightly constricted at the septa. Septa thickened around the approximately 1.5 µm wide pore.
- Spores of *Neurospora* (Type 55C) indicate local bog fires. Spores ellipsoidal, non-septate, brown, 22–28 × 15–18 µm, with two protruding apical pores, about 1 µm wide; with about 16 fine, longitudinal grooves.

Some algal spores:

The hyaline spores of *Mougeotia*, *Spirogyra* and *Zygnema*-type are produced by filamentous green algae inhabiting shallow, stagnant, oxygen-rich freshwater pools. Spores of *Mougeotia* are square, laterally straight to concave; retuse angles often with depressions. Surface smooth or with small pits.

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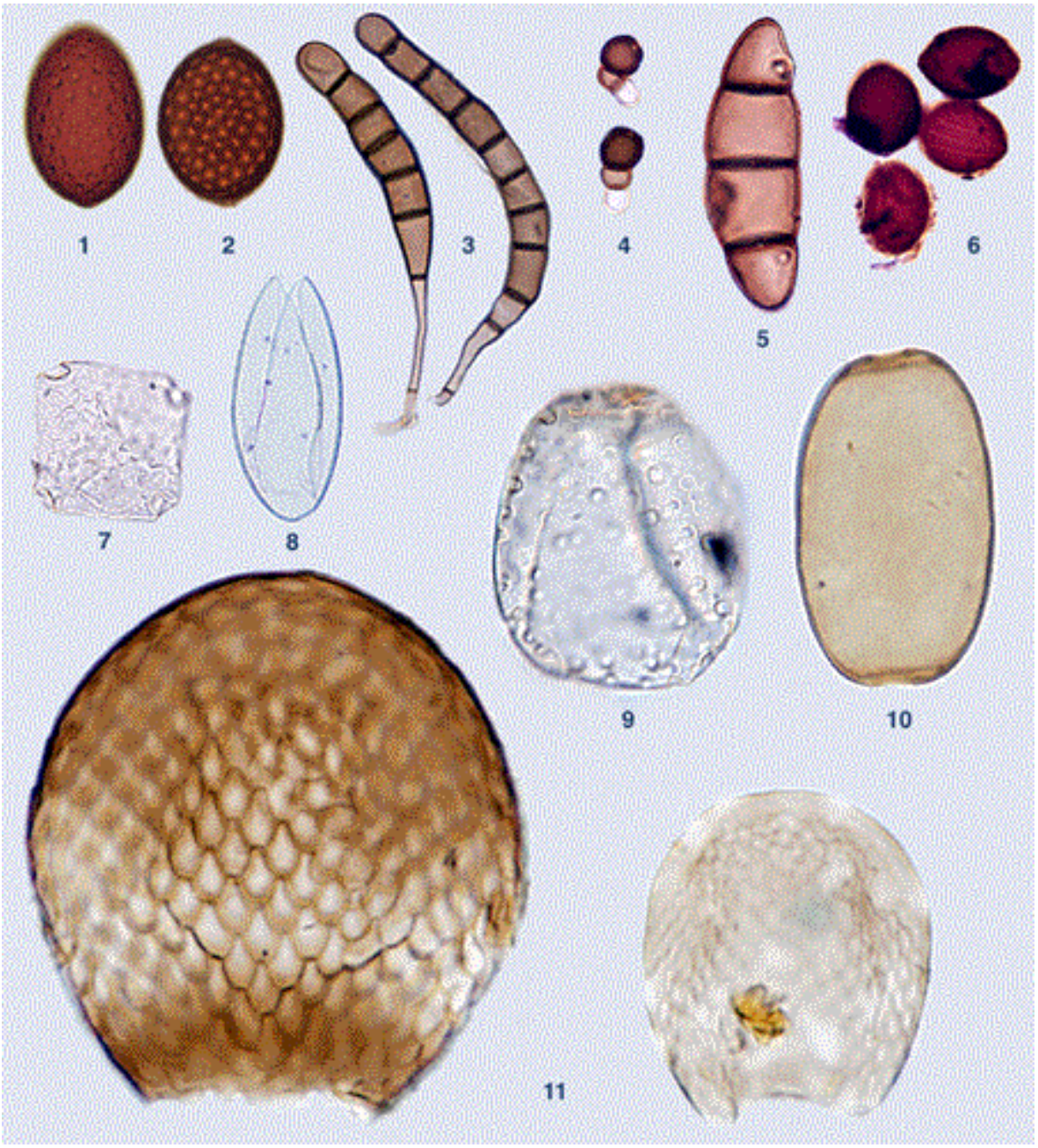

Figure 2. Selection of non-pollen palynomorphs from raised bog peat. 1: *Gelasinospora* sp. (ascospore; Type 1). 2: *Gelasinospora* cf. *reticulispora* (ascospore; Type 2). 3: Spore Type 10. 4: Spore Type 12. 5: *Meliola ellisii* (ascospore; Type 14). 6: *Neurospora* sp. (ascospores; Type 55C). 7: *Mougeotia* (zygospore). 8: *Spirogyra* (zygospore or aplanospore). 9: *Zygnema*-type (zygospore or aplanospore). 10: *Amphitrema flavum*. 11: *Assulina* sp.

Spores of *Spirogyra* are ellipsoidal. Walls are smooth or reticulate; spores show a longitudinal furrow which often encircles almost the whole spore. Spores of *Zygnema*-type are spheroidal and flattened, with pits all over the surface.

9. CONCLUSIONS

Pollen analysis is a powerful and widely applied technique in peatland palaeoecology. In acid bogs, pollen usually preserves very well and is easily

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identifiable. Pollen preparation, identification, counting and plotting methods are now well established. Some recent research has focused on simulation methods for quantitative landscape-scale vegetation reconstruction using pollen data. However, augmentation of pollen data from bogs through the counting of NPPs can assist in palaeoecological interpretation, especially for studies of palaeoclimate and of grazing activity.

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