Chapter 11
Fossil Pollen and Spores in Paleoecology

Luke Mander and Surangi W. Punyasena

Abstract The discipline of paleoecology is a multidisciplinary field that uses geological and biological evidence to investigate the past occurrence, distribution and abundance of species and populations on timescales ranging from hundreds to hundreds of millions of years. In this way, paleoecology is broadly concerned with the ecology of the past. In this article, we discuss how paleoecological data are derived from assemblages of fossil pollen and spores, which are dispersed by plants as part of their life cycles, and how this material can be used to reconstruct paleoenvironments. We outline how pollen and spores can be analyzed and classified, and explore the potential strengths and weaknesses of paleoecological data, before considering technological and methodological developments that may play a role in the future development of this discipline.

Keywords Vegetation history • Plants • Morphology Taxonomy • Microscopy • Climate

Background and Overview

The discipline of paleoecology is concerned with the ecology of the past. It uses geological and biological evidence to investigate the past occurrence, distribution and abundance of species and populations on various timescales (Birks and Birks 1980; Seddon 2012). Paleoecology is a multidisciplinary field, and researchers working in this discipline gather data from a variety of geological and biological sources in order to investigate ancient ecology. This includes paleontological data that provide physical evidence of the organisms that were living in the past, sedimentological data that speak to the environments in which these organisms lived and were fossilized, and geochemical data that provide a window into ancient climates and can track the passing of time.

In this review, we are concerned with the paleoecology of ancient plant life as reconstructed using fossil pollen and spores. Pollen and spores are dispersed by plants as part of their life cycle. They are abundant and well preserved in the fossil record, and provide extensive, continuous records of ecological and environmental change. Paleoecologists who undertake this research aim to reconstruct the diversity, composition and abundance of past vegetation. Paleoecologists also use plant macrofossils, including vegetative structures such as leaves and their fragments, and rarer reproductive structures, such as cones. Although these larger fossils are not as abundant in the geologic sediments, when available, they provide detailed taxonomic, structural, and physiological data that may not be available with pollen and spores alone. Macrofossils, therefore, serve a counterpoint to pollen and spore records of Earth's past vegetation. More recent advances in metagenomic analyses of ancient DNA provide similarly detailed taxonomic occurrence data that cannot as of yet be replicated with pollen (Pedersen et al. 2016).

Pollen and spores are extracted primarily from sedimentary deposits. These samples are often unconsolidated sediments that have accumulated at the bottom of lakes, but researchers also examine sediment from peat bogs and sedimentary rock outcrops. Pollen is preserved in a wide range of environments. Non-sedimentary sources of recent pollen material include pollen trapped within moss polsters, packrat middens, ice cores, and within permafrost. In order to extract pollen and spores from these samples, they must be macerated in the laboratory, and this involves using

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chemicals to dissolve minerals and excess organic material. The taxonomic identification of these pollen and spores involves visual analysis by a human expert using a microscope. Pollen and spores are classified by using specialist terminology to describe the morphological features that characterize each specimen, and each specimen is compared to formal taxonomic descriptions in monographic works, as well as reference material (pollen and spores extracted from the reproductive structures of taxonomically identified plants collected in the field or herbarium).

Paleoecological data can be derived from any period in Earth history that contains fossil pollen and spores. This stretches from the first assemblages of spores produced by land plants in the Ordovician/Darriwillian (Strother et al. 2015) to the present day. Although paleoecological analyses are undertaken on assemblages of pollen and spores throughout Earth history (e.g., Traverse 2007), the discipline of paleoecology has centered on analyses of material from the Quaternary period ($\sim$2.6 Ma–present). Similarly, paleoecological analyses have been undertaken on deposits all over the world, from the tropics to the poles, but there has been a historical focus on North America and northwest Europe, and this is reflected to a degree in our review.

Foundational Work and Current Themes

The use of pollen and spores to reconstruct vegetation history, as it is practiced today, dates back to von Post (1916). He showed that pollen in samples of peat could pinpoint the position of modern vegetation zones, such as the southern spruce border in Sweden, and demonstrated that fossil pollen grains could be used to reconstruct their position through time (von Post 1916). The subsequent work of Potonie (1934), Wodehouse (1935) and Erdtman (1952) made attempts to erect a formal system of nomenclature for describing the morphology of pollen and spores, and the legacy of this work echoes in recent syntheses of descriptive terminology that aim to ensure paleoecologists use the same terms to describe the same morphological features (Punt et al. 2007). During this phase of the development of paleoecology, techniques for examining critical features of pollen and spore morphology were developed, such as the Lux-Obscuritas method of analyzing patterns of the organization of pollen and spore walls (Erdtman 1952). The widespread adoption of electron microscopy by paleoecologists in the latter part of the mid-20th century allowed finer morphological details of pollen and spores to be investigated, and this facilitated an increase in the taxonomic precision of pollen and spore records of Earth's vegetation (Fægri et al. 1989). Textbooks outlining paleoecological methods, including instructions on how to sample different environments, how to extract pollen and spores in the laboratory, as well as how to describe them and relate the composition of assemblages of pollen and spores found in sediments to the source vegetation that produced them, began to appear with the publication of Erdtman's *An Introduction to Pollen Analysis* (Erdtman 1943). Today there is a wide selection of such texts available that exhaustively document the discipline of pollen and spore-based paleoecology (e.g., Birks and Birks 1980; Fægri et al. 1989; Traverse 2007).

Many current methods in paleoecology are almost identical to those outlined in early texts. These include many of the laboratory methods used to extract fossil pollen and spores from sediments and reference pollen and spore material from flowers and other reproductive structures. Additionally, the practice of a human analyst classifying pollen and spores into taxonomic groups using morphological features observed through a microscope has remained largely unchanged for the last century. The results of these visual classifications – "pollen counts" – are often summarized in "pollen diagrams" (visual representations of pollen taxon abundances as consecutive vertical line graphs) that are broadly similar to the diagram presented by von Post (1916). Today pollen diagrams are constructed using software such as Tilia (Grimm 1991–2001), C2 (Juggins 2007), and the R-package rioja (Juggins 2015), which allows for the statistical analysis of count data as well as visual interpretation. Over the last forty years, numerical approaches to pollen analysis, advocated by Davis (1963) and Gordon and Birks (1972), have evolved into increasingly sophisticated computational models to quantitatively reconstruct past vegetation cover and paleoenvironments (e.g., Jackson and Williams 2004; Dawson et al. 2016).

Paleoecological data today are used in a wide variety of settings. In addition to the reconstruction of vegetation and climate history, which is a traditional mainstay of paleoecological research, pollen data finds application in comparing the output of Earth system and land surface models to empirical data (e.g., Cowling et al. 2008) and in the identification of ecological baselines that are useful in conservation biology (e.g., Gosling et al. 2009). Pollen data are also used in the provision of data to test phylogenetic hypotheses generated by molecular work such as the use of fossil pollen to constrain the evolutionary origins of plant taxa (e.g., Barreda et al. 2015).

From Plant to Sediment: The Biology, Dispersal and Preservation of Pollen and Spores

The Raw Materials: What Are Pollen and Spores?

Pollen and spores represent a discrete phase in the plant life cycle, which is characterized by a sequence of reproduction known as an alternation of generations (Jarzen and Nichols 1996). This sequence typically alternates between a sporophyte generation carrying a double set of chromosomes (diploid; 2-*n*) and a gametophyte generation carrying a single set of chromosomes (haploid; *n*). There is an evolutionary trajectory towards the elaboration of the sporophyte generation and the reduction of the gametophyte generation (Willis and McElwain 2002). This can be observed in the anatomy of fossil plants, and also in comparative studies of the reproductive characters of primitive plants. In the following sections, we outline the life cycles of major plant groups, and highlight when pollen and spores are produced in these life cycles. This outline of plant life cycles represents a synthesis of information from Playford and Dettmann (1996), Willis and McElwain (2002), Raven et al. (2005), Rudall and Bateman (2007) and Traverse (2007).

Homosporous plants: Extant members of this plant group include non-vascular embryophytes such as liverworts, hornworts and mosses. In these primitive non-vascular plants, the haploid (*n*) gametophyte is generally emphasized while the diploid (2*n*) sporophyte is reduced in size and remains attached to the gametophyte, on which it is dependent for nutrition (Fig. 11.1). More complex extant homosporous plants include most ferns and some lycopods, and in these vascular plants the diploid sporophyte is generally emphasized while the haploid gameophyte is separate and reduced in size (Fig. 11.2).

The generalized life cycle of a homosporous plant is as follows. The haploid gametophyte bears an antheridium and an archegonium. The antheridium produces a male haploid gamete by mitosis and the archegonium produces a female haploid gamete by mitosis. These two gametes fuse when the male gamete swims to the archegonium in a film of water and encounters the female gamete. This results in the production of a diploid zygote. Germination of the zygote results in the production of a diploid sporophyte. Upon reaching maturity, the sporophyte undergoes meiotic division resulting in the production of haploid spores. In primitive non-vascular plants such as liverworts, hornworts and mosses, spores are produced in a capsule (Fig. 11.1). In more advanced homosporous vascular plants, such as certain ferns and some lycopods, spores are produced in sporangia (Fig. 11.2). These spores are released and, upon germination, grow into haploid gametophytes.

Heterosporous plants: Extant members of this plant group include the vascular embryophytes, including some ferns such as *Azolla* and *Marselia*, and in these plants the diploid sporophyte is emphasized. In these plants the diploid sporophyte bears a microsporophyll with a microsporangium, and a megasporophyll with a megasporangium. Meiotic division within the microsporangium results in the production of haploid microspores (Fig. 11.3), which germinate to produce a male microgametophyte with an antheridium. Meiotic division within the megasporangium results in the production of haploid megaspores (Fig. 11.3),

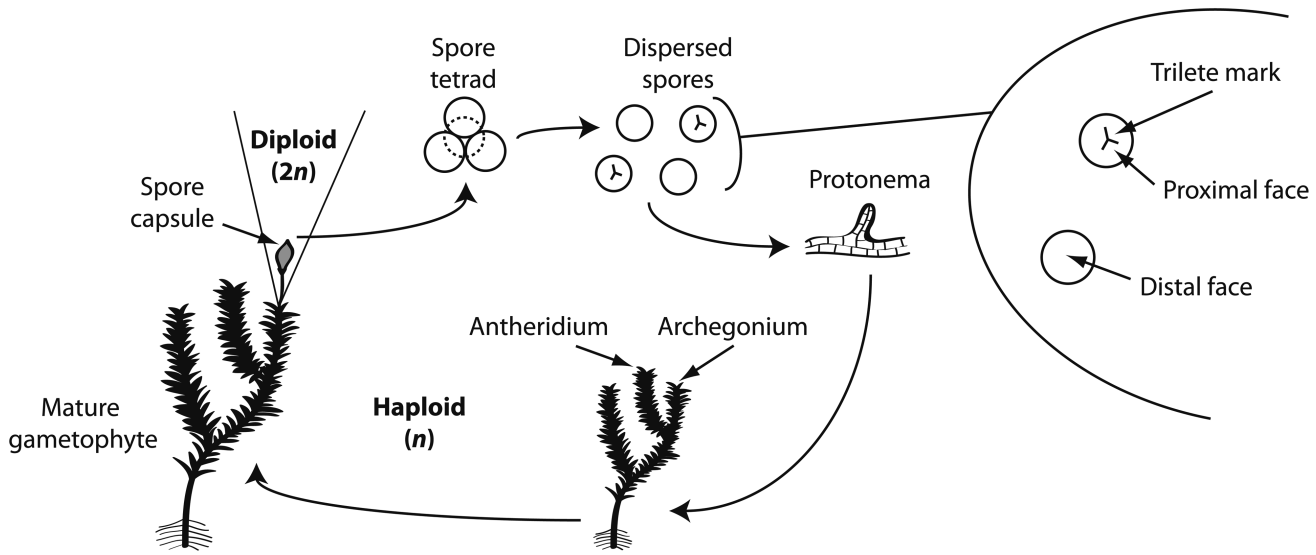

Fig. 11.1 Schematic life cycle of a non-vascular homosporous plant such as a moss in which the haploid gametophyte is emphasized and a diploid spore capsule remains attached to the gametophyte. Not to scale. Based on Playford and Dettmann (1996)

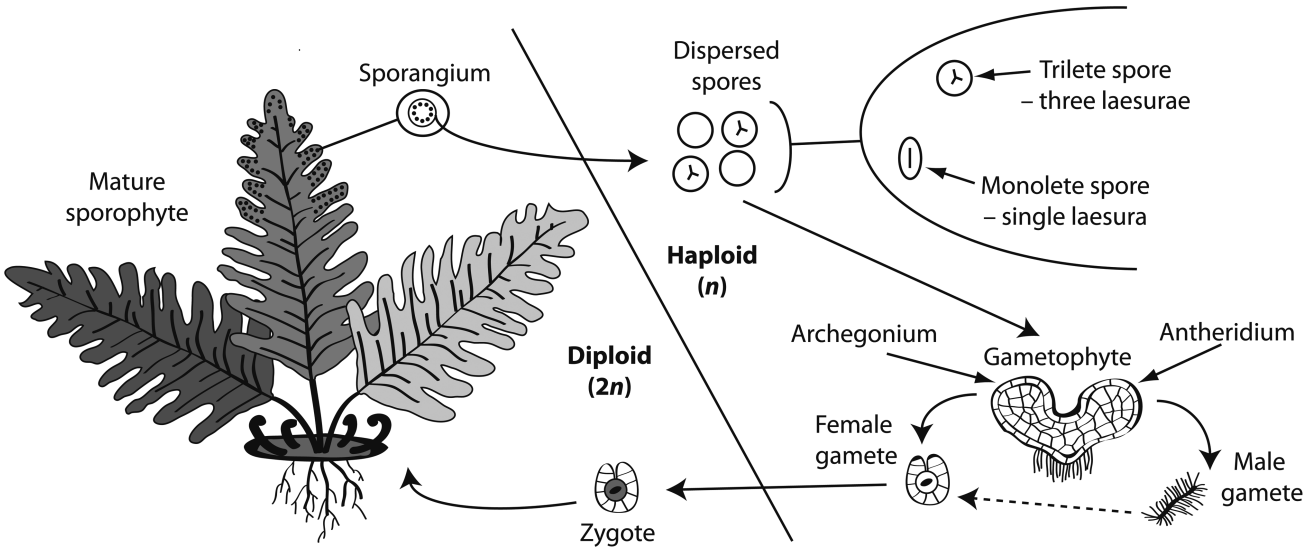

Fig. 11.2 Schematic life cycle of a homosporous fern in which the diploid sporophyte is emphasized and the haploid gametophyte is separate and reduced. Not to scale. Based on Playford and Dettmann (1996)

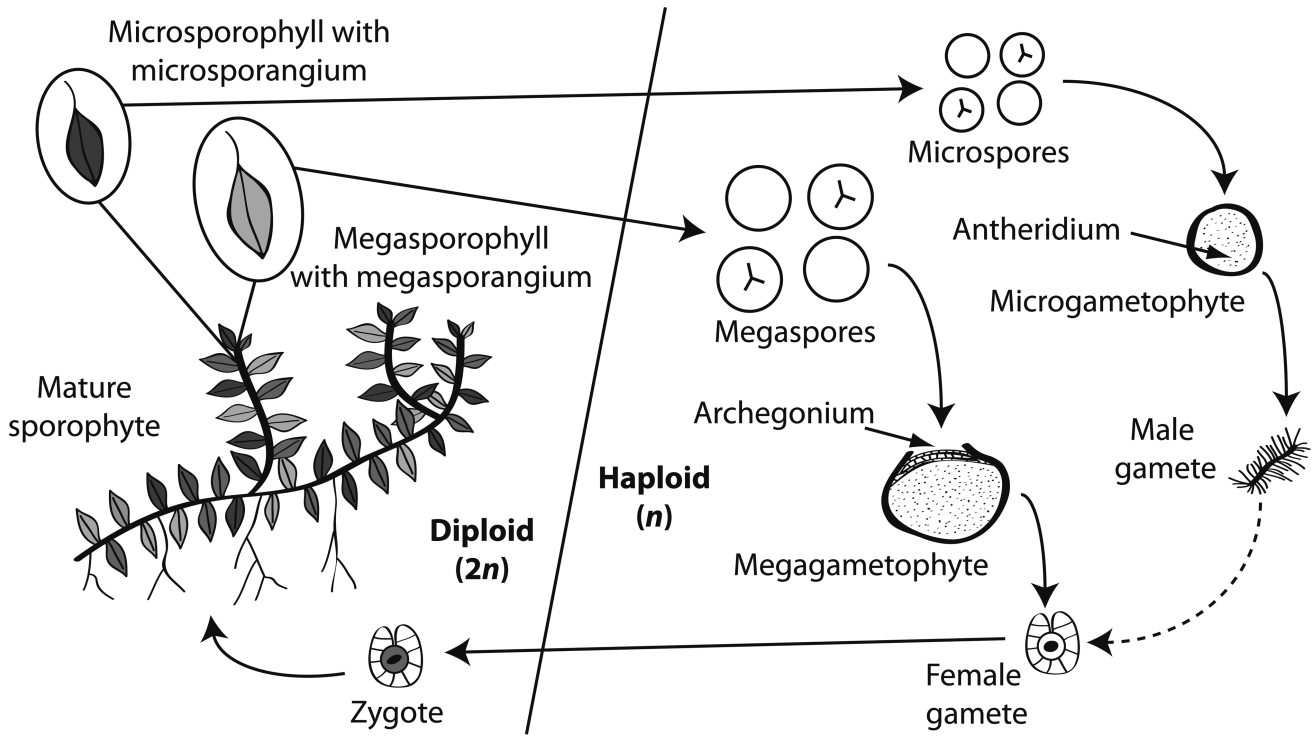

Fig. 11.3 Schematic life cycle of a heterosporous plant, in this case a lycopod, in which the diploid sporophyte is emphasized. Paleoecologists use both microspores and megaspores to reconstruct ancient vegetation. Not to scale. Based on Playford and Dettmann (1996)

which germinate to produce a female megagametophyte with an archegonium. Paleoecologists use both microspores and megaspores to reconstruct vegetation history. The antheridium of the microgametophyte produces a male haploid gamete by mitosis and the archegonium of the megagametophyte produces a female haploid gamete by mitosis. These two gametes fuse when the male gamete swims to the female gamete in a film of water. This results in the production of a diploid embryo. Germination of the embryo results in the production of a diploid sporophyte.

Gymnosperms: Extant seed plants form a monophyletic group with five major clades: cycads, ginkgos, conifers,

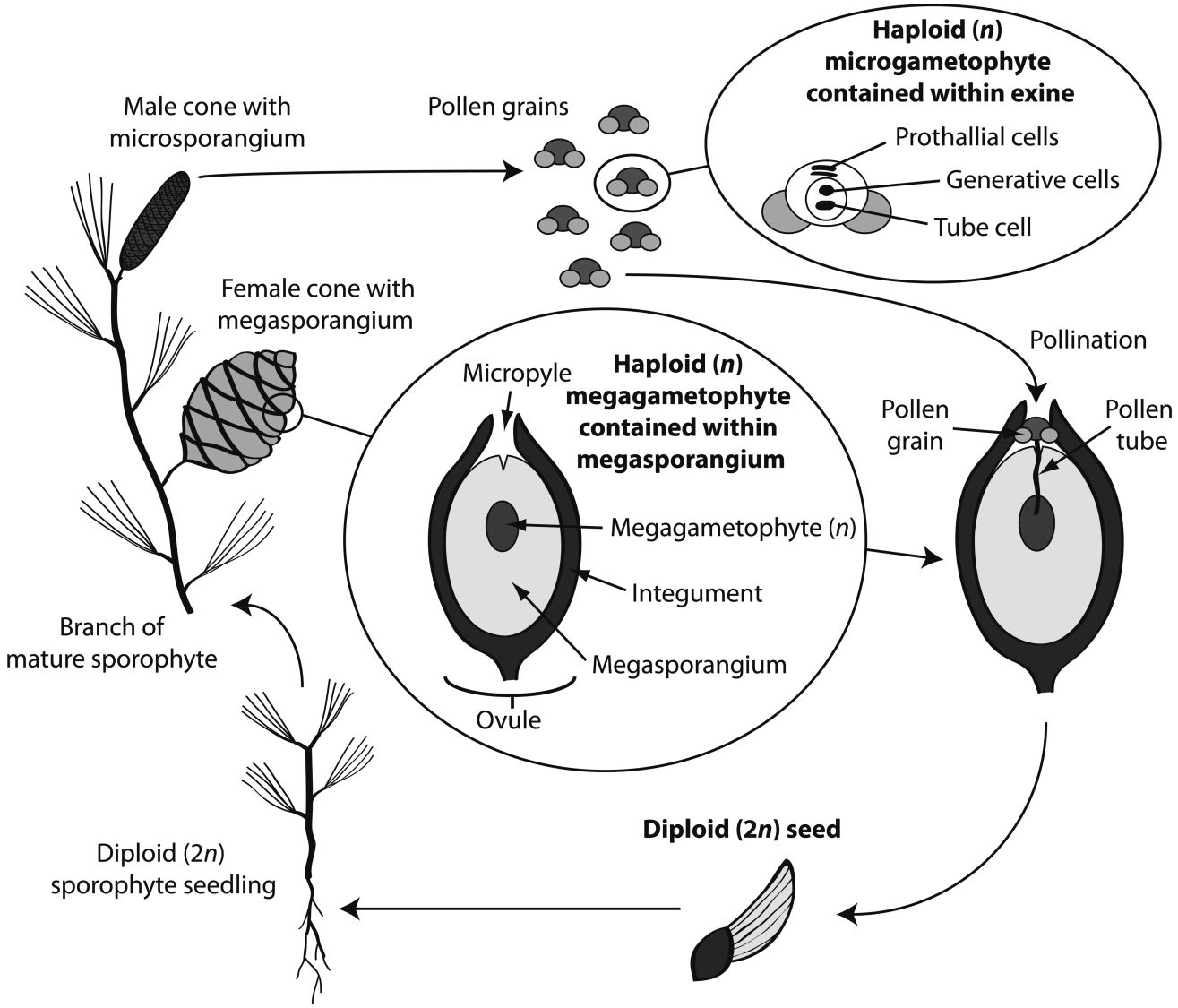

Fig. 11.4 Schematic life cycle of a gymnosperm, in this case a pine tree, in which the diploid sporophyte is emphasized. Not to scale. Based on Raven et al. (2005)

gnetophytes and angiosperms. The first four of these are called gymnosperms and the seeds of these plants are not enclosed in a carpel as they are in angiosperms. In these plants the diploid sporophyte is emphasized, and their generalized life cycle is as follows (Fig. 11.4). The diploid sporophyte bears a male cone with a microsporangium (a pollen sac), and a female cone with a megasporangium. In most gymnosperms male and female cones are borne on the same sporophyte, but in others such as *Ginkgo*, male and female cones are produced by different sporophytes and do not occur on the same sporophyte. Meiotic division within the microsporangium results in the production of haploid microspores, which comprise a microgametophyte contained in a microspore coating, the exine. These microspores are known as pollen grains (Fig. 11.4). The microgametophyte consists of two prothallial cells, a relatively small generative cell with two sperm and in some groups, such as conifers and gnetophytes, there is also a vegetative tube cell that directs the growth of a pollen tube. The megasporangium is contained within an integument and remains attached to the parent sporophyte as an ovule. Meiotic division within the megasporangium results in the production of a haploid megagametophyte with an egg cell.

Upon reaching maturity, the pollen grains are dispersed and alight near the opening of the megasporangium. Pollen is carried through an opening in the integument of the ovule (the micropyle) in a liquid drop and into the vicinity of the megagametophyte. In some groups, such as conifers and gnetophytes, a pollen tube introduces non-motile fertilizing spermatozoids into the vicinity of the egg (siphonogamous

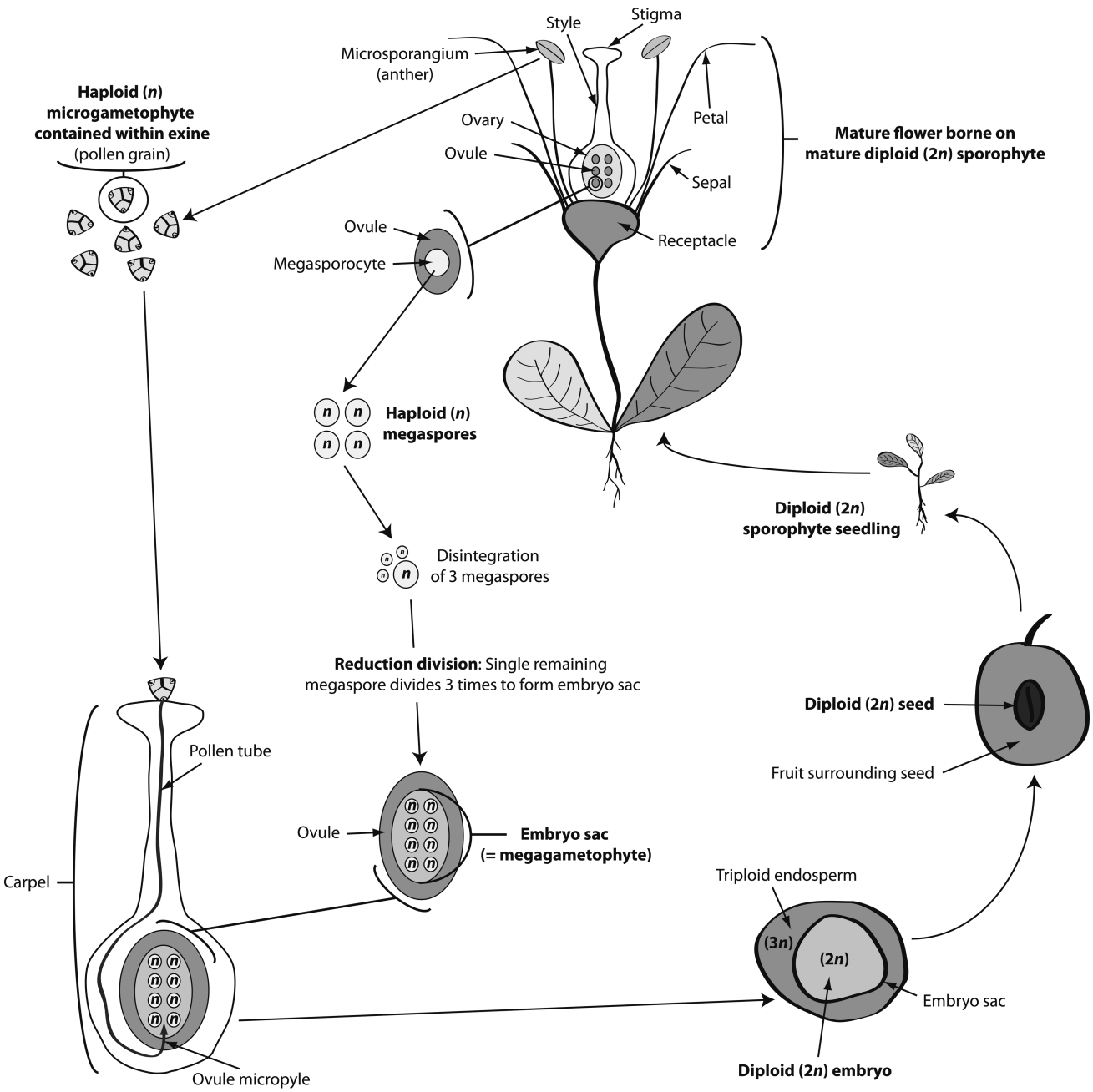

Fig. 11.5 Schematic life cycle of an angiosperm in which the diploid sporophyte is emphasized. Not to scale. Based on Jarzen and Nichols (1996)

fertilization; (Chaloner 1970; Poort et al. 1996)). In other groups, such as cycads and *Ginkgo*, a pollen tube that serves a solely haustorial function develops and absorbs nutrients from the tissues of the megasporangium. After several months of growth, the end of the haustorial pollen tube closest to the pollen grain enlarges and bursts, releasing motile spermatozoids that swim to the egg (zooidogamous fertilization; (Chaloner 1970; Poort et al. 1996)). Fertilization of the egg by the fusion of male and female gametes results in the production of a diploid embryo, which is contained in a coating formed from the ovule integument. This structure is known as a seed. Germination of the seed results in the production of a diploid sporophyte (Fig. 11.4).

Angiosperms: The flowering plants are the fifth major clade of extant seed plants. In these plants the diploid sporophyte is emphasized, and their generalised life cycle is as follows (Fig. 11.5). The diploid sporophyte bears a flower with a microsporangium (an anther) and a megasporangium (an ovule). Meiotic division within the microsporangium results in the production of haploid microspores, which

comprise a microgametophyte contained in a microspore coating, the exine. These microspores are known as pollen grains (Fig. 11.5). Each pollen grain contains three nuclei, one of which is the pollen tube nucleus and the other two are non-motile sperm nuclei. The megasporangium is enclosed by the tissues of the carpel. Meiotic division within the megasporangium results in the production of four haploid megaspores. Three of these disintegrate and the remaining functional megaspore undergoes reduction division to form a megagametophyte or embryo sac that has eight nuclei. One of these nuclei is the egg and two of these nuclei are the polar nuclei.

Upon reaching maturity, the pollen grains are dispersed, usually by wind or an animal vector, and alight on the stigma. The pollen germinates on the stigma and a pollen tube is produced. The pollen tube grows down the style (for $\sim$50 cm in the case of *Zea mays*) and enters the ovule via the ovule micropyle. One sperm nucleus fuses with the egg to produce a diploid embryo. The other sperm nucleus fuses with the two polar nuclei to produce a triploid ($3n$) primary endosperm nucleus, which is a source of nutrition for the developing embryo. The diploid embryo develops within the embryo sac. The integuments of the ovule become the seed coat and the tissues of the ovary become the fruit. Germination of the seed results in the production of a diploid sporophyte.

General Relationships Between Pollen and Spores and Vegetation

Pollen and spores are dispersed as part of the life cycles of plants and accumulate in a variety of depositional settings, including lakes, fluvial environments, swamps, and offshore marine sediments (Gastaldo 2001; Traverse 2007). For angiosperm pollen, pollination mechanism (wind-, animal-, or self-pollination) influences the amount of pollen produced and released into the environment, with wind-pollinated species generally producing the most pollen per individual. (Spores and gymnosperm pollen are primarily wind dispersed).

The transport and deposition of pollen and spores in a forested area has a composite nature, with aerial transfer taking place partly through the vegetation (the trunk space), partly above the canopy, and partly through rainfall (Tauber 1967). A primary assumption that is made when using pollen and spores for the purpose of paleoecology is that an interpretable relationship exists between these dispersed pollen and spores and the vegetation that produced them (Birks and Birks 2000). Studies that compare the composition of the source vegetation and assemblages of modern dispersed pollen and spores in surface samples indicate that this assumption is reasonable in many situations, but also highlight that reading patterns of vegetation change from fossil assemblages through time is not straightforward.

Surface sample studies: Mapped patterns of present-day tree pollen percentages in temperate regions of Europe and North America correspond broadly with geographic patterns of relative tree abundance (e.g., Webb 1974; Delcourt et al. 1983, 1984; Huntley and Birks 1983; Prentice et al. 1987). These observations support the idea that, as a first approximation, changes in pollen and spore percentages in samples through time can be interpreted in terms of roughly comparable changes in relative abundances of taxa in the source vegetation (Prentice and Webb 1986). Similarly, although offshore sediments are thought to present a more blurred picture of the source vegetation (Scott et al. 2004), the distribution of pollen and spores in surface samples in marine sediments offshore West Africa broadly corresponds with the spatial distribution of vegetation growing on the land. For example, "the distribution of Poaceae pollen in the marine surface sediments [offshore southern West Africa] shows a good correspondence with the regions of savannah and desert biomes on the adjacent continent" (Dupont et al. 2007, p. 91).

However, the percentage of a given pollen or spore taxon in a sample depends not only on its own abundance, but also on the abundance of one or more others. This leads to relationships between the percentage of pollen and spore taxa in samples and the percentage of their parent plants in the source vegetation that are non-linear (Davis 1963; Prentice and Webb 1986); such non-linearity is known as the "Fagerlind effect" after Fagerlind (1952). Additionally, a general finding from surface sample studies is that although patterns in the relative abundance of different plant taxa are broadly reflected in pollen and spore data, the representation of certain taxa is biased at the expense of others. In the pollen record, certain taxa are commonly overrepresented (e.g., *Pinus*), whereas others are commonly underrepresented (e.g., *Acer*). This is because pollen spectra are biased towards taxa with high pollen productivity and/or slow deposition from the atmosphere (Prentice 1985).

How much space? When assemblages of pollen and spores are examined, the results of surface sample studies allow a researcher to interpret the assemblage in terms of the broad composition of the vegetation. However, these studies provide less information on the source area of the pollen and spores. In particular, it can be extremely difficult to interpret the size of the area from which the pollen and spores under investigation were recruited (Jacobsen and Bradshaw 1981). This is important because if assemblages of pollen and spores were recruited from very small local habitats, then changes in their composition through time could reflect the vagaries of local ecology (Davis 2000). In contrast, if

assemblages of pollen and spores were recruited from a much larger area, then changes in their composition may reflect changes in regional climate (Davis 2000).

Empirical surface sample studies have demonstrated that a general relationship exists between the size of the basin into which pollen and spores are deposited and the size of the source area. Most of these studies have focused on the pollen of trees and they show that larger basins collect pollen from a larger area than smaller basins (Janssen 1973; Bradshaw and Webb 1985; Jackson 1990; see Sugita 1994). Pollen grains can be transported thousands of kilometers, but it is thought that the abundance of such long-distance grains in samples is low (Bakker 1985). This general relationship has been confirmed by simulation studies (e.g., Prentice 1985; Sugita 1993), which have highlighted that the pollen loading per unit area of lake surface from local trees declines as lake size increases because the ratio of perimeter to area decreases as lake radius increases (Sugita 1994). Such modelling work has been used to examine the source area of pollen for idealized depositional basins with different radii. For forest hollows of radius 2 m, 30–45% of the pollen deposited is derived from within 50–100 m (Sugita 1994). For small lakes of radius 50 m this source area increases to 300–400 m, and for medium lakes of radius 250 m this source area is 600–800 m (Sugita 1994). In dense tropical forests where the majority of plants are insect pollinated (Bush 1995) and pollen and spore spectra are not always dominated by canopy trees (Fægri 1966), pollen and spores may travel only 10–50 m from the source plant (Kershaw and Strickland 1990; Bush and Rivera 1998).

Plant macrofossils: Macrofossils (leaves, cones, twigs, shoots and other vegetative and reproductive structures) are extremely useful as paleoecological records in themselves (Jackson and Booth 2007; Taylor et al. 2009). For example, paratropical leaf litter deposits have been shown to reflect the heterogeneity of the source vegetation (Burnham 1989), while temperate deciduous forest litter corresponds closely in composition to the source forest and reflects the relative abundance of species comprising more than 10% of the stem-basal area (Burnham et al. 1992). Relative abundances of modern leaf litter of coniferous trees from temperate forests in North America are also significantly correlated with their basal areas in nearby forests (Dunwiddie 1987). In the context of this review, which is focused on the use of pollen and spores in paleoecology, data from macrofossils are complementary to pollen and spore data and highlight certain vegetation-sensing properties of pollen and spores.

In particular, macrofossil data show that some taxa are generally absent or poorly represented in the pollen and spore record. For example, *Abies*, *Pseudotsuga* and *Larix* are typically well represented in macrofossil assemblages, but these taxa are generally absent or poorly represented in assemblages of pollen and spores extracted from the same samples (Dunwiddie 1987; Jackson and Booth 2007). Additionally, they highlight that pollen and spores generally record vegetation at low taxonomic resolution (Jackson and Booth 2007; Mander and Punyasena 2014). For example, macrofossils can demonstrate that the source vegetation at a given site contains several species within a particular genus, or several genera within a particular family, but these multiple taxa may be represented by a considerably smaller number of pollen and spore morphotypes. There are several classic examples of this, including *Picea* (spruce) and *Quercus* (oak) pollen, which are both extremely difficult to classify below the genus level, and Poaceae (grass) pollen, which is very difficult to classify at a taxonomic rank below family (Mander et al. 2013) (see Mander and Punyasena 2014 for a general review of taxonomic resolution).

The preservation of pollen and spores: The outer layer of pollen and spores, the exine, is composed of an organic compound called sporopollenin. This substance is extremely inert and is resistant to the effects of a variety of acid treatments. The tissues that are contained within this tough exine coating, including genetic material, are extremely labile and consequently are rarely found as fossils. Fossilized pollen tubes have been reported as far back in time as the Pennsylvanian (323–299 Ma) (e.g., Rothwell 1972), but pollen and spores that are found as fossils generally represent only the outer exine layer.

However, sporopollenin is susceptible to degradation by strong oxidants in a laboratory setting such as H_2O_2 and CrO_3 (Traverse 2007), and pollen and spores are vulnerable to a variety of damage types on their journey from parent plant to sediment. These include chemical damage, which can involve oxidation as a result of deposition in sediments prone to wet/dry cycles, and microbial attack, and mechanical damage such as tearing, compression and folding (Mander et al. 2012) (Fig. 11.6). Once a pollen grain or spore is sealed in sediment, it is vulnerable to further microbial attack and compression, as well as thermal maturation due to processes such as igneous intrusion (Mander et al. 2012) (Fig. 11.6). Taxa differ in the amount of sporopollenin that is invested in the exine, and thin-walled taxa such as *Populus* may be destroyed by these processes, while thicker-walled taxa may survive. As a result, changes in the relative abundance of thin-walled taxa may reflect taphonomic, rather than ecological factors.

The sensitivity of sporopollenin to oxidation means that certain rock types, such as sediments with a reddish color that is indicative of oxidation, should generally be avoided because these rocks are unlikely to contain pristine pollen and spores. Pollen grains range in size from $\sim$4.5 μm to $\sim$200 μm, and this means that they are likely to be winnowed away during the deposition of relatively coarse-grained rocks such as sandstones. Consequently, such coarse-grained sediments should generally be avoided, and

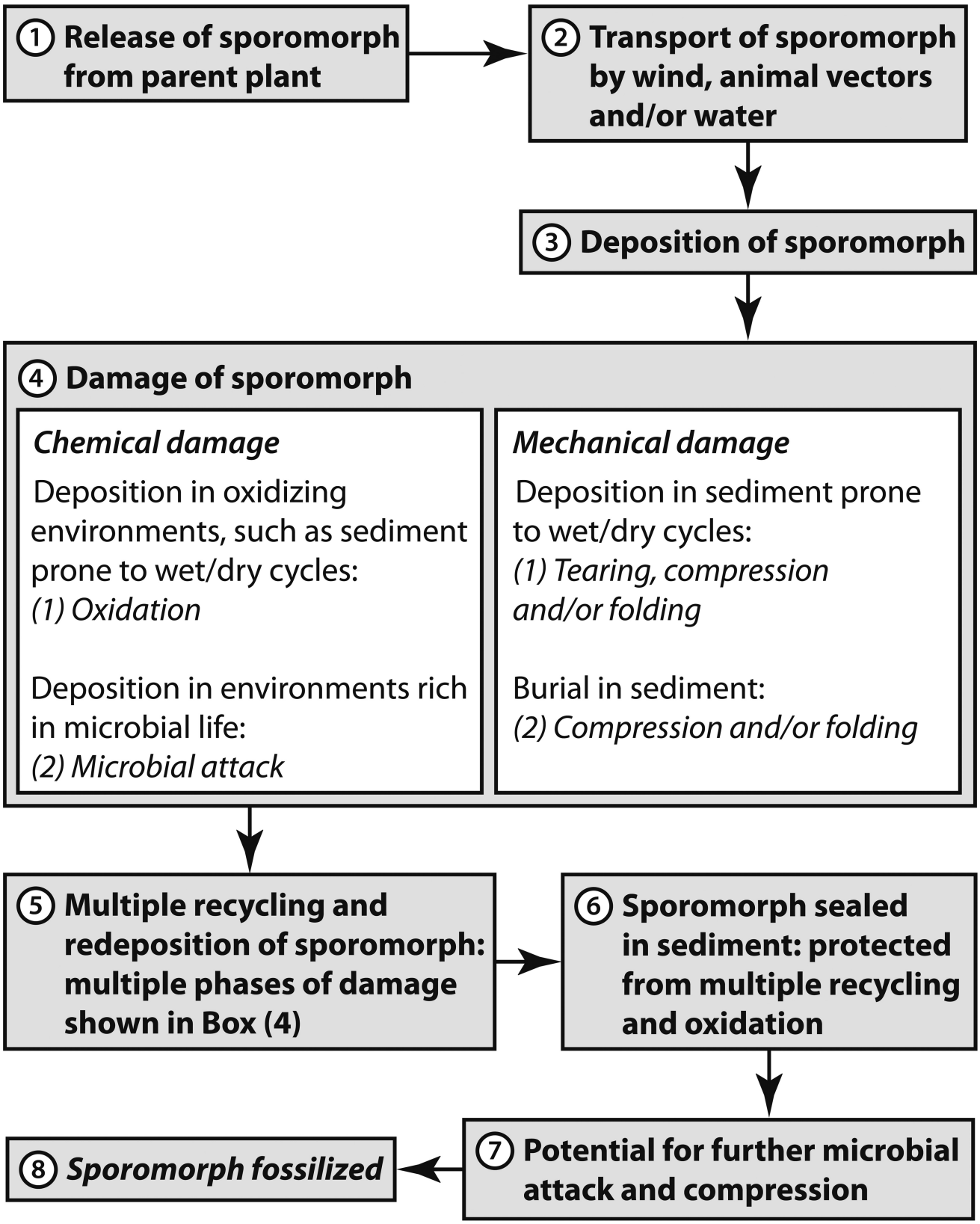

Fig. 11.6 Flow diagram illustrating the types of damage that can affect pollen and spores (referred to in this figure collectively as sporomorphs) in the period between their release and eventual fossilization (from Mander et al. 2012)

finer grained siltstones and mudstones should be targeted instead.

Palynological preparations invariably contain a variety of other fossils that are also tough and resistant to decay. These include plant cuticles, fungal spores, charcoal, and non-pollen palynomorphs. These other fossils can provide additional information on the paleoecology of a sample, and this information is generally complementary to the information derived from pollen and spores.

The Description and Classification of Pollen and Spores

Description

In most paleoecological studies, pollen and spores are classified into taxonomic groups on the basis of their exine morphology. This involves using specialist terminology to describe the morphological features that characterize different specimens. Increasingly, workers are taking a standardized approach to the description of pollen and spores, using published glossaries such as that of Punt et al. (2007), which contain hundreds of specialist terms for the features encountered by the paleoecologist. Morphological features that are typically used to describe pollen and spores include, but are not limited to, their shape, dispersal unit, exine stratification, the nature and arrangement of any apertures, and the nature of any surface ornamentation (Punt et al. 2007).

Some of these morphological features may have functional significance. Pollen apertures, for instance, represent sites where the pollen tube can easily exit the pollen grain, but they also allow for the ingress and egress of water and serve as pathways that guide the folding of pollen grains during desiccation (Wodehouse 1935; Katifori et al. 2010). Additionally, there are suggestions that coarse surface ornamentation may represent an adaptation for insect pollination and that very fine surface ornamentation represents an adaptation for wind pollination, or that surface ornamentation may be involved in the production of static electrical charge to assist the transfer of pollen from vector to stigma (Punt 1986; Chaloner 2013). However, it is not necessary for morphological features that are used to describe a particular pollen grain or spore to have any known biological function.

Classification

Classification of pollen and spores into taxonomic groups involves comparing the morphology of specimens, as recorded by descriptive terminology (Punt et al. 2007), with published identification keys. These keys include broad syntheses such as the *World Pollen and Spore Flora* and *The Northwest European Pollen Flora* (Punt et al. 2003), as well as more locally specific monographic works such as *The Pollen and Spores of Barro Colorado Island* (Roubik and Moreno 1991). These keys do not give complete morphological descriptions, but serve to narrow down the range of possibilities during the classification process. Paleoecologists also make extensive use of reference collections. These are microscope slides of pollen and spores extracted from extant plants, usually from vouchered herbarium specimens, and allow the paleoecologist to establish a robust link between the morphology of a particular pollen grain and its parent plant. It is this link that allows paleoecologists to establish a relationship between the pollen and spores found in sediments and the parent plants that produced them. Consultation of reference material is a crucial step in the classification process, and "any identification must be considered tentative until tested against an actual specimen in the pollen herbarium" (Fægri et al. 1989, p. 237). Reference material that encompasses the full range of natural morphological variation among taxa allows the paleoecologist to compare the morphology of fossil pollen and spores with a wide spectrum of extant taxa (Birks and Birks 1980; Jarzen and Jarzen 2006). Monographs of reference material allow workers to establish pollen and spore types and also provide a basis for work comparing the morphology of pollen and spores from different regions and time periods (Punt et al. 2003).

The classification of pollen and spores into taxonomic groups underpins the discipline of paleoecology, and it produces raw data that allow changes in vegetation diversity and composition to be examined in time and space. Generally speaking, classification at a high taxonomic level, such as the rank of family, is an easier task than classification at a low taxonomic level, such as the rank of species. For example, it is quite straightforward to discriminate the pollen of oak and grass from each other, oak having three colpi and grass having a single pore (Fig. 11.7). However, it is very difficult to discriminate different species of oak from one-another and to discriminate different species of grass from one-another (Fig. 11.7). In certain situations, such as the case of oak and grass pollen, the difficulty of classifying at the species level arises because the pollen of different species is very similar morphologically.

A Prototypical Paleoecological Analysis

In this section of our review, we provide a skeleton of a typical paleoecological analysis. We begin by considering the types of sites that paleoecologists investigate, outline the methods used to release pollen and spores from sediment

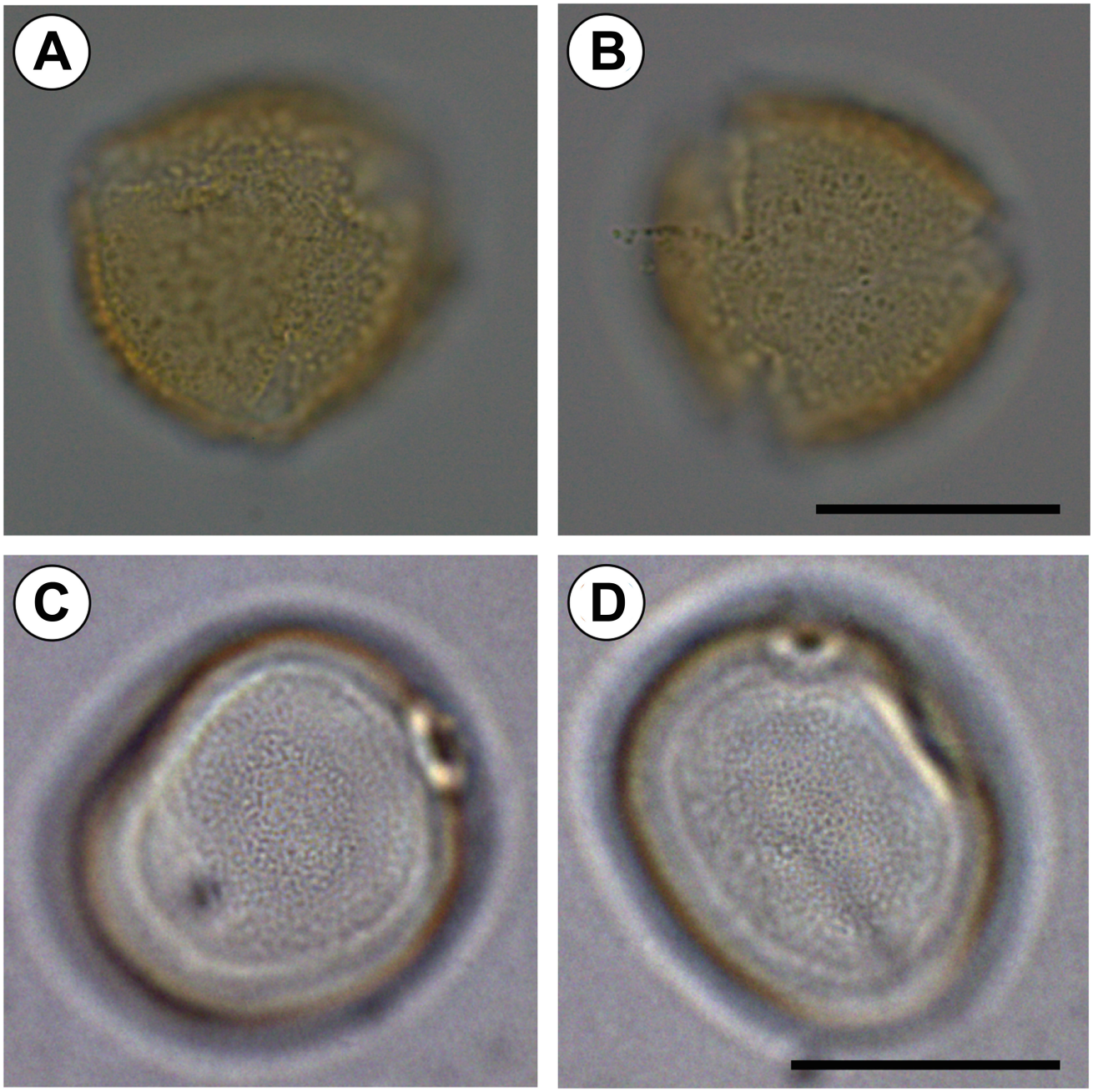

Fig. 11.7 Comparison of pollen produced by two species of oak and two species of grass. Oak pollen: A, *Quercus englemannia*; B, *Quercus virginiana*. Grass pollen: C, *Stipa tenuifolia*; D, *Dactylis glomerata*. Scale bars in B and D represent 20 μm

samples, and highlight the analytical techniques that are routinely used in paleoecology. We also consider the areas of potential strength in paleoecological data, and areas of potential weakness in paleoecological data.

Typical Locations

Almost any location in which pollen and spores accumulate is available for the paleoecologist to study. These include

tiny moss polsters within forested areas, peat bogs, sediments in lakes, sediments in abandoned river channels, offshore marine sediments, as well as rocks exposed at outcrop. The choice of location depends on the type of paleoecological study that is intended, and also on the availability of the locations themselves. For example, a classical investigation of the vegetation history of an area over the last few thousand years or so could involve gathering sediments from any of the 304 million natural lakes that cover 4.2 million km^2 of the Earth's surface (Downing et al. 2006). In contrast, a researcher aiming to study pollen and spores preserved in rocks hundreds of millions of years old deep in the geological past is likely to have a more limited set of potential localities from which to gather data.

For classical studies of vegetation history in the Quaternary, a locality that gives a broad integrated picture of the vegetation of the area should be selected. With the results of empirical surface sample studies in mind (Janssen 1973; Webb 1974; Delcourt et al. 1983, 1984; Bradshaw and Webb 1985; Prentice et al. 1987; Jackson 1990), together with guidance from theoretical work (Prentice 1985; Sugita 1993, 1994), the primary sampling site should be large enough to capture pollen and spores from the whole area and not be overwhelmed by pollen and spores produced by local plants (Fægri et al. 1989). Large lakes, such as the 107 ha Rogers Lake in southern Connecticut (Davis 1969), or smaller lakes ($\sim$1 ha) with a simple shape and bathymetry, are the typical localities selected for this purpose. Investigators may choose to study two or more lakes in order to identify patterns that are local versus regional in extent, and so build up a picture of regional vegetation change.

Samples from each of these locations must be taken during a fieldwork campaign. In outcrop situations, samples may be gathered by isolating them from the outcrop using a rock hammer and chisel, and storing them in sample bags. These situations are typical in pre-Quaternary studies where samples are taken from sedimentary successions exposed in cliffs or on the foreshore. Samples gathered in this fashion must be accurately located on a sedimentary log, which provides a graphical representation of the rocks under investigation. Samples gathered from peat may involve a similar process of taking discrete samples, cutting a peat monolith, or may be taken using a coring device. Sampling from a typical lake involves gathering sediment cores from the lake bottom. To do this, researchers construct a transportable raft with scaffolding to support a coring device such as a Livingstone sampler (Wright et al. 1984), and float onto the lake in order to collect sediment cores. A detailed treatment of sampling and coring is provided in Chap. 4 of Fægri et al. (1989). The advantage of this approach is that coring equipment can be taken to remote locations using aircraft or other vehicles, assembled in the field, and then dismantled and returned to the laboratory together with the cores.

Contamination and Reworking

Samples must be cleaned and sealed effectively after sampling to prevent contamination. This contamination can involve transfer of material between different samples and also the inclusion of modern pollen and spores within samples. Contamination with modern pollen can be easy to spot in deep time material; an angiosperm pollen grain in a 350 million-year-old rock sample is unlikely to be anything but contamination, and it can be spotted quickly in countries where there are many exotic plants. However, other forms of contamination can be difficult to detect and has the potential to confound the results of a paleoecological analysis (Scott and Bonnefille 1986). Standard approaches to preventing contamination of more recent geologic samples include working in room free of dust and plant debris. The ideal space is well ventilated but windowless, limiting the introduction of pollen from the atmosphere. Careful sample preparation methods also critical. The exterior layer of sediment must be cleaned or removed before processing to remove modern material that may have come in contact with the sample. Cross-contamination also occurs among samples when sample preparation test tubes and other instruments are not thoroughly cleaned between uses.

Another form of contamination arises from the erosion and redeposition of pollen material. These reworked pollen grains are older than the sedimentary deposits in which they occur. Reworked material can be recognized by the quality of preservation (Mander et al. 2012) and by the identification of morphological types that are biostratigraphic outliers (e.g., Cushing 1964). These outliers should be removed from any paleoecological or evolutionary analysis.

Chronologies

Establishing a well-defined chronology is the basis of paleoecological analysis, as the timing of vegetation changes play an important role in hypothesis testing. In studies of the Late Quaternary, researchers are able to use radiocarbon dating of organic material. Radiocarbon within living organisms is at equilibrium with the atmosphere; the radiocarbon clock begins upon their death. Because the concentration of radiocarbon in the atmosphere varies over millennial timescales, radiocarbon dates must be calibrated in order to provide a chronology in calendar years. These regularly updated calibration curves (IntCal13, SHCal13, and Marine13) are derived from carbon isotope measurements of tree rings, varves, marine foraminifera, uranium-thorium-dated corals and speleothems (Reimer et al. 2013).

Contamination from older or younger sources of carbon is a potential source of error. Best practices include avoiding

reliance on small amounts of organic material, which are most easily contaminated by exogenous carbon, and bulk sediments, as algae and aquatic plants may derive carbon from radiocarbon-depleted carbonate rocks (Grimm et al. 2009; Blois et al. 2011). The most accurate age estimates will be derived from the fossil remnants of short-lived herbaceous plants (Grimm et al. 2009).

High-resolution dating can identify periods in time that were characterized by the deposition of large amounts of sediment, and intervals where much time has elapsed but very little sediment has been deposited. Beyond 50,000 year BP, however, the utility of radiocarbon dating diminishes, and it becomes increasingly difficult to gain knowledge of exactly how much time is represented by a given succession of sedimentary rocks. Researchers undertaking paleoecological studies in pre-Quaternary time often report compositional change in vegetation relative to the appearance or disappearance of biostratigraphically sensitive organisms such as ammonites, or relative to some geochemical signature of paleoenvironmental change such as carbon or oxygen isotopes. Absolute dates in such settings can be established using radiometric dating techniques such as U/Pb geochronology (e.g., Schoene et al. 2010).

Laboratory Methods to Release Pollen and Spores from Samples

In order to examine pollen and spores from samples taken from cores, or taken from discrete field samples, it is necessary to macerate the samples in the laboratory, and to prepare the macerated sample residue for viewing under the microscope. These maceration techniques have changed very little over the last 50 years or so, although there is some variation between laboratories, and detailed guidance can be found in standard textbooks (Fægri et al. 1989; Traverse 2007). In this review, we outline standard and widely used methods for releasing pollen and spores from (*i*) soft lake sediments of Quaternary age, and (*ii*) from well-cemented, hard, sedimentary rocks of pre-Quaternary age.

Quaternary lake sediments: The following is adapted from Fægri et al. (1989) we have used these methods with only minor deviations to extract pollen and spores from Quaternary sediments. The choice of mounting medium varies (steps 32–40), and can include silicone oil, glycerine jelly, glycerol or other media. Many Quaternary palynologists prefer the use of viscous liquid media, such as silicone oil, to allow the manual rotation of grains. However, the highest-resolution optical imaging requires solid media with a refraction index of glass (Sivaguru et al. 2018). The following should only be undertaken in a fully equipped laboratory with trained personnel.

(1) Place 0.5–2 cm^3 sample into 50 ml polypropylene tube.
(2) Fill tube with 20 ml 5–10% HCl and place in hot water bath for 20 minutes.
(3) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(4) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(5) Fill tube with 10 ml 10% KOH and place in hot water bath for 5 minutes.
(6) Remove samples from water bath and top up with deionized water.
(7) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(8) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(9) Fill tube with 20 ml deionized water and sieve sample through 180 μm mesh into second tube, retaining pollen and spores.
(10) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(11) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(12) Fill tube with 10 ml 5–10% HCl.
(13) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(14) Fill tube with 10 ml 40% HF. Leave for 12 hours in fume hood.
(15) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(16) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(17) Fill tube with 20 ml 5–10% HCl and place in hot water bath for 20 minutes.
(18) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(19) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(20) Fill tube with 20 ml glacial acetic acid.
(21) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(22) Repeat (19) and (20).
(23) In a measuring cylinder, combine 9 parts acetic anhydride with 1 part sulphuric acid (H_2SO_4).
(24) Add 10 ml of this mixture to the sample and place in a hot water bath for 3 minutes.
(25) Remove from water bath and top up with glacial acetic acid.
(26) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(27) Fill tube with 20 ml glacial acetic acid.
(28) Centrifuge at 3000 rpm for 3 minutes, decant supernatant.

(29) Wash sample with deionized water, centrifuge at 3000 rpm for 3 minutes, decant supernatant.
(30) Transfer sample to test tube.
(31) Centrifuge sample for 5 minutes at 2000 rpm.
(32) Decant sample.
(33) Add glycerine-water (80% glycerol 20% water) to sample.
(34) Agitate sample using 'lab dancer' test-tube shaker.
(35) Transfer sample to 20 ml test tube.
(36) Centrifuge sample for 5 minutes at 2000 rpm.
(37) Pipette sample residue onto slide.
(38) Add glycerine jelly to residue.
(39) Melt glycerine jelly using hot-plate set at 80°C.
(40) Add cover slip and label; leave to dry overnight.
(41) Clean slide using razor blade, seal edges with clear lacquer, leave to dry overnight.
(42) Inspect pollen and spores using a microscope.

Pre-Quaternary rocks: The following is adapted from Traverse (2007), and one of us (LM) has used this protocol to extract pollen and spores from a variety of Mesozoic and Cenozoic rocks. The choice of mounting medium varies (steps 39–47), and can include silicone oil, glycerine jelly, glycerol or other media. Again, however, the highest-resolution optical imaging requires solid media with a refraction index of glass (Sivaguru et al. 2018). The following should only be undertaken in a fully equipped laboratory with trained personnel.

(1) Crush the rock sample in pestle and mortar.
(2) Dry at 65°C overnight.
(3) Record dry weight and add two *Lycopodium* spore tablets (these tablets contain a known number of spores, and this allows a calculation of pollen concentration).
(4) Transfer sample to polypropylene beaker.
(5) Add ~30 ml 30% cold HCl to sample, wait until reaction has calmed.
(6) Top up beaker with water and leave overnight.
(7) Decant supernatant into waste container.
(8) Add ~30 ml 38% cold HF to sample.
(9) Place sample on orbital shaker, in fume cupboard, and leave (shaking) for 2 hours.
(10) Leave sample to settle overnight.
(11) Decant supernatant into waste container.
(12) Add ~30 ml 30% cold HCl to sample.
(13) Top up beaker with water.
(14) Leave sample to settle overnight.
(15) Decant supernatant into waste container.
(16) Add ~30 ml 38% cold HF to sample.
(17) Place sample on orbital shaker, in fume cupboard, and leave (shaking) for 2 hours.
(18) Leave sample to settle overnight.
(19) Decant supernatant into waste container.
(20) Add ~30 ml 30% cold HCl to sample.
(21) Top up beaker with water.
(22) Leave sample to settle overnight.
(23) Decant supernatant into waste container.
(24) Sieve sample using 250 µm mesh – retain residue.
(25) Sieve sample using 15 µm mesh (10 µm where angiosperms are present).
(26) Transfer residue to small ceramic dish – float in ultrasonic bath to loosen particles.
(27) Sieve sample using 15 µm mesh.
(28) Transfer sample to test tube.
(29) Centrifuge sample for 5 minutes at 2000 rpm.
(30) Decant supernatant.
(31) Fill test tube (two thirds ~10 ml) with $ZnCl_2$ (specific gravity 2.1).
(32) Centrifuge sample for 15 minutes at 800 rpm.
(33) Pipette supernatant sample into 15 µm mesh sieve and wash away any $ZnCl_2$.
(34) Transfer residue to small ceramic dish – float in ultrasonic bath to loosen particles.
(35) Sieve sample using 15 µm mesh.
(36) Transfer sample to test tube.
(37) Centrifuge sample for 5 minutes at 2000 rpm.
(38) Decant sample.
(39) Add glycerine-water (80% glycerol 20% water) to sample.
(40) Agitate sample using 'lab dancer' test-tube shaker.
(41) Transfer sample to 20 ml test tube.
(42) Centrifuge sample for 5 minutes at 2000 rpm.
(43) Pipette sample residue onto slide.
(44) Add glycerine jelly to residue.
(45) Melt glycerine jelly using hot-plate set at 80°C.
(46) Add cover slip and label leave to dry overnight.
(47) Clean slide using razor blade, seal edges with clear lacquer, leave to dry overnight.
(48) Inspect pollen and spores using a microscope.

Data collection and analysis: Routine paleoecological analyses that aim to reconstruct vegetation history involve inspecting pollen and spores using a transmitted light microscope with brightfield illumination. This is a standard workhorse microscopy technique that allows paleoecologists to examine hundreds of specimens quickly. Additionally, the descriptive terminology that is used to describe pollen and spores is mostly composed of terms for morphological features that can be seen using brightfield illumination. This facilitates standardized descriptions, and ensures that researchers use the same terms for the same features. However, there are a variety of other microscopy techniques available to the paleoecologist (Sivaguru et al. 2012). These include the scanning electron microscope, which is useful for examining the surface of pollen and spores at a higher resolution than can be achieved using a light microscope, transmission electron microscopy, which allows high-resolution investigation of internal pollen structures, and fluorescence

techniques such as confocal microscopy, which can be useful for viewing some internal structures of pollen and spores, and for reconstructing the three-dimensional shape of specimens. Recent advances in optical super-resolution microscopy additionally allow for unprecedented high-resolution investigations of the internal structure of palynomorphs in three dimensions (Sivaguru et al. 2018).

Researchers will typically count a large number of specimens per sample in order to generate data on the relative abundance of the constituent taxa. The number of specimens counted (known as the "pollen sum") varies. Some workers choose to count a set number of specimens, others count until a certain number of the non-dominant type have been counted, while some count all specimens in an aliquot of residue from a known volume (or weight) of sediment to produce direct measurements of pollen and spore concentrations (see laboratory protocols above; Bennett and Willis 2001). Pollen data can also be reported as pollen influx, which is the pollen concentration normalized by the sediment accumulation rate (grains deposited per year per cm^2 of sediment) (Thompson 1980; Bennett and Willis 2001).

The choice of what is or is not included in the pollen sum and whether percentages, concentrations, or influx is reported is critical, as this will affect paleoecological interpretations and future meta-analyses of these data. Best practice dictates that both the raw numbers and summarization method be documented, alongside the final interpreted pollen spectra. With the proliferation of online databases, such as the North American Pollen Database (http://www.ncdc.noaa.gov/paleo/napd.html), the European Pollen Database (http://www.europeanpollendatabase.net), and the Latin American Pollen Database (http://www.ncdc.noaa.gov/paleo/lapd.html), there are multiple repositories for this critical metadata. To date, the most comprehensive site for data archival is the Neotoma Paleoecology Database (http://www.neotomadb.org), which catalogs both plant and animal fossil abundance data for the Neogene.

Paleoecologists use a variety of techniques to display the results of pollen counts. The use of "pollen diagrams", which display the percentages of taxa through time, date back to von Post (1916), and are still in widespread use today. They are useful for providing an immediate graphical overview of vegetation change through time, and similar diagrams are used to display the accumulation rates of pollen and spores (e.g., Davis 1969). However, over the past 50 years, palynologists have increasingly moved away from subjective interpretations of pollen spectra toward more rigorous, repeatable, quantitative analyses aimed at testing specific ecological or environmental hypotheses. Paleoecologists now use software such as Tilia (Grimm 1991–2001), C2 (Juggins 2007), and Rioja (Juggins 2015) not only to plot pollen diagrams, but also to statistically analyze count data. Multivariate analyses such as detrended correspondence analysis (DCA) and non-metric multidimensional scaling (NMDS) allow the patterns of compositional change in a suite of samples to be visualized in an ordination (Legendre and Birks 2012), while stratigraphically constrained cluster analyses can be used to quantitatively define biozones in a succession (Gordon and Birks 1972; Grimm 1987). These biozones can then be used for the purpose of stratigraphic correlation. Recently developed maximum likelihood methods produce quantitative estimates of the stratigraphic position of isolated samples, and also quantify the level of certainty in those estimates, and this provides a quantitative means of correlating single samples (Punyasena et al. 2012a).

Climatic Interpretation of Pollen Data

Pollen most directly represents the vegetation from which it was derived, with the relative abundance of each taxon influenced not only by the number of mature individuals producing pollen, but the pollen productivity of each individual, and the dispersal mechanism of the species (Fægri et al. 1989). Therefore, there is not a one-to-one representation between pollen abundance and the vegetation from which it was derived (Davis 1963). Despite these ecological and taphonomic filters, however, the vegetation changes captured by pollen are widely accepted as indirectly capturing underlying changes in environmental and climatic conditions.

Reconstruction of paleoclimates from pollen counts can be done in several ways. The simplest approach is through qualitative assessments of analogue biomes. This method requires identification of analogue taxa within a pollen record. Interpretations can be based on the presence/absence of key indicator taxa (e.g., Mayle et al. 2004) or based on the composite pollen spectrum (e.g., von Post 1916).

Most recent applications of an indicator taxon approach derive quantitative climatic values from pollen data by applying knowledge of modern analogues with well-defined biogeographic distributions (Kershaw and Nix 1988; Birks and Birks 1980). A classic example is the study of European holly, ivy, and mistletoe by Iversen (1944). This approach requires substantial knowledge of the autecology of multiple taxa. This data-intensity has become less of a limitation as databases of bioclimatic distributions have accumulated (e.g., Kershaw and Nix 1988) and probabilistic methods have been developed to work with sparse biogeographic data (Bush et al. 2004; Punyasena et al. 2008).

Composite assemblage approaches have similarly moved from qualitative assessments of the similarity of a sample's taxonomic makeup to modern biomes, to quantitative comparisons of pollen spectra between fossil and surface pollen assemblages. Comparison to surface sediments rather than standing vegetation implicitly incorporates many of the

taphonomic filters present in fossil pollen assemblages (Wright 1967). Classical ordination methods, such as correspondence analysis (CA) or non-metric multidimensional scaling (NMDS) (Legendre and Birks 2012) allow for fossil assemblages to be matched to their closest modern biome (Williams 2003). Canonical ordinations, such as canonical correspondence analysis (CCA), can be used to directly relate pollen composition to climatic or environmental data (Legendre and Birks 2012). Another widely adopted approach is the use of regression-based transfer functions (Sachs et al. 1977), which use surface samples to relate the abundances of widespread pollen taxa to vegetation abundance. These transfer functions serve as a "correction" of the taphonomic biases of pollen dispersal and preservation, and can then be used to reconstruct paleovegetation. Taken one step further – by mapping these data into climate space and generating pollen-climate response surfaces – regression-based transfer functions can also produce direct estimates of paleoclimate (Bartlein et al. 1986). Other approaches have included the process of "biomization", where pollen spectra are interpreted as plant function types, and these functional types are associated with a specific global biome (Prentice et al. 1996; Prentice and Webb 1998). Biomization allows global comparisons of pollen spectra by focusing on the ecology of the represented plant community rather than its taxonomic composition.

The latest generation of pollen-vegetation models incorporate Bayesian hierarchical models (Paciorek and McLachlan 2009; Garreta et al. 2010; Dawson et al. 2016). Bayesian and related maximum likelihood models are capable of explicitly accounting for and reporting all sources of uncertainty in their environmental reconstructions (Punyasena et al. 2008; Dawson et al. 2016). Although computationally more intensive than traditional regression-based or multivariate approaches, modeling all sources of uncertainty – from the identification of individual grains to climatic distributions of species – should lead to the most transparent and reproducible interpretations of paleoclimate.

Strengths and Weakness of Pollen-Based Paleoecology

Areas of Potential Strength

The consistency of pollen analysis methods over the last century means that pollen data can be aggregated and compared in large meta-analyses. This is particularly true for Late Quaternary samples from well-studied regions like North America and western Europe – the time period and areas for which we have the most existing data. The wide dispersal of pollen and spores, together with the robustness and chemical resistance of the sporopollenin-rich exine (susceptibility to oxidation and the fragility of thin-walled species such as *Populus* notwithstanding), also means that pollen and spores are an ideal fossil group with which to track species in time and space. Additionally, they exhibit a large amount of morphological variation, and although there are a handful of long-standing and difficult classification problems at fine taxonomic levels (Fig. 11.7), pollen and spores in general have sufficient morphological diversity to enable classification.

One of the central strengths of paleoecological data is the provision of information on ecological changes that occur over timescales that are longer than a single human life. This provides information on how life responds to long-term climatic changes that are outside the natural conditions on Earth today. Paleoecological data can also allow scientists to examine past vegetation in pre-industrial times, and these data contribute materially to on-going debate about the biotic effects of anthropogenic climate change. We do not consider the generation of age models in this review, but we note that radiocarbon dates derived from organic matter released from sediment samples can provide accurate dating of sediments, particularly over the last 50,000 years. This provides a means of comparing the temporal patterns of vegetation change from multiple locations and regions, and interpreting these patterns in light of regional and global climate, which allows scientists to examine the rates of long-term ecological processes.

As surface-sample and simulation studies have shown, paleoecological data also have the potential to yield information at multiple spatial scales. At one extreme, paleoecological data gathered from moss polsters and small bogs can allow researchers to reconstruct very local vegetation, while pollen and spores in offshore marine sediments can be used to reconstruct the latitudinal boundaries of entire biomes (Dupont et al. 2007). Such a broad range of spatial scales is amenable to synthesis, and this allows for the reconstruction of vegetation change in relation to climate over entire continents such as North America (Overpeck et al. 1992; Jackson and Williams 2004).

Areas of Potential Weakness

Despite these strengths, there are some inherent weaknesses in paleoecological data. One of these is that the process of classification as it is currently practiced is reliant on the expertise of the analyst. The taxonomic identification of pollen and spores is a highly specialized skill, and the variability in analyst experience means that it is difficult to assess the degree to which different analysts have confidence in the classifications they produce. Similarly, when presented with a pollen diagram that displays fluctuations in the abundance of taxa through time, it is difficult for an

independent observer to examine the distribution of any errors made during the classification process, and this undermines, to a degree, the robustness of paleoecological results.

The issue of taxonomic resolution is a substantial challenge for paleoecologists (Mander and Punyasena 2014). The lack of taxonomic precision in the classification of pollen and spores means that most paleoecological data are not reporting patterns at the species level, which is the fundamental unit of evolution. Perhaps more interestingly, the potential taxonomic precision of pollen and spore classifications varies between clades. Some speciose families, such as the Rubiaceae and the Papillionoideae, produce large numbers of pollen morphotypes, some of which are diagnostic to the genus or species level, whereas other large families such as the Poaceae and the Asteraceae contain many species that produce very similar pollen morphotypes. This means that the use of data from pollen and spores to reconstruct plant diversity, even during the Holocene (e.g., Birks et al. 2016) should be interpreted with caution.

Another challenge is the assumption inherent in all paleoenvironmental reconstructions that climatic thresholds for taxa have remained the same over time (i.e., evolution has not occurred). This is very likely not true, as Davis and Shaw (2001) have argued, since a changing climate can potentially serve as a strong selective factor on plant populations. Despite recognizing that autecological preferences may have changed over time, all paleoclimatic interpretations of pollen data largely rely on the assumption that these evolutionary changes are not significant enough to alter our interpretation of pollen data.

There are other problems to paleoenvironmental reconstruction that relate to whether our modern analogues and fossil samples are capturing ecosystems in equilibrium (i.e., climax communities). There is evidence that vegetation and climate have not been in equilibrium for most of the Holocene (Davis 1976). This is further complicated by the recognition that our current landscape has also been significantly altered by anthropogenic influences, so the potential distributions and environmental tolerances of many plant species may be dramatically underestimated. The latter aspect of anthropogenic disturbance is being circumvented by research using historic – rather than current – vegetation records (Dawson et al. 2016). The potential for disequilibrium within post-glacial communities requires that, as is the case for evolution, our interpretations of past environments based on vegetation carry an inherent degree of uncertainty.

Future Developments

In our view, there are two broad areas that are having an immediate impact on the current development of paleoecology. The first is technological advances that are being incorporated into paleoecological research. Microscopy techniques such as super-resolution fluorescence techniques that can gather data on structures as small as $\sim$120 nm (e.g., Sivaguru et al. 2012) can increase the amount of morphological information that is recovered from individual specimens, and this has the potential to increase the taxonomic precision of classifications (e.g., Mander and Punyasena 2014). Similar advances in the technology used to analyze data have the potential for similar advances in the speed, accuracy and repeatability of classifications. The use of computational image analysis and machine learning has demonstrable potential for solving classical classification problems in paleoecology, with recent examples including spruce (Punyasena et al. 2012b; Kong et al. 2016) and grass pollen (Mander et al. 2013). However, these approaches require the construction of substantial image libraries and a barrier to progress in this area is the rate of image acquisition. For example, the images in the library of SEM images of grass pollen constructed by Mander et al. (2013) were gathered by a human analyst, and this limited the speed of data acquisition. Nevertheless, the development of scanning electron microscopes that are capable of automated imaged acquisition (e.g., the Zeiss EVO MA microscope) and the development of software to segment and analyze image from pathology scanning microscopes (Tcheng et al. 2016) highlight that technological advances have real potential to speed up the data acquisition process. This is also demonstrated by the integrated system developed by Holt et al. (2011), which uses a robotic microscope to automatically collect images of pollen and software to automatically classify each specimen.

The second is the construction of cyber infrastructure that increases the connectivity between researchers and promotes synthetic data analysis. A key development is the building of databases, including the expansion of the Neotoma database. Unlike the Paleobiology Database (pbdb.org), Neotoma is structured to handle abundance data like pollen counts. However, with the development of advanced programming interfaces (APIs) for both databases, Neotoma and the Paleobiology Database are moving toward data sharing across platforms. As these databases expand their functionality, the hope is that data sharing and public data archival

becomes the norm among paleoecologists. These databases provide platforms that allow paleoecologists to synthesize and analyze data from wide geographic areas, and share taxonomic information about groups of organisms. They provide an extended life to paleoecological data, beyond the original paper and analysis. Such databases also increase the temporal scope of paleoecological investigations. Much paleoecological research is still focused on the Quaternary, where pollen and spore data are most abundant and accessible. However, as paleoecological methods are adopted by researchers in deep-time, recent and ancient ecosystems can be compared using comparable data. Such expansion reflects the on-going development of the discipline of paleoecology.

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