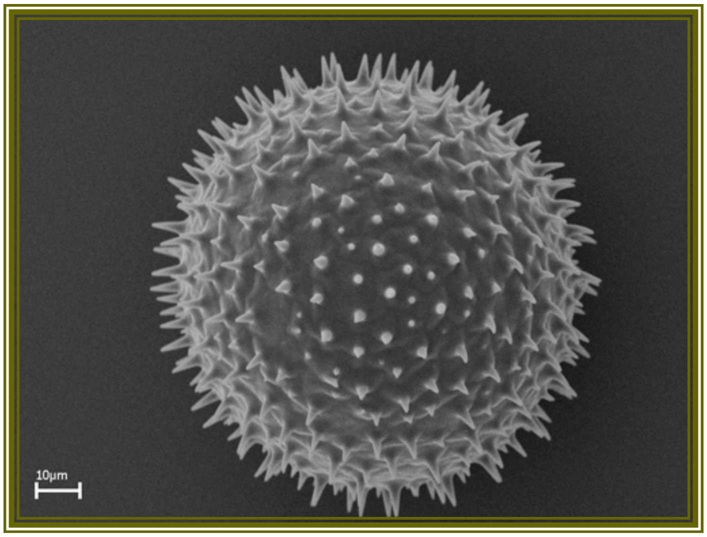
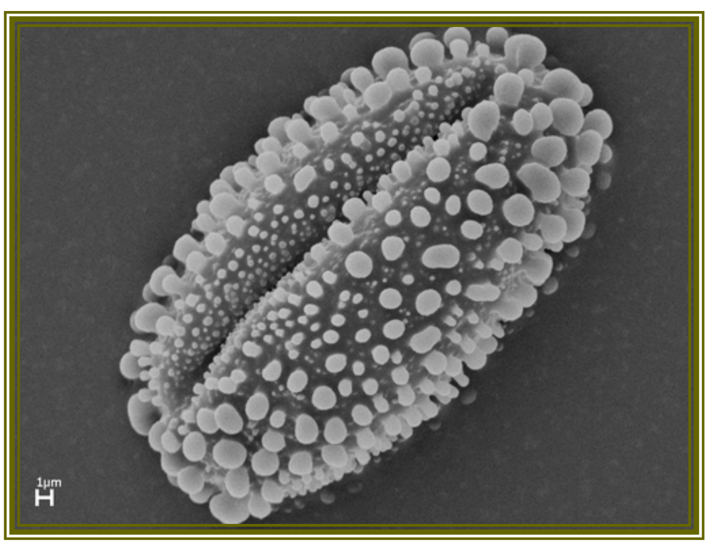
Michael S. Pascoe

SEM Pollen images of selected British flora:towards development of a national database.





SEM Pollen images of selected British flora:towards development of a national database.

Michael S. Pascoe

Submitted in partial fulfillment towards the MSc Plant Conservation (seed banking)

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Front cover. Top: *Malva sylvestris* pollen and flower, Bottom: *Ilex aquifolium* flower and pollen.

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Preface

The world of pollen was for me, before I began this research, the fertilization of a flower to produce seed; it was essential to the plant world and I knew to human survival. After five months of field work, peering into the reproductive cycle of nature, delicately removing the anthers and analyzing the pollen under the scanning electron microscope (SEM) I now realize that these are the missiles of botanical warfare. The flowers the political attractant for the pollinators yet the tax for that syrupy drink are the transport of these sculptured wonders.

In undertaking this work I have been assisted by many great people. I sincerely mean that since I am not a research scientist but a horticulturist with a love for nature and a curiosity of science. Thus, to those who assisted me, all of you exhibited great patience and understanding; thank you.

In particular I would like thank the following for their guidance and participation:

Dr. Peter Scott, head of the MSc plant conservation program and my advisor, for help in developing this project, a pleasurable combination of field work where I saw much of southern England and the 'right' mix of science. The long walks to Ferring Rife, Castle Hill and the Adur Valley are etched in my mind as are our office discussions where I took more than my share of your time than I was due. The casual presentation of a sample you collected, crumpled and dried, which contained some

Preface

botanical treasure, elicited many a smirk as I tried to extract pollen from their desiccated forms late into the night.

Dave Randall, scanning electron microscopy unit manager, the SEM genius, for his incredible patience, especially in those first few weeks when I did many a foolish 'thing' with the microscope which caused me untold anxiety but which he fixed with a click of the mouse: most times. His support, good humour and lively discussion helped the many days spent at the machine pass that much more quickly. Dave, 'I hope the images are in focus' as I do not have your 'Midas' touch.

I would like also to thank the following who contributed, all in no small way. Christian Mong from the University of Bergen, for, while working at the Millennium Seed Bank spent many patient hours in the field with me collecting and classifying plants and who sent the *P. sylvestris* pollen from Bergen; a plant I omitted to collect when in bloom. David Hardman, Head of Horticulture and Public Education at the Royal Botanical Gardens, Wakehurst Place, for allowing me to collect pollen from such a wonderful garden and landscape. Rachel Neville for providing many SEM images and sources of information that formed part of her undergraduate studies and Dr. Fergus Massey and David Barnham-Fisher for their participation in the forensic pollen analysis exercise.

SEM Pollen images of selected British flora

The development of a SEM database for British flora

The rapidly evolving and expanding world we live in has an insatiable quest for knowledge founded both in the need to know for purely intellectual reasons and for the acquisition of knowledge and its application to practical problems of a 'growing' yet problematic world. International cooperation and research in decision making both on a local and global perspective requires this knowledge to initiate informed policy decisions both within their immediate environment and on a global perspective. Collecting information has existed for many , many years and in fact the first databases were produced during industrial revolution. The early databases consisted of ledgers and journals that articulated production costs, inventory, salaries and other miscellaneous expenses (Wikipedia, 2007). However these early database or collections of information were difficult to share as they were often, heavy cumbersome tomes of paper. With the advent of the computer, information sharing has become instantaneous, what would a hundred years ago have taken us weeks if not longer to obtain can now be done in a few key-strokes. The development of botanical databases have benefited from this rapid sharing of information, ePIC (electronic plant information centre) is a collaboration between the Royal Botanic Gardens Kew, the Royal Botanic Gardens Edinburgh, The Missouri Botanical Garden and several other global organizations; this open database provides a wealth of current botanical information garnered from across the world that is readily available to both the scientific and public communities. Through the national botanic garden and the national herbarium of Australia the Australian Database Resources provides information on most Australian plant species including cultivated plants; this is achieved both through images, herbarium scans, plant names and records, catalogues of living collections and even common names of plants. The popularity of plant databases is further enhanced with the vascular plant collection hosted by the University of British Columbia, this database obviously revolves around Canadian plant species and provides a wealth of current information on the subject. The flora of North America (United States and Canada) is presented in a database called MOBOT hosted by the Missouri Botanical Garden. MOBOT is one of the most comprehensive and current plant databases on North American Flora and involves a number of individual and institutional contributors of note.

But what of pollen databases, concise collections of information regarding the identifying characteristics of pollen grains and the familial relationships that may or may not exist? There is information available both in hard copy and electronically, however compared to botanical databases and the availability of current plant information the quantity and clarity of information on pollen morphology and related plant floral characteristics is nominal and as of yet not as globally comprehensive as the above mentioned plant databases demonstrate. The development of a pollen database as a source of collected and shared information is not new; there are many examples of pollen databases with one of the largest being the Northwest European Pollen Flora (NEPF) that involves 56 of the 130 families and 596 of the 2500 species known to that particular region (Joosten and de Klerk, 2002). The African Pollen Database is another such entity that is gradually developing but focuses more on the dissemination of informational sources rather than the presentation of specific information. Work began on this database in the early 1990's and continues with 1145 taxa classified to date (2006) with a focus on tree and shrub pollen types (Vincens *et al,* 2006) . The Canadian Association of Palynologists also hosts a website database but again like the African Pollen Database is mainly a collection of information sources and is lacking in either pollen and floral images or sources for such. While locally (West Sussex) we have the work of Larry MacDonald at Wakehurst Place, a satellite garden of the Royal Botanic Gardens Kew. MacDonald's work utilizes light microscope images and pollen sampling through traps, pollen is not collected from identifiable sources (see Fig. 1). Thus concise pollen information is difficult to find that fits the regional area such as West Sussex or for that matter England. In fact in a brief survey of the internet the following results were obtained when images for a specific genus of pollen were searched using Google®. As demonstrated in Table 1 . There is a dearth of scanning electron microscope images of pollen grains available on the internet. Light microscope images are a little more common but even for some of the more frequent species such as *Fuchsia* no results were returned.

Botanical Name	***LM***	***SEM***
Agrostemma	1	0
Clematis	2	0
Eranthis	0	0
Fritilaria	2	0
Fumaria	2	1
Fuschii	0	0
Lamium	2	0
Muscari	0	0
Nigella	1	1
Polygonum	6	0
Ribes	2	0
Stellaria	0	0

Table 1. When a random selection of pollen grain images was searched on the internet using Google® the following results were obtained. The results are based on the image option of Google® and include totals from the first two pages of the search only. Searches were conducted at the genus level only. Light microscope (LM) and scanning electron microscope (SEM) columns denote the total number of images found for that specific genus; searches were conducted at the species level initially but in almost all cases returned no results.

The next logical question it would seem then, is why is there a need for a regional pollen database of SEM images and associated floral characteristics. To answer that question a background understanding of palynology needs to be realized to appreciate the 'fit' of such a data Base. There are an estimated 300, 000 species of plants in the world where their classification and organization is in a constant state of flux as new and improved scientific techniques are employed (Royal Botanic Gardens Kew, 2000).

Fig. 1. An air pollen trap at the Royal Botanic Gardens Kew, Wakehurst Place. Three such traps exist in the garden. Pollen is carried by the wind and blown into the traps where is sticks to a glass slide that has been coated with an adhesive. Pollen is then stained and examined under LM and catalogued.

Palynology is the study of pollen grains, spores and other minute natural detritus. The principle focus of palynolgy however is the microscopic examination of pollen and spores. The two groups, pollen and spores, differ considerably in their actual function but both result from cell division which results in a halving of their chromosome numbers, there are a few exceptions to this rule with some of the algae and fungi. Most pollen and spores need to be transported to execute their function; it is this mobile aspect of pollen function that can provide a wealth of information to botanists, entomologists, biologists, forensic balneologists, climatologist, archaeologists and many other associated and related fields. The pollen grain, the focus of this paper is a vessel that contains the male gametophyte generation of the angiosperm or gymnosperm. Successful pollination occurs if the pollen grain arrives at the stigma of a plant of the same species and germinates and completes the fertilization of the egg. Although palynology is concerned both with the structure and formation of the pollen grains and their history and movement this research focused on the structure and classification of pollen grains, and how these unique 'vehicles' can and are used as botanical fingerprints in understanding both plant and human interaction; both in the past and the modern world.

The flowchart in Fig. 2 on the following page outlines the main points of pollen analysis with the dominant characteristic of the chart the target plant or plant communities. As demonstrated in this schematic interpretation there are three principle sampling points used in modern palynological studies. Collection of pollen at the flowering stage of the plant, during dispersal (pollen rain) or during and after deposition, usually fossilized pollen. Each of these facets of pollen analysis develops different pictures of pollen ecology with the later two, pollen rain and pollen preservation (fossilization) relying on information developed from analysis of the pollen at the production point; comparing unknown pollen to a known entity. In developing a database of pollen images in Britain this research focused on the analysis of pollen at production from identifiable source plants.

The pollen production model

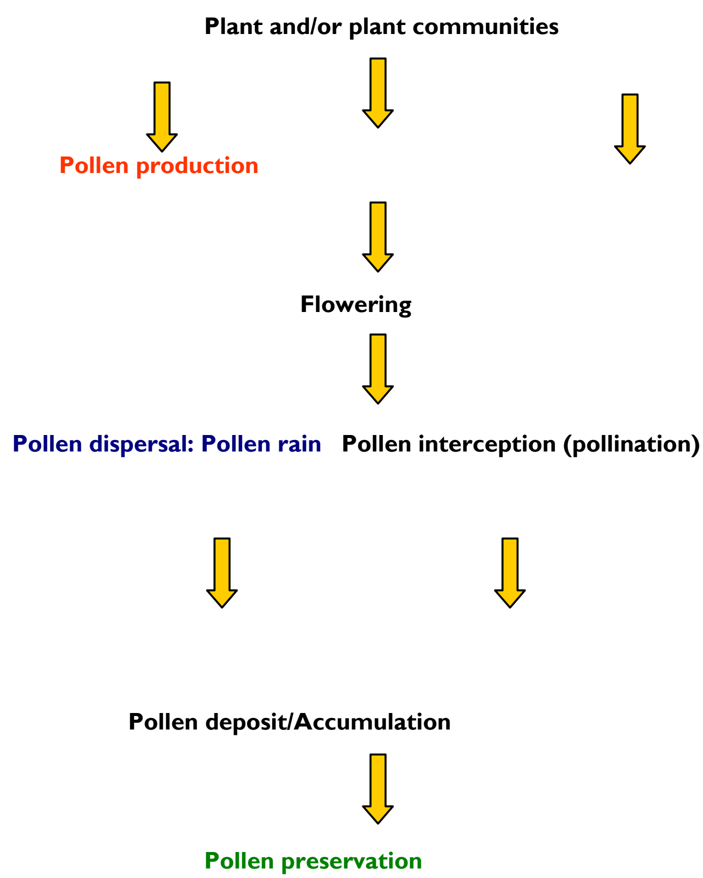


Fig. 2. The pollen production model outlining the possible dispersal and collection points of pollen in modern palynology. The research undertaken at the University of Sussex focused on collecting pollen during pollen production from identifiable plants (red).

The essence of pollen is transport, the movement of pollen from the anther of the plant to the stigma of the same or different plant. The modes of pollen movement as demonstrated in later sections of this paper are many and varied and the adaptation of plants to them are fundamental both for the survival of the species and the analytical studies of modern palynological methods. The transport of pollen is summarized in Fig. 3. a view of the pollen budget is presented.

This budget is maintained both from local pollen production and pollen from the outside, pollen leaving the community and through deposition depletes the community resource. Much of the pollen 'pool' is lost through decay in deposition however some may be retrieved through fossilized remains.

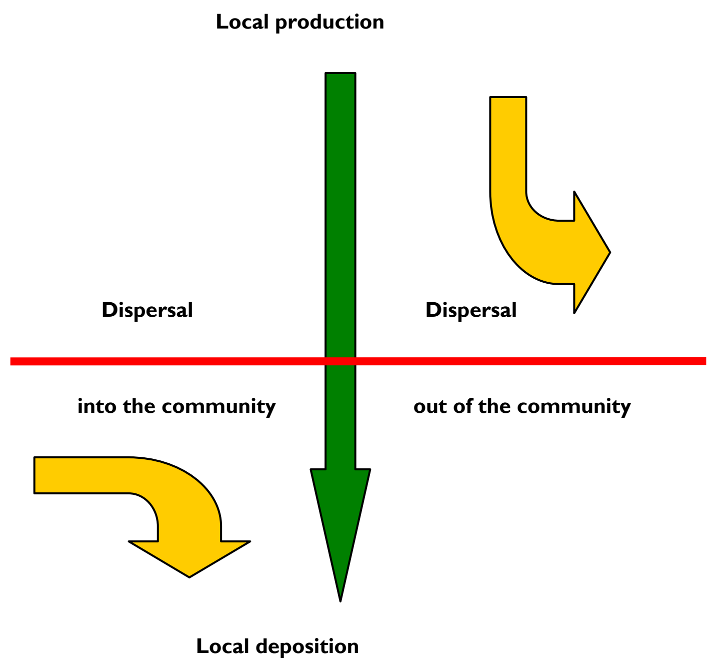


Fig. 3. The pollen budget model demonstrating pollen movement in and out of the natural community. This research focused on local production although the field of palynology addresses all areas of pollen dispersal and movement.

An understanding of the pollen model is essential in appreciating the pollen resources available for palynological studies. In appreciating the amount and type (fresh vs. preserved) of pollen available for analysis and the way it is disbursed allows us to investigate many other ecological fields using these miniscule botanical fingerprints.

Pollen can tell us a lot about the world both past and present, pollen grains can tell us what plants were around thousands of years ago, how our ancestors used them how plants have moved across the region, country and globe, what plants produced a certain variety of honey or even who may have committed a crime. A case in New Zealand that involved drug trafficking was resolved through the use of pollen analysis. The suspect *Cannabis* was thought to come from a large plantation some distance away from where the arrest occurred, if this was the case the person would be have been charged with trafficking; a serious crime. However pollen evidence found on the *Cannabis* did not match the pollen statistics found on the plantation grown plants but did match local pollen characteristics for the home grow operation which the accused had initially stated was the case; this resulted in a charge of possession but not trafficking (Horrocks and Walsh, 1996).

Analysis of iron age vessels found in a hill fort in south-western Germany allowed researchers to understand how people in that era moved and possibly traded. The pollen analysis of the vessels remnant contents reinforced the hypothesis that the people in the fort had travelled or traded from an area over 100km away since the pollen grains found in the vessel were from plants that were specific to that area and not the local environ:100km was the distance between iron age forts in the area (Rösch, 1999).

It is because pollen grains are so small, they can range In size from 7-200µm (Mildenhall, Wiltshire and Bryant 2006) that they can travel and be easily embedded in lake sediments, preserved and thus provide a biological and historical record thousands of years hence. The extremely small size allows the pollen to travel great distances and 'hitch' rides employing all manner of materials and methods, pollen from the great lakes region of Canada has been found in Greenland carried by wind currents (Rousseau *et al* , 2004). Pollen can also rely on other forms of transport, it can be carried by insects, animals, birds , humans and other transient materials such as planes, trains and boats; practically any moveable material.

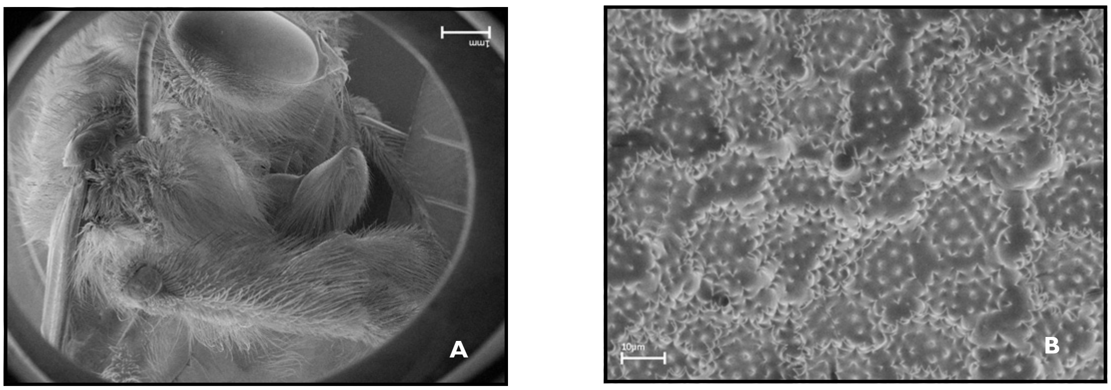


Fig.4. *Andrena fucata* (A) captured at the Quinta De Sáo Pedro, Portugal, collecting nectar and pollen on an a *Euroyps abrotanifolius,* an ornamental plant . Image (B) is a close-up view of the pollen basket, full of grains collected from *E. abrotanifolius.* The bee, one of the most commonly recognized pollinators carries large quantities of pollen both back to the hive and onwards, unknowingly to fertilize plants.

The durability of pollen as a component of vascular plants in comparison to other vegetative plant tissue is very high, the exine affords the pollen grain a greater degree of protection than most other vegetative components of flowering plants especially those of a herbaceous nature (Moore *et al,* 1991). The complex chemistry of the exine can render the pollen grain resistant to decay in moist, wet, saline, low oxygen, and dry environments and in many instances ensures their survivability. Pollen can in most cases withstand the rigours of the environment for many, many years without visible external deterioration. Although some species of pollen grain may collapse both over time and within a particular species as an occurrence of either both environmental and or biological process individual grains can still in many cases be identified to an accurate taxonomic level. *Taraxicum* and *Hieracium* according to Joosten and de Klerk, 2002 (cited Punt and Clarke, 1984) commonly produce abortive or malformed pollen that can still be easily identified as *Lacutaceae*.

Since pollen is small, durable, can attach itself to almost all types of materials, travel on the wind, water or adhere to other mobile opportunities and can often be identified in most instances to the genus level and in many cases the species level, it can become a natural indicator for work in the following sub-fields of palynology (Moore *et al*, 1991)

1 Plant taxonomy
2 Genetic and evolutionary studies
3 Melissopalynology
4 Forensic science
5 Aeropalynology
6 Vegetation history (Past human interaction on vegetative patterns)
7 Climatic change studies (global warming)
8 Monitoring genetically modified crops (GMO's)

Palynology and plant taxonomy

Plant taxonomy is concerned with evolutionary relationships between extant plant populations and communities and developing a level of hierarchal organization. Taxonomic classification once used a method that relied predominantly on easily recognized aspects of plant vegetative parts as a method of classification and grouping; predominantly flower parts. Carolus Linneaus was the principal behind binomial nomenclature and began in the 1700's to systematically organize the natural world by classifying plants, animals and birds under this hierarchal structure with his first published work, *Species plantarum* in 1753 and based the organization of the natural world upon shared physical characteristics (Royal Botanic Gardens Kew, 2000). Today that order based only on morphological characteristics has changed and few of Linnaeus's original classifications remain although many plants still carry the 'L' after their name in recognition of his efforts such as *Agrostemma githago* L (Stace, 1997, pg. 174). Even though much of the work of Linnaeus has today been re-examined he is still credited with the hierarchical sexual structure of plant classification that is currently used and that modern scientists still work towards albeit with new tools and knowledge (Stearn, 1960).

In the twenty first century, with the use of DNA analysis, radically improved microscopy including the use of scanning electron microscopes, an accurate and concise picture of species relationships in the biological word is rapidly developing and being refined. Where in the past, flower morphology played a primary role in the classification of plants today that responsibility is shared with DNA sequencing and tools such pollen morphology .

The phylognenetics of the orchid family have been under active study through the use of DNA sequencing for the past ten years, prior to the advent of DNA analysis the most current classification of this species was based entirely on morphological data and was completed by Dressler in 1993. Professor Mark Chase in the conference proceedings of *Orchid Conservation* incorporated new DNA information on the orchid family that resulted in the re-classification of many species and the introduction of a new subfamily for the *Vanilla* orchid and its relatives (*Vanilloideae*). Pollen analysis also came into play with regard to the classification of several 'primitive' genera of orchids in which the pollen was not tightly bound into masses (pollinia) as is typical with many genera, these 'primitives' based on this identification characteristic (loose pollen) were consequently moved into the subfamily *Epidendroideae* (Dixon *et al*, 2003, pg. 69).

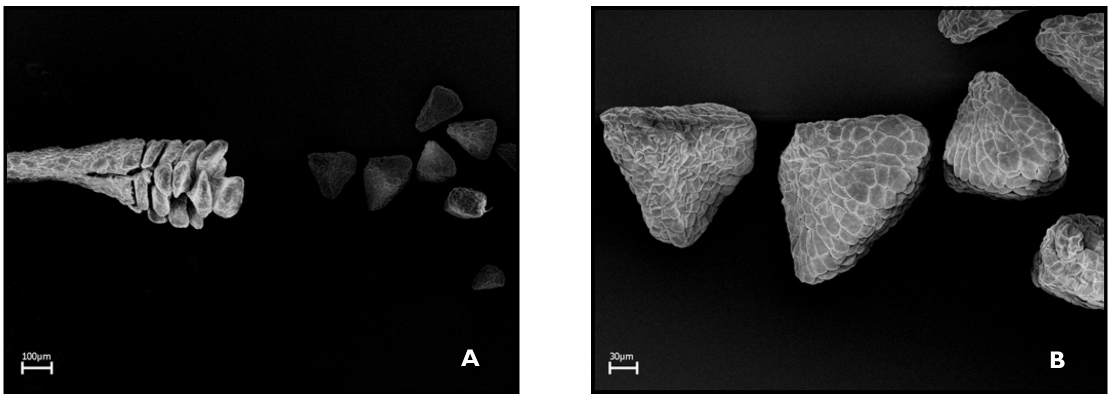


Fig. 5. Image (A) is the pollinia of *Anacamptis pyramidalis*, a native British orchid. Image (B) are the clusters or granules of pollen. The reclassification of some species of orchid by Dixon *et al* (2003) was based on the fact that some species did not demonstrate this common characteristic of the *Orchidaceae* family, instead of granules of pollen they released individual pollen grains from the pollinia.

Pollen analysis has played and continues to play a role in plant classification producing more character reference points for refined plant classification and groupings than the traditional external morphological characteristics of a plant alone.

The role of pollen analysis is further reinforced in taxonomic classification based on the work of Ackerman and Williams (1981) in their study entitled *Pollen morphology of the Chloreinae (Orchidaceae: Durideae)* and related subtribes. Ackerman and Williams found that the hesitant taxonomic classification of the *Chloreinae* group of plants that were wide spread geographically, was in fact sound based on their analysis of the pollen characteristics of the five species represented in that particular genera. However they found that two other genera, within the same area geographically, *Corybas* and *Acianthus* have quite different pollen characteristics and that their current taxonomic classification based in the *Chloreinae* grouping should be questioned further using DNA sequencing.

The work of Herber (2001) using pollen analysis (morphological characteristics of pollen grains) has questioned the current subfamilial taxonomic classification of members of the *Theymelaeaceae* family in relation to their past taxonomical classifications undertaken by Domke in 1934, which was based on external morphological characterises only; predominantly floral. Herber (2001) believes that several changes are warranted at the subfamilial level of *Theymelaeaceae* based on his observations of pollen grains and suggests that the following changes in current classification occur: *Octolepis* should be transferred from *Aquilarioideae* to the *Gonystyloideae* subfamily, *Aquilaria* should be transferred from the *Aquilarioideae* subfamily to the *Synandrodaphnoideae* subfamily and *Gyrinops* should move from *Aquilarioideae* to the *Thymelaeoideae* subfamily, Herber qualifies this reclassification in part by stating that "the shared presence of the crotonoid pattern (on the pollen grain) and other characters (further reference to his paper) in *Thymelaeoideae*, *Synandrodaphnoideae* and the re-circumscribed *Aquilarioideae* indicates that these three subfamilies form one monophyletic group".

The genus *Strobilanthes* is a species rich, taxonomically complex group of plants that has proven problematic and confusing to classify due to the high level of conflict based on morphological characteristics. However the work of Carine and Robert (2000, pp. 380) used pollen character reference points to attempt to resolve several species relationships and in fact were successful in clarifying some taxonomic classification problems using a few rigorously examined pollen morphological characteristics

The pollen role in genetic and evolutionary studies

Evolution according to Charles Darwin is the gradual change in biological forms to adapt to an evolving environment. Plants do this for if not they face the risk of extinction. However there are a few plants on earth that have remained the same physiologically . Plants that have resisted change at the risk of their demise. *Wollemia nobilis* found in 1995 as two isolated stands of a few trees west of Sydney, Australia is one such example (Jones *et al*, 1995) . Another prehistoric individual that has defied evolution is *Metasequoia glyptostroboides* discovered as a fossil record in 1941 and found as a living plant in 1944, in Madaoxi, China.

When a new species is discovered , botanists and taxonomists using morphological evidence will be able to place it to a reasonable degree of accuracy in a family, much as Linneaus did in the 1700's. However today , it appears that almost all new discoveries at some point in their cataloguing or placement in the hierarchy of the botanical world will undergo further and more intense investigation of familial relationships using methods other than observable morphological characteristics with the naked eye. The recently discovered plant *W. nobilis* is one such plant that recently underwent such scrutiny. Upon examination of the pollen grains of *W. nobilis* Dettmann and Jarzen (2000, pg191) found that the grains closely resembled the fossilized pollen of the species *Dilwynites* previously identified by Harris in 1965, the extant and pollen fossil shared the same morphological characteristics with regard to size and shape although there were slight differences in some of the sculptural elements, which were found to be finer on *Dilwynites;* the conclusion was that the two plants species were related. Dettmann and Jarzen (2000, pg 199) concluded that *W. nobilis* and *Dilwynites* are closely related when compared to other members of the *Araucariaceae* family and that the pollen from the species *D. granulatus* is almost an identical match to *W. nobilis,* having similar sculptural elements to *W. nobilis* than to other members of the genus *Dilwynites.* Thus suggesting a close familial relationship to *Dilwynites* and proposing that the placement of *W. nobilis* in the *Araucariaceae* family is indeed correct based on this evidence, eliminating other possible family relationships of *Cupressaceae*, *Taxodiaceae*, *Pinaceae*, *Cephalotaxaceae* and *Taxaceae*.

It is believed that land plants have evolved from a primitive ancestor but have adapted to the changing environment. Lampert *et al* (2007) suggests that the ability of plants to adapt from pollen tubes walls to woody plants depended on cellulosic, pectic and protein networks and that these networks must be ancestral, *Coleochaete,* a primitive prototype liverwort that also has these networks thus establishes the possibility of a link between evolved and primitive plant forms. Thus molecular biology is also a factor in understanding plant evolution.

Recent studies have demonstrated the importance of genetic adaptation in species response to quaternary environmental changes (Guinet, 1996; Huntley *et al*., 1997; Davis and Shaw, 2000). This summation was based on what is known about the modern genetic variability of current plant species and on theoretical reasoning. Davis and Shaw (2001) argued that a plant or plant communities response to a changing physical environment on an interglacial–glacial scale was probably not based solely on species migration, but on both migration and the ability of the species to genetically adapt. Huntley *et al*, (1997) however suggest that the ability of plant adaptation to a specific climate change incident is limited but the species migrates with favourable climatic progression. Modern biological populations of many plant species carry a considerable amount of genetic variability in their DNA , this variability has been the basis of past evolutionary change and according to Davis and Shaw (2001) played a role in past plant adaptation to environmental change.

It is further argued by Davis and Shaw (2001) that as a plants distribution changed during the late– quaternary period populations also underwent genetic changes. Seedling survival was greater in those individuals with the desired genotype that favoured local conditions and that over time natural selection would encourage the growth of populations with desirable genes adapted to the changed climate.

DNA analysis of modern plant species provides insights into past migration and distribution patterns (Burga and Hussendörfer, 2001; Comps *et al*., 2001), suggests surprisingly that some measures of genetic diversity are higher in newly colonized areas than in glacial refugia (Widmer and Lexer, 2001; Comps *et al*., 2001).

The *Acacia* genus in Australia is a very complex group of plants that interbreeds and produces numerous hybrids and crosses, as a result of its botanical promiscuity, and has always suffered from confusion regarding its morphological taxonomic classification . According to Guinet (1986) an analysis of pollen from *Acacia* in Australia has found a progressive morphological change in pollen characteristics following the north/south gradient and historic movement and colonization and spread of this species over time. The pollen data analysis however did concur with the current taxonomic standing and reconfirmed that a progressive evolutionary breeding program evolved with species movement based on the evolutionary characteristics of the species.

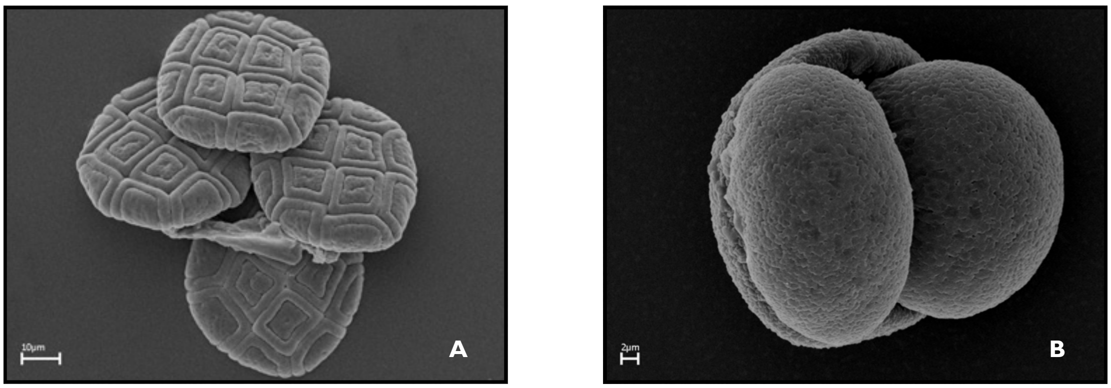


Fig. 6. Guinet (1986) studied the pollen morphology of *Acacia* species in determining their adaptation and progressive movement across Australia. Image (A) Is the pollen grain from *Acacia cyanophylla* collected in Portugal. Suyama *et al* (1996) successfully extracted DNA from fossilized pollen of *P. sylvestris,* (B) is a fresh pollen grain of *P. sylvestris*.

Plant DNA has been successfully extracted from fossilized pollen, specifically that of *Pinus sylvestris* (Suyama *et al.* 1996). With such groundbreaking work attempts in the future might lead to important developments and refinements in plant classification, this technology could for example be applied to the fossilised pollen of *D. granulatus* and confirm or deny its relation to the recently discovered *W. nobilis.* It may also have greater implications in tracing the origins of plant species as they dispersed across continents including post glacial dispersal and may be able to confirm plant population dynamics to a species or even local population level.

It would appear from the previously presented three examples, that of *Wollemia, Acacia,* and *P. sylvestris* that pollen analysis has a definite role in clarifying taxonomic classification problems but varies from genera to genera and family to family in its ability to refine outstanding taxonomic issues, this flaw in its use is based primarily in the fact that botanical sexual promiscuity of a species results in numerous hybrids and interspecific crosses. Detailed pollen morphology using SEM and LM requires precise application and should be used as an additional tool, not a stand alone one in the resolution of such classification issues and should be coupled with DNA analysis, molecular biology and taxonomic studies as equal partners in plant taxonomy.

Melissopalynology

According to Macintyre (2007) Einstein was purported to have said " If the bee disappeared off the surface of the globe then man would have only four years of life left. No more bees, no more pollination, no more plants, no more animals, no more life". This apocalyptic concern is shared today even more so than in Einstein's time through the 1920's, 30's, 40'4 and 50's. Today there is concern for the honeybee as an epidemic of decline is sweeping through hives. Colony collapse disorder (CCD) which started in the United States has already crossed the ocean and wiped out colonies in Europe(Macintyre, 2007). Of the 200, 000 species of animals that act as pollinators only 1, 000 are non-insect (or beetle) pollinated, clearly if the bee disappears then we face a calamitous problem; already lack of pollination has resulted in lower than expected yields in of blueberries in Maine, and almonds in California (Lammer-Helps, 2007).

Bees require four natural ingredients to survive, pollen, nectar, water and resin : resin is used to reinforce the hive, water to cool the hive and dilute the honey, nectar supplies the carbohydrates for energy while pollen is the source of proteins, fatty substances, mineral and vitamins (Canadian Association of Palynologists, 2007). Thus pollen and bees have developed a very intense productive symbiotic relationship, pollen is moved from plant to plant by bees ensuring pollination and hopefully subsequent seed set (plant benefit) but pollen is also lost or given up to the bee as a necessary food source for hive survival.

Since bee visits to specific plants are difficult to track on a large scale; a group of undergraduates tracked bees at the Quninta São Pedro research station in Portugal in March 2007, but managed to follow only a few; the study of pollen and bees is critical in understanding both the movement of bees and the functioning of the hive; this may help us understand and hopefully reverse such symptoms as CCD. The study of pollen and honey is called melissopalynology and although not a new science it is crucial in understanding the crops and plants visited by bees but more important to the apiculture industry: the specific pollen content of honey utilised in developing premium marketable honey and honey products.

Pollen can be incorporated into honey in many ways by

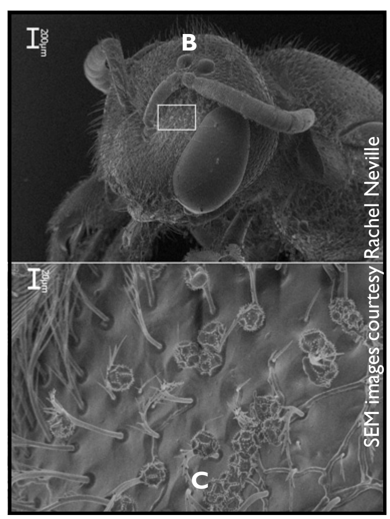


Fig. 7. A bee dusted with pollen from a member of the *Asteraceae* family. Note that only one type of pollen is usually found on a bee at any time. Thus pollen analysis can determine the pollen content of honey. If the pollen analysis of the honey determines that it is made from one genus of plant then the apiculturist may market that honey as a unifloral product which commands a premium price. Image (C) an enlarged area outlined in by the box in image (B) shows pollen grains caught in the hairs of the bee.

bees , when bees suck nectar while visiting plants they can ingest pollen at the same time, pollen can become attached to the fine hairs on the bee visiting flowers (see Fig. 7. pg. 21) while some bees have a specific function in the hive: to collect pollen as a food source. Once the bee returns to the hive the ingested pollen is regurgitated along with the nectar and deposited in the combs, bees groom themselves releasing externally attached pollen and of course those whose specific function it is to collect pollen empty these baskets into cells within the combs of the hive, thus a botanical fingerprint through pollen exists of the bee's behaviour.

Thus pollen becomes a critical tool in melissopalynology and helps us determine the workings of a hive; we can tell which plants were visited and the percentage frequency of such visits by analysing the content pollen of the honey and comparing the pollen extracted to a pollen catalogue. Pollen analyses of honey has been used since the 1970's as an industry tool in determining the floral nectar harvested by bees to produce honey (Moar, 1985, Soria *et al*, 2003). Arrone *et al* (2004) used spectra pollen analysis to determine the origin of several unifloral produced honeys in Southern Italy and found that spectra pollen analysis was a powerful tool in determining the origin of honey from several distinct geographical areas. Barth (2004) provides specific details about which plants were visited by comparing locally collected pollen samples and the pollen grains found in the honey of local hives; during comparative analysis over the period of research they found that the bees had visited *Eupatorium, Ricinus communis, Sapindaceae, Cecropia, Borreria, Gochnatia, Panicum, Spondias, Triumfetta* and *Veronia.* An accurate picture emerged of specific hives correlated with specific plant species visits. In all the research however it was necessary to have a comparative factor for pollen analysis, Barth and Luz had to collect and record local pollen sources, developing their own regional pollen catalogue to support their research; this reinforces the proposal for the development of regional pollen databases.

Forensic science and pollen

Undoubtedly one of the more exciting areas of palynology is forensic science. Television shows such as Crime Scene Investigation (CSI) have done much to glamorise the role of forensic palynology, before shows such as CSI, microscopic images of pollen were most likely unknown to the general public but now are recognised as a tool in criminal investigations.

Not all pollen however is useful in forensic palynology it depends on the pollen dispersal method and the quantity of pollen that is produced or dispersed: plants can be divided into three main groups with regard to pollen production and dispersal: autogamous, zoogamous and anemophilous.

Autogamous pollen are very efficient pollinators because they are self pollinating. As such they produce little pollen that is rarely dispersed into the atmosphere. However since it is not readily dispersed and it is produced in such small quantities it has little value in forensic palynology since it is rarely found outside of its immediate floral environment.

Zoogamous pollen is probably the most valuable type of pollen in forensic palynology. Plants that are zoogamous depend on pollinators (animals, birds, insects and beetles) to transmit the pollen. This efficient method of pollen distribution does not require massive amounts of pollen (anemophilous) but a greater quantity is produced than in autogamous plants. This type of pollen is valuable in forensic palynology since it has a resilient exine as it must be transported by the carrier across distance and variable environmental conditions; pollen grains of this type are often long-lived. The quantity produced is also a valuable factor since its deposition is usually specific whereas with anemophilous distribution the potential for broadcast contamination is great (Bryant and Mildenhall, 2007, pp. 8-9).

Pollen that is dispersed by wind is called anemophilous. We have all seen the clouds of pollen released from a pine tree in spring stirred by the gentlest breeze. Huge quantities of pollen are produced since the plant relies on wind to carry it, much of the pollen will be lost. The pollen grains produced must be small and light weight to travel on wind currents and as a result the grains can travel great distances; pollen from the Great Lakes basin in North America has been found in Greenland confirming that pollen grains can travel extreme distances (Rousseau *et al*, 2004). Anemophilous pollen though has little value in forensic palynology for it is difficult to ascertain with any degree of certainty its source according to Bryant and Mildenhall (2007, pp. 11).

One of the earliest cases of forensic palynology resulting in a criminal charge was that of a murder in Austria. Pollen samples collected from the accused clothing included both modern (fresh) and fossilized grains, the fossilized pollen being very rare and known to only one location in the country allowed the prosecution to pinpoint a location outside of the city where they suspected the murderer had been, when confronted with this evidence the shocked murder confessed to the crime and told them where the body was; in the area that the palynologist had indicated (Bryant and Mildenhall, 2007).

Forensic palynology is still not widely used today as one might think even though the technique has been available for many years. According to Horrocks *and Walsh* (1998) New Zealand is possibly one of the few countries in the world that routinely uses forensic palynolgy in criminal prosecution cases. Bryant *et al* (1990) in a survey of law enforcement agencies in the United States noted that few knew little about such techniques or had it employed it in assisting the resolution of criminal investigations.

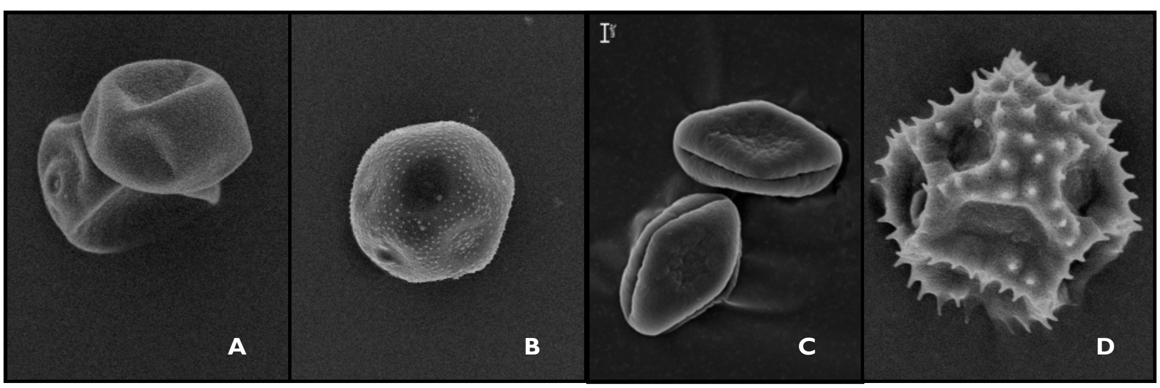


Fig. 8. A-D are pollen grains removed from the clothing of Dr. Fergus Massey and David Barnham-Fischer as part of a forensic exercise while they were conducting field work at Castle Hill Nature Reserve, West Sussex. The aim was to attempt to resolve identification issues when source plants are not known. A degree of identification can occur here based on information gleaned from the database. Pollen grain (A) is from the *Poaceae* family, (B) most likely the *Caryophyllaceae* family, (C) is unknown since there is no comparative data and (D) from the *Asteraceae* family. Further assumptions can be made knowing some of the characteristics of the site: a grazed (managed) chalk grassland, part of the Sussex Downs. With location information we can further propose but not verify without a site visit that (B) is most likely a *Stellaria* sp. which is endemic to this area and (D) is a *Leontodon* sp. also endemic to the area. Further resolution could occur with a site visit and the collection of pollen from flowering plants that would then be identified and compared to the unknowns.

The development of tools such as a "crime pollen calendar", where pollen was collected and catalogued from over 26 corpses in Parma (Northern Italy) are advancing the field of forensic palynology: pollen analysis is becoming recognised as a valuable tool in criminal investigations and the need for the development of databases of information where none has existed is recognised (Montali *et al*, 2006).

Palynology although not yet widely accepted across the globe continues to be used to solve criminal cases. Horrocks and Walsh (1998) describe a sexual assault case in New Zealand where the accused denied being in the area the witness said he was, pollen samples were collected from both the perpetrator, the victim and both locations (where the victim said the assault occurred and where the perpetrator said he was at the time of the assault); the pollen of *Cymbalaria muralis* was found on the perpetrators clothing: this plant is quite rare in New Zealand and was found growing in the location that the victim stated the assault had occurred at, other local pollen grains from this area also matched both victims and perpetrators clothing pollen samples: the perpetrator was charged with and convicted of sexual assault.

According to Horrocks and Walsh (1998) the use of 'Bayes Theorem' in the assessment of palynological evidence permits such evidence to be assessed in an un-biased structured environment that is presentable in a manner that is understood by the average juror. Pollen samples used as a tool in crime scenes are not absolutely conclusive, since pollen is variable in dispersion methods, quantity produced and longevity. For a criminal investigation to prove conclusively that a specific grain or type of pollen originated from an exact location is unreasonable, however, when combined with other forensic and tertiary evidence (including other pollen genera) it may paint a picture beyond reasonable doubt.

Aeropalynology

According to Rogers (1996) approximately 17% of the population suffers from allergies due to airborne pollution caused by pollen from anemophilous plants. Milkovska *et al* (2006) indicates in their research that 10-20% of the population of Northern and Central Europe suffer from allergic distress to their airways resulting from pollen released by anemophilous plants with the primary agitant being *Betula* pollen, responsible for 19% of these reactions. *Betula* is considered to be the main cause of pollinosis not only during actual pollination but prior and post local pollen dispersal since it can travel great distances (Jato *et al* 1999). Tsou *et al* (1996) states that the 84. 43% of the pollen irritants in Taiwan are from the following anemophilous plants: *Broussonetia, Cassuarina, Trema orientalis, Poaceae*, and *Humulus scandens* with *Broussonetia* comprising the main allergen at 70.37%. Hence the study of pollen release and distribution and movement in the environment is a primary health concern.

Forecasting pollen rains becomes an important tool in public health, Milkovska *et al* (2006) states that to prevent or reduce allergic suffering doctors need precise information about the timing a and intensity of pollen incidents particularly those that cause severe distress and require medical attention. Rogers (1996) revealed in her study of pollen rain in Toronto, Canada, that over a period of five years (1985-1990) the pollen of *Ambrosia,* a major airway irritant, was released in a regular seasonal progression and that most of the other pollen dispersed from anemophilous plants occurred over a 2-3 year cycle; these releases were 20days for trees, 5 days for grasses and 2 days for ragweed (*Ambrosia*).

Predicting local pollen rain intensity as a preventative medical treatment tool however is not all conclusive, pollen travels great distances as previously mentioned studies by Rousseau *et al* (2004) indicate; pollen was found in Greenland from the Great Lakes basin in North America . This fact was previously reinforced by Salas (1983) cited Moar (1959) that a major constituent of local pollen was transported great distances into the Tasman and Pacific regions of Australia and that local pollen rain was assumed to contain a large proportion of 'non-local' pollen types.

Rogers (1996, pp. 169-223) lists 54 major pollen groups analysed during her study, 14 were organised to the family level only, while the remainder were classified to the genus; none were classified to species level. I expect some confusion over the identification of pollen grains in this study also existed because of lack of comparative morphology. Rogers listed *Castanea* as one of the constituents of pollen rain, this suspect grain could have been confused with similar pollen types. The reasoning behind this observation is that *Castanea dentata* is essentially an extinct tree in Canada, eradicated by chestnut blight over 100 years ago; the only evidence of this plant that exists today are suckers growing from stumps These suckers are low in the forest canopy, personal observation indicate less than 3m in height and seldom flower; thus bringing to question their role as a major constituent (1 of 54 recognised) in pollen rain in the Toronto area. Rogers study indicates a need for accurate pollen grain identification, as she herself states, this argument is reinforced by pollen production responses to climate change that are evolving rapidly; development of efficient sampling and automated pollen counting methods will great assist and advance of our understanding of the constituents and movement of pollen rain (Rogers, 1996, pp. 138).

Tracing vegetation history

Analyzing past species vegetative patterns and comparing them to current plant ranges allows us to predict species movement due to climate and cultural influences. It can also provide historical glimpses into the way a certain area or vegetative district may have appeared and evolved helping us to understand plant evolution and dominance, species movement, historic human interactions and current aesthetic, evolutionary and commercial impacts.

Nakagawa *et al* (1999) used quaternary palynolgy to compare the past vegetative range to the current *in situ* growing range of *Pinus cembra* around the Lake Cristol area in the French Alps. In using this technique Nakagawa and associates were able to map the extent of decline for this particular species of plant. Using fossilized pollen samples they observed the pollen of Himalayan Pine with SEM and succeeded in reconstructing a more reliable vegetation history than with LM (Nakagawa *et al*, 1999).

New Zealand, the world leader In forensic palynology is also very progressive analysing preserved pollen to predict past vegetative patterns. In the Dew Lakes area on the South Island, New Zealand, pollen analysis was used to confirm that 10, 500 years ago a *Nothofagus-Podocarpus* mixed forest existed and is identical to what remains today, researches noted however that *Nothofagus* species increased while *Podocarpus* and other mixed species of shrubs declined (Dodson, 1978). Thus pollen analysis confirmed Dodson's suspicions and that of many silviculturists, that *Nothofagus* was in fact excluding other valuable lumber species but that the time frame for such exclusion was over a period of many thousand s of years, negating the commercial impact substantially (Dodson, 1978).

McGlone and Moar (1998) discovered that a Maori settlement in the Lake Pukaki region on the South Island of New Zealand had a direct influence on the current remnant vegetation species, *Halocarpus bidwilli*, a large shrub. An extinct Maori village over a period of years transformed this area from scrub to grassland through extensive use of fire land clearing practices to the point that the current vegetation (indigenous grassland) has not permitted the reestablishment of *H. bidwilli* over the past 600-800 years (McGlone and Moar, 1998). Hence through fossilized pollen analysis and radio carbon dating what was seen to be natural, undisturbed grass land was in fact a severely altered and at one managed landscape that had changed dramatically from what it was 800 years ago.

Lacourse and Gajewski (2000) had formulated several questions about the direction of black spruce (*Picea mariana*) migration in the southwest area of the Yukon, Canada's most northerly territory. Through quaternary pollen analysis they were able to discover that *P. mariana* migrated north from British Columbia, eventually moving up the Mackenzie Delta some 9000 years ago. Their findings concur with Cwynar and Spear (1991) who discovered that *P. mariana* arrived in neighbouring Alaska 9000 years ago, but in fact found evidence on Antifreeze Pond on the Alaskan-Yukon border that *P. mariana* was a dominant species in that area 8700 years ago.

Through pollen analysis, predominantly, quaternary palynology combined with other scientific tools such as radio carbon dating we understand how plant movement has changed, how past human interaction has changed vegetation patterns and how possible future human, cultural and climatic influences may alter the landscape.

Climate change studies

The amount of seed produced is often an indication of plant health or environmental conditions, in years of drought, frequently in the year following the drought, a woody plant species such as *Betula* or *Picea* will produce a heavy seed set: in response to extenuating environmental conditions a plants ultimate goal is survival. Greater seed set is the direct result of greater pollen production, and thus a anemophilous plant leaves a botanical fingerprint which is often deposited in lake sediment: preserved, providing us with a record of past ecological and environmental incidents . Delph *et al* (1997) states the growing conditions of the plant will influence both the quantity and quality of its offspring which thus can be tied to pollen, seed and plant populations following an ecological incident. Modern pollen rain is often modelled to develop a picture of past vegetation patterns which are compared to pollen found in lake sediments, the pollen analyses permits us to glimpse plant movement, environmental and cultural influences and develop a comparative picture of past and present vegetative patterns Seppa and Bennett, 2003).

Paleoclimatological use of pollen records according to Seppa and Bennett (2003) has advanced dramatically over the past number of years , improved classification and automatic pollen counting has done much to refine the quality of information drawn from this field and permitted the development of past climatic and vegetation patterns with what they hope is a reasonable degree of accuracy. The need for long term paleoclimatological data is great, since today we are concerned with the human influence on the environment and the effects of global warming. However for accurate climatic modelling to occur pollen data requires , credible, careful assessment based on comparative meteorological data and even then pollen based climatic reconstructions are likely to remain approximate in nature providing at best insights in past weather patterns (Seppa and Bennett, 2003).

GMO crops and pollen

Genetically modified crops (GMO's) foothold in traditional agriculture is gaining. In the United States (US.) it is estimated, according to a report by the Agricultural Policy Analysis Centre based at The University of Tennessee, that 86% of all soybeans, 76% of all cotton and 46% of all corn planted in the US. last year were genetically modified; an increase over the previous year of 5%, 3% and 6% respectively (Ray, 2004). Corn acreage dedicated to GMO's is increasing faster than that of soybeans and cotton, the other two main US. GMO crops, over 36.5 million acres were dedicated to GMO corn in 2003 (Pew, 2007)

Pollen drift from GMO crops is now a major concern with many farmers and environmentalists alike. Those farmers who work certified organic farms fear loosing their certification if their crops become contaminated with pollen from neighbouring GMO crops. While environmentalists are concerned about the decline in butterfly and bee populations that may be attributable to GMO crops.

According to Thomison (2004) farmers who grow identity-preserved (IP) grain crops risk loosing the IP premium priced product status if their grain becomes contaminated by GMO's. A significant portion of IP grain products are destined for exclusive overseas markets, such as Japan, and as such they are subjected to rigorous testing to ensure that they are not contaminated (Thomison, 2004).

Colony collapse disorder of bee hives is a growing critical phenomena of the apiculture industry. Dively (2007) suggests that the public perception of CCD is possibly linked to GMO corn, specifically *Bacillus thuringiensis* (Bt) modified plants. According to Dively (2007) this has been disproved in her research, bees were fed pollen from GMO sweet corn, with a higher concentration of Bt than would normally be obtainable from field grown GMO's and naturally foraging insects, yet the bees in the lab showed no adverse effect or weight loss due to this diet.

Cornell University researcher John Losey, in the journal *Nature* sparked controversy in 1999 when he reported that laboratory findings confirmed that monarch butterfly larvae died after eating milkweed (*Asclepias* sp.) that was dusted with wind blown pollen from GM corn (Pew, 2007). The controversy still rages today about the safety of GM crops and although current research states that the effect of Bt corn on Monarch populations is minimal the safety of GMO's has struck a chord with the environmentally conscious public.

Thus the question raised is who is responsible for pollen drift? Ray (2004) suggests that since the very early days of agriculture no responsibility was assigned for pollen drift, if a farm product could not be contained by a fence then it was not the responsibility of the owner. Obviously attempting to contain pollen from large GMO fields would be impractical, however does this dissuade the owner of a GMO crop from affecting the income of another individual whether it be someone next door or a distant neighbour.

The Danish government disagrees with the opinion of Ray and have enacted legislation to protect Danish farmers crops from GMO contamination. Paragraph 9 (1) of *Act No. 436* (2004) states that "the Minister for Food, Agriculture and Fisheries shall pay compensation to any farmer who suffers a loss due to the occurrence of genetically modified materials in his crops".

Pollen analysis will obviously continue to play a major role in the monitoring of GMO's, pollen traps like those pictured on page 6 are expected to provide the analytical data to further investigate the effects or apparent lack of, on crops but also on the environment.

Process and methods

The identification of an unknown pollen grain is only possible if it is compared to a known entity, an identified grain from source. The bench marks for the development of this database are

Process and Methods

all known or identified plants with the pollen collected from the flower structures of the plants.

Pollen was collected from plants, beginning on February 7, 2007 , dates for pollen collected by Rachel Neville are not known although the SEM date is given; pollen collection continued until the end June, 2007. Pollen was collected from known plants with identification confirmed according to three main reference books: *The Wild Flowers of the British Isles* (Garrard and Streeter, 1983), *The Wild Flowers of Britain* (Phillips, 1972) and *New Flora of the British Isles* (Stace, 1997), the later text was considered the most current and comprehensive reference of the three.

A data base of possible plants was developed using these reference materials which identified all indigenous, native and naturalized or commonly occurring plants in the United Kingdom. This was necessary to focus the research and resources on a specific target group of plants. Limited work was previously undertaken by an undergraduate student Neville, who collected and scanned approximately 35 pollen samples. As a result of Neville's work it was anticipated that the resources (time and money) available to this project would permit the collection and scanning of 100-150 samples . At the conclusion of the field work towards the end of June 2007 over 300 samples were scanned, 267 SEM images and associated plant photographs are presented on pages 41-235.

Different vegetative zones were visited with the expectation that a broad cross-section of plants representing the United Kingdom flora would be collected. Plants from aquatic, woodland, alkaline grass downs, forest, acid forest, hedgerow, meadow, bog, arable land, and garden were targeted.

It was noted that reviewing the work Neville (SEM images) that several pollen grains had collapsed when photographed during SEM, this may have been a characteristic of a particular species or the analytical process (the ways the samples were handled or timing; the length between collecting and analyzing). Therefore one of the objectives of this research was to collect fresh pollen and produce SEM images as expediently as possible since the viability characteristics (pollen collapse) of many of the pollen samples collected for this project were and are still unknown.

Where possible samples were collected from source following the target lists and analyzed within one to two weeks; dates of collection and analysis are given under each of the SEM images in the main body of the text.

As previously mentioned each pollen sample was collected from source through varying locales in the Sussex area; collection locations are also noted under each of the SEM images, thus helping to develop a picture of local distribution and conditions. Once a target species was identified in the field several blooms were removed from the plant, these were placed in labeled (date, location and plant if known) paper collection bags, the tops of the bags folded to reduce cross contamination and refrigerated until prepared for SEM analysis, see Fig. 9A. Once

35 samples were collected identification was confirmed using the outlined texts and keys if necessary: this information was written on the outside of the appropriate collection bag.

Numbered aluminium SEM stubs with adhesive tape were prepared (14 stubs to a plastic case, Fig. 9B) .The anthers were removed from a target plant and wiped across the adhesive on the stub, the stub was then placed in the case and closed, while the procedure was repeated for each of the target plants; it was imperative that cross contamination be avoided of both the stubs, samples and working area. Only one sample was removed from the collection bag and prepared for SEM at a time, between sample preparations the working surface and tools (scalpel and tweezers) were wiped with a 5% bleach solution.

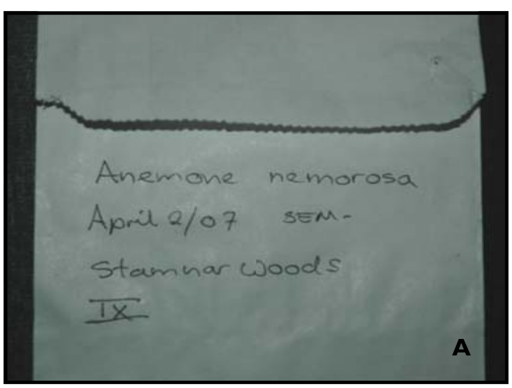


Fig. 9. The purity of a pollen sample must begin in the field as cross contamination can produce dubious results or at least consume valuable time and resources. Image (A) represents a collection bag, noting plant name, collection date, location and a number corresponding to the appropriate SEM stub. SEM date was also added once completed as the collection bags and samples were retained for the duration of the project. Additionally, samples were prepared for freezing (-20° C) with the same information on the Ziploc bag. Image (B) shows a prepared set of 14 SEM samples in their case, these have been sputtered coated and are ready for SEM. SEM stubs were not retained, since it was suspected that extensive pollen grain collapse would occur because of sputter coating and SEM.

The stubs were sputter coated with gold palladium to induce conductivity in the SEM sample (Fig. 10A). Three images of each sample were taken, in varying magnifications. This technique allowed the confirmation that the pollen was in fact from a particular target species (a majority of similar grain types) since in several samples, especially anemophilous trees, foreign grains were found within the flower structure and had been transferred to the SEM stubs.

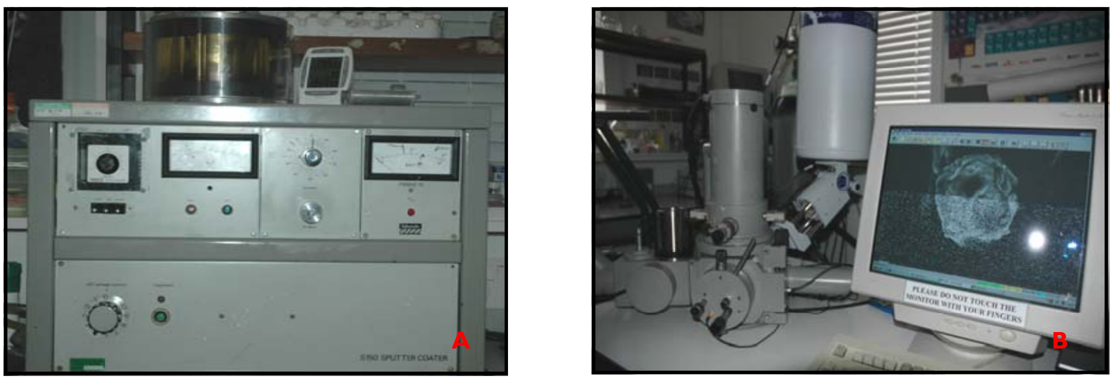


Fig. 10. There are two simple steps to SEM imaging. The first is to coat the SEM stub so that it is conductive and thus will produce an image when scanned. (A) is the sputter coater, with the chamber at the top holding specimens for coating: coating used was gold palladium (see Fig. 9B for sputter coated SEM stubs). Image (B) shows a scan in progress, the actual scanning has stopped and the image is now being verified on the monitor, hence the partial image. SEM can be an efficient process, about 35 SEM stubs could be scanned (including sputter coating) in an average day producing three images for each stub: 105 SEM images a day.

Some flower colour variations within a species were analyzed on a comparative basis for possible differences in their pollen characteristics. Plants such as *Centranthus ruber* where the predominant species has a red flower but where white flowered forms are commonly found were collected and analyzed. *Symphytum officinale* which is normally white flowered also has a purple coloured form and *Verbascum thapsus* usually yellow flowered, a white flowered form , that is rare. In all three instances the pollen from each of the different flower colours was collected and analyzed, for the comparative analysis of colour variations see each of the family summaries for that particular species.

Additionally *Primula vulgaris* has two flower types, pin eyed and thrum eyed. Pollen was collected from both and analyzed, the results are presented on page 184.

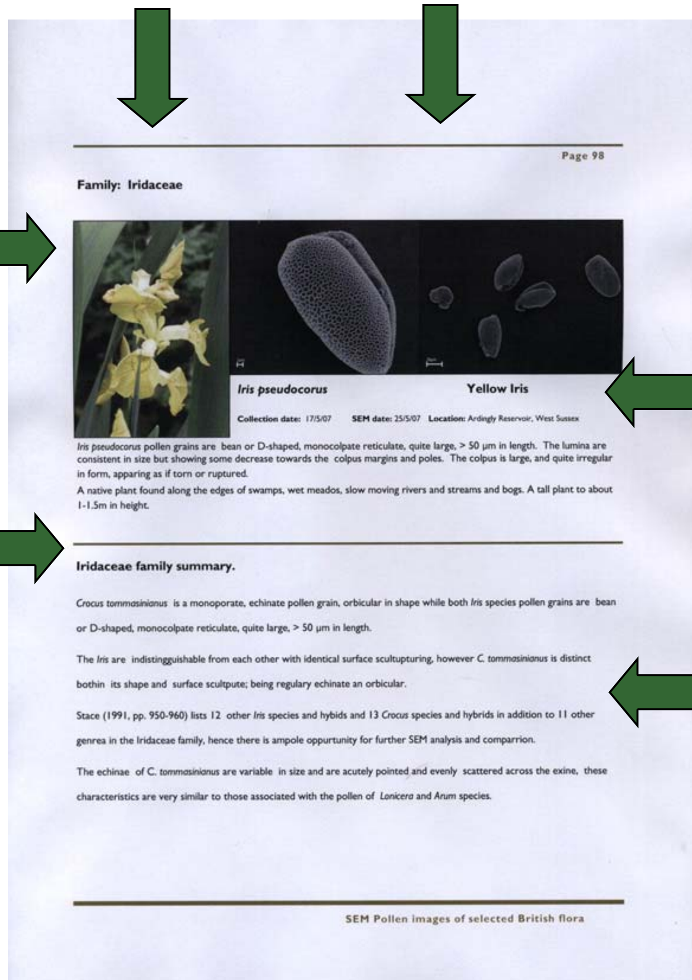
After the preparation of SEM stubs the remaining vegetative floral parts were placed in Ziploc ® bags, labeled with botanical name, collection location and date and frozen at -20ºC for later possible comparative analysis (future undergraduate work) using a light microscope.
The SEM and floral images are organized alphabetically within their respective families and constitute the majority of this work; 194 pages.. A pollen morphological analysis is given under each species as well as brief notes about preferred growing locations of each plant. At the end of each family a summary recounts the morphological characteristics of each pollen grain analyzed but also compares and contrasts them within and outside the family. Recommendations for future work are also given in this section.

Pollen data base: Page interpretation.

Fig. 11. A sample page from the pollen database project. This page includes a family summary in addition to the basic fields of information presented for each of the plant species examined.

Family name

SEM images. Three images were taken of each sample although two are presented here. The third was used for comparative morphology. The two images presented are the closet focal range presenting the greatest detail.



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Family: Iridaceae

Iris pseudocorus **Yellow Iris**

Collection date: 17/5/07 **SEM date:** 25/5/07 **Location:** Ardingly Reservoir, West Sussex

Iris pseudocorus pollen grains are bean or D-shaped, monocolpate reticulate, quite large, > 50 µm in length. The lumina are consistent in size but showing some decrease towards the colpus margins and poles. The colpus is large, and quite irregular in form, apparing as if torn or ruptured.

A native plant found along the edges of swamps, wet meados, slow moving rivers and streams and bogs. A tall plant to about 1-1.5m in height.

Iridaceae family summary.

Crocus tommasinianus is a monoporate, echinate pollen grain, orbicular in shape while both *Iris* species pollen grains are bean or D-shaped, monocolpate reticulate, quite large, > 50 µm in length.

The *Iris* are indistingguishable from each other with identical surface scultupturing, however *C. tommasinianus* is distinct bothin its shape and surface scultpute; being regulary echinate an orbicular.

Stace (1991, pp. 950-960) lists 12 other *Iris* species and hybids and 13 *Crocus* species and hybrids in addition to 11 other genrea in the Iridaceae family, hence there is ampole oppurtunity for further SEM analysis and comparrion.

The echinae of C. *tommasinianus* are variable in size and are acutely pointed and evenly scattered across the exine, these characteristics are very similar to those associated with the pollen of *Lonicera* and *Arum* species.

SEM Pollen images of selected British flora

Image of bloom for comparative flower morphol-

Pollen grain morphology is presented here and is based on the key from Moore *et al* (1991). A glossary of terms is given at the back of the manual also based on Moore *et al*'s work.

A brief plant description is also given, Basic growing conditions are noted as well as plant height. Stace (1991) was the basis for this information along with field notes.

Plant information and location should assist those interested in viewing or collecting the plants .

Botanical and common names (Stace, 1991) Below the botanical and common names are found the collection statistics, pollen collection and SEM date as well as the collection location. This information builds a regional picture of plant distribution. The dates may help explain pollen collapse issues.

Family summary.
At the end of each family section a summary is presented, comparing and contrasting the pollen characteristics for that particular family. In addition opinions are presented on further taxonomic and pollen morphological issues.

Discussion, summary and conclusions.

Discussion and Summary

In the family summary section of this paper I have encapsulated the pollen morphological characteristics for each species depicted and compared and contrasted them to other family members. In addition plants from one family may have been compared and contrasted to another family if they identical morphologic characteristics. Additionally the summary section following the family has suggested preliminary conclusions regarding the effectiveness of SEM in pollen analysis. This has resulted in what I consider to be some potentially outstanding taxonomic issues regarding pollen morphology of some plants relative to their floral attributes when subjected to scrutiny in their familial relationships. Technical problems such as heavy coatings (tectum) on some pollen types rendering detailed examination of sculptural detail difficult are also explored. Mention in also made to type collection comparison for species that show some variability within single collections but also lack detail that may distinguish them to a class in only one sampling interval. Hence, for a summary review and detailed conclusions for a specific genus, species, variety or cultivar refer to the appropriate family summary. Presented here is a summary of the results regarding the development of a SEM database, the role of SEM in plant science relationships and what the future may hold for the field of palynology.

The summary section of this research will endeavor to discuss and answer the following five questions regarding the role of SEM and its relationship to palynology:

1. Is SEM the only choice in pollen analysis?
2. What is a type collection and is it necessary?
3. Automatic counting and pollen recognition, why?
4. Is there a need for the development of further palynology resources?
5. Is a pollen key useful in identification?

It is fortunate that most genera analyzed under SEM show distinct and recognizable morphological differences thus enabling classification to the species level. There are several instances though where SEM does not resolve pollen morphological differences to a degree that is acceptable: a genus level of classification only is a common occurrence. Some pollen grains where a extensive tectum covers underlying sculptural detail the morphological characteristics of that pollen grain are hidden, not permitting the identification to possibly even the genus level let alone further refinement to the species level. Simple tools are available to easily resolve this issue, in the form of an acetolysis wash. Further details about this technique are presented in the family summary where appropriate (*Euphorbiaceae).*

However there are many genera that do not have detectable differences even when surface sculpturing is clearly visible, examples are the *Poaceae* and *Taxaceae* families, where classification based on pollen morphology stops at the genus level. Technology such as confocal miscoprosy and modern light miscoprosy need to be included here to possibly further refine and define pollen morphology. Skvarla *et al* (2003) through transmission electron microscopy

(TEM) has examined the annulus –pore of several members of the *Poaceae* family in the hope of resolving some of these outstanding classification issues and even though this approach has worked on another complex and confusing family, *Plantaginaceae*, additional detailed study is warranted to determine if such analysis could be used on all members of the family. Even then we may not be able to definitely classify all pollen grains to the species level and I suspect certainly not to the variety, subspecies , cultivar or interspecific hybrid levels.

Is SEM the only choice in pollen analysis?

Thus there is no one piece of technology or tool that will provide all the answers in understanding pollen morphology, instead we must rely upon a combination of them. The majority of the images available in databases and on the internet are LM stained slides of pollen and although these provide necessary detail about cell wall, pori circumference and sculptural thicknesses these tools still do not produce a clear 3 dimensional or easily understandable visual image of the pollen grain: SEM does. Thus the combination of the two tools, or even three, TEM, may provide a comprehensive understanding of a particular pollen grain. Again this may not always be as conclusive as we would like it to be, we only have to look once again at such families as *Poaceae* and *Taxaceae* where grains appear similar under both imaging regimes and even with TEM (*Poaceae),* morphological classification issues are still unresolved.

Science is part of a process where we focus on a specific detail or issue in relationship to a much more complex 'picture', This process occurs in pollen analysis, where we develop an interest in the structure of the pollen grain but may not include or relate it to the larger picture, where did it come from and how did it get here? These are questions we need to ask ourselves especially when trying to analyze and understand pollen morphology. I believe it is important to realize that part of that 'big' picture is to understand the relationship between the pollen grain and its source; the flower. In other words, if pollen grains characteristics are similar within a given family shouldn't the pollen morphology be also? I believe the answer to that question should be yes; but a yes which requires further clarification. If we examine the *Rosacace* family, one of the largest plant families in the world , a group that is very complex and produces many natural hybrids yet with numerous subfamily levels, we begin to understand the complexity with which we are faced; some plant taxonomy and pollen morphological characteristics do not relate to members of even the same genus (Stace, 1991) . Does this mean that we need to reclassify plants? In some cases I think we do, as we are at present and will continue to do so as new technologies and our understanding of plant familial relationships continue to grow. The International Code of Botanical Nomenclature (ICBN) is a work in progress, we only have to look at the recent re-classification of the *Aceraceae* and *Hippocastanaceae* families to the *Sapindaceae* family. These two groups of plants I learned, not so long ago were organized in their separate families (*Aceraceae* and *Hippocastanum*) and although they remain in separate subfamilies they are now in the same family. What caused this familial upheaval, was it pollen morphology? No. Was it DNA analysis? Yes, partially, but it was also a review of the morphological characteristics of the plant itself. If we examine the two types of pollen grains (*Acer* and *Hippocastanum*) f we see similarities in pollen size, shape and sculpturing elements but we can also detect characteristics that may be also common to other families.

What is a type collection and is it necessary?

This research has made repeated reference to type collections. Moore *et al* (1991) in the book *Pollen analysis, second edition* which is primarily concerned with quaternary palynology recommends the establishment of a type slide collection as a matter of common procedure. Since Moore's work relies primarily on LM images and was written before the rapid advancement of digital technology such a collection the importance of such a resource could be understood. However the primary disadvantage of holding a static collection of slides is that it would not have be readily accessible unless of course one visited Oxford. Referring to a type collection in this research it should be assumed that it refers to a database of images. It is agreed that both LM and SEM and possibly TEM images are tools that should be employed in pollen analysis and hence images from all technologies should be included in such a database. Through the process of this research readily accessible images that could be compared to current SEM images developed at the University of Sussex have been difficult if not, in many cases impossible to find. The simple summary of internet searches for SEM pollen images on page reinforce the lack of material accessible. Additionally the forensic exercise on page 25 where one pollen grain is shown that has no comparative data is yet another example of a dearth of reference material available; although this issue is easily resolvable through a site visit and interviews with the onsite researches such resources may not always be so readily accessible.

Throughout this research efforts have been made to collect pollen at source, from verifiable plant populations so that reliable SEM images are developed. However, flower character and pollen morphological relationships should not be ignored, the *Brassicaceae* family is an example of pollen/flower morphology relationships since the pollen is identical to is relatives as is the flower structure. *Mentha aquatica* shares the attributes of the *Brassicaceae* family even though it resides in the *Lamiaceae* family: should this plants familial relationship be questioned based on both flower and pollen morphology?

Automatic counting and pollen recognition, why?

Developing a map of past vegetative patterns, assessing climate change and plant interactions and predicting pollen counts and potential sources of allergens requires both an intricate knowledge of pollen morphology but additionally massive amounts of information to support the conclusions of such research: the greater the pollen numbers analyzed the sharper the picture realized. Quaternary palynology must look at thousands of pollen grains to build an accurate picture of a small area. Lacourse and Gajewski viewed over 5000 pollen samples that had to be manually identified before they could begin to understand the past plant species range around Sulphur Lake in the southwest Yukon Territory, Canada. In 35 core sample of lake sediment over 50 pollen grains per sample were observed under SEM in the analysis of the Lake Cristol area in the French Alps in an attempt to understand and map the range of Pinus species and compare it to present day ranges (Nakagawa *et al*, 2000).

Thus we can see that the argument for automatic recognition and counting of pollen samples would play a significant role not only in this one field of palynology but others. Consider aeropalynology; subject collected samples from wind traps (page 6) analyzing with SEM and an automatic recognition tool and waiting for the results. We could conceivable build a very accurate if not daily picture of pollen rain and the species producing it producing accurate regional warning and alerts.

Automatic pollen grain recognition is not a new tool, it has been successful in a number of cases already. Rodriguez *et al* (2003) has use it successfully on members of the Urticaceae family, with over 80% accuracy. Jato *et al* (1999) has used automatic recognition and counting in aeropalynology studies looking at allergens in Japan, their research attributes an 84.43% success rate in grouping pollen to five genera.

Automatic pollen grain recognition needs a model or a set of models that the compute can map and produce the 'blueprint' that is required in automatic recognition. To be as accurate as possible pollen types need to mapped that are cover all of the potential (or as many as are known) variables for a specific genus or species of plant; thus a source of information is required. The development of a database of information could be that source, a source not only using the University of Sussex's library of pollen images but might also include individual researches work or other institutions work. Such institutions might include; The Royal Botanic gardens, Kew, The Royal Botanic Gardens, Edinburgh and Dublin Botanic Gardens to name a few.

Is there a need for the development of further palynology resources?

There are as previously stated few resources available on the internet that deal will pollen imaging in any credible way, however there is also a significant lack in printed resources dealing in a more general nature with pollen analysis. What little there is deals principally with quaternary palynology and not with current pollen imaging and floral relations and the modern world of pollen. Both the University of Sussex and the Royal Botanic Gardens, Kew and Wakehurst have very limited printed resources available. The principal sources relate primarily to quaternary palynology and include the primary reference work by Moore *et al* (1991) entitled *Pollen analysis: second edition*, *Textbook of pollen analysis* (Fageri and Iversen, 1989) and the rather slim pictorial book *Atlas of airborne pollen grains and spores in Northern Europe* (Nilsson, *et al*, 1977).

New and fresh resources are needed, the *Atlas of airborne pollen grains and spores in Northern Europe* is a good pictorial resource although limited in scope but even though it contains photography that is outdated it is one of the few texts to compare and analyze pollen grains through both SEM and LM. *Pollen analysis: second edition*, seems to be today, the main reference book on pollen, frequently cited in palynology papers and websites. It has an excellent key (although directed towards fossilized pollen) and a limited but useful section of LM and SEM images. Its limitation is that it is based primarily in quaternary analysis. A fresh approach I believe is needed , a combination of the two, new and more comprehensive images of SEM and LM are required, include an analysis of flower morphology and pollen relationships, a revised key that is simple to use and a discussion on current issues and their relationship to palynology such as climate change, plant evolution relationships, aeropalynology and allergens. Of course this will all depend upon the intention and audience who would use such a resource!

Is a pollen key useful in identification?

A comprehensive pollen key is a critical tool In the field of palynology. The pollen key in *Pollen analysis: second edition* by Moore *et al* (1991) has been indispensable during the course of this research and although a key is included in *Textbook of pollen analysis* (Fageri and Iversen, 1989) the key in *Pollen analysis: second edition* is simple to use and provides much more descriptive detail on pollen grain morphology. That said however, it took almost a week of repeated use to even begin to fully comprehend the complex terminology involved. It is understood that such terminology must exist to provide clear and concise relationships between itself and the pollen morphological characteristic it is depicting however for the casual "lay person' I believe such terminology would be overwhelming and discouraging.

A pollen key is essential but however I believe at times overly complex and needs to be adapted from user to user. If for example a lawyer called an expert witness to describe incriminating pollen evidence in a criminal prosecution and used the terminology common to palynology (see glossary) in describing pollen evidence to a jury I am sure they would be confused at best if not overwhelmed. This is confirmed by Horrocks and Walsh (1996) who state that "the court is assisted by having the scientist assess the evidence and present it in a manner which is more readily understood by lay people".

Conclusion

From clouds of pollen produced by anemophilous trees, to the traditional zoogamous route where these tiny projectiles are carried on the backs of 'beasts' to the incestuous, miserly, autogamous self pollinators it is in the quiet woods, on the chalk downs, or along the rivers edge that the world becomes a botanical battlefield for self-preservation and acquisition of new territory.

It is in the garden trenches of nature that pollen, deposited, transported or preserved produces a botanical fingerprint that can yield so much knowledge both now and of lives lived. Pollen has aided our understanding of plant evolution, the work of bees, solved crimes, caused allergies, told us what people ate and where they traveled thousands of years ago, predicted wind currents, traveled across oceans and assists us in understanding and predicting the effects of climate change.
Pollen does so much, it does what it is supposed to do; fertilize plants resulting in the continuation of life on earth, but unknowingly it does much, much more. However, are understanding of pollen morphology and its use in modern science is still very limited and in its infancy, few well developed, accessible databases exists and even fewer books; we need to know more and we are ready to learn, hence towards a national pollen database.

Glossary of palynology related terms.

Apocolpium: The area at the end of a zonocolpate pollen grain delineated by the terminus of the colpus

Aeropalynology: The study of pollen and spores in the air (often relating to allergies)

Anemophilous: A plant that produces massive amounts of pollen and is wind pollinated.

Autogamous: Plants that are self pollinating and as such produce little pollen.

Baculum: A sculptural element that is longer than broad and always higher than 1µm: Bacula, singular. Baculate-to have.

Clavae: A series of projections on a pollen grain which is higher than it its broad and narrower at its base than its terminus: Clava-singular, Clavate-to have.

Colpate: More than one colpi.

Colpus: A furrow or groove that traverses the pollen grain, the length must be => than 2 X width: Colpi-singular.

Columellae: Small rod like elements that are attached to the tectum at the top and at the bottom to the nexine.

Columellate: With columellae.

Costa: A collar or thickening, maybe ridge-like around an aperture.

Dizonocolpate: In equatorial view having two colpi.

Echinae: Pointed or conical elements around the outside of the grain.

Echinate: Having echinae.

Endoaperture: Aperture in nexine.

Endocracks: Fissures, cracks or gaps in the nexine.

Endoporus: An endoaperture that may be faintly circular or elliptic: length/width ration <2.

Equatorial Bridge: An interruption of the colpus at the equator where the mesocolpia joins together over the colpus.

Glossary of palynology related terms continued.

Equatorial Girdle: A circular Endoaperture circumventing the equator of a zonocolporate pollen grain.

Eureticulate: The tectum is partially dissolved with the arrangement of the columellae often corresponding to that of the muri.

Foveolae: Large perforations in the tectum, but narrower than the area between each Perforation: Foveolate-to have.

Gemma: A sculptural element that is has a width the same as the height but is narrows at its base.

Hecterocolpate: Colpi may be apertures while other apertures are colpi and pori.

Hexangular: The shape of a six sided polygon, referring to pollen grain shape.

Hexapantocolpate: Scattered over the surface in no apparent arrangement are six colpi.

Hexaptocolporate: Scattered over the surface in no apparent arrangement are six colpi with a porus to each colpi.

Hexapantoporate: Scattered over the surface in no apparent arrangement are six pori.

Hexazonocolpate: Six colpi arranged in the equatorial zone.

Hexazonolcolporate: Six colpi arranged in the equatorial zone with each having a pori.

Hexazonoporate: Six pori arranged in the equatorial zone.

Infratectal bacula: Slim rods which traverse the bases of the echinae and the thickness of the tectum.

Intectate: No tectum is present, the sculptural elements are free, i.e.: *Geranium* pollen grains.

Lacuna: In echinolophate pollen grains it is a large gap, or tall ridge.

Lumen: The gap between the walls of a structure of a reticulate, striate or rugulate grain: Lumina-plural.

Melissopalynology: The study of pollen in honey often relating to unifloral honey certification

Meridional: the features of a grain that run from the distal to the proximal pole.

Glossary of palynology related terms continued.

Mesocolpium: The surface area between two adjacent colpi.

Microechinate: Echinae less than 1µm in height.

Microreticulate: With lumina less than 1µm in diameter.

Microrugulate: With rugulae less than 1µm in diameter.

Monocolpate: Having one colpus.

Monoporate: having one pori.

Muri: A wall or ridge separating lumina of a reticulate, striate or rugulate grain.

Nexine: An unscupltuered portion of the inner exine.

Operculum: A thick membrane over the porus of colpus, often forming a ridge: Operculate-to have.

Pentapantocolpate: Scattered over the surface in no apparent arrangement are five colpi.

Pentapantocolporate: Scattered over the surface in no apparent arrangement are five colpi with a porus to each colpi.

Pentapantocolporate: Scattered over the surface in no apparent arrangement are five pori.

Pentazonalcolpate: Five colpi arranged in the equatorial zone.

Pentazonocolporate: Five colpi arranged in the equatorial zone each with a pori.

Pentazonoporate: Five pori arranged in the equatorial zone.

Perforate: Small holes, less than 1µm in diameter piercing the tectum.

Pila: A rod like element with a swollen end, i.e.: Ilex: Pilate-to have.

Pollinosis: An allergic medical condition prevalent in some individuals caused by a reaction to pollen grains in the air.

Pollen rain: Pollen grains that are found in the air and can be carried great distances by air currents.

Glossary of palynology related terms continued.

Polypantocolpate: Scattered over the surface in no apparent arrangement are more than six colpi.

Polypantocolporate: Scattered over the surface in no apparent arrangement are more than six pori.

Polyzonocolpate: More than six colpi arranged in the equatorial zone.

Polyzonalcolporate: More than six colpi arranged in the equatorial zone each with a pori.

Porus: An aperture with a length/breadth ratio less than 2: Porate-to have.

Porus membrane: A thin layer of exine covering the pori.

Psilate: Smooth, lacking sculpture.

Rectangular-obtuse: An oblong pollen grain with rounded corners.

Reticulum: Mesh like sculptural elements.

Rugulate: Sculpturing elements with the length/breadth ratio of 2 or greater.

Sacci: Sack like elements i.e.: *Pinus* sp.: Saccate-to have.

Scabrae: Sculptural elements less than 1µm high: Scabrate-to have.

Semitectate: Partially absent tectum.

Sexine: The outer part o fhte exine that is sculptured.

Simplicolumellate: Columellae in one row under each murs.

Striate: Pattern of parraell muri and lumina ,more or less running in rows.

Suprareticulate: Sculpturing that is independent of collumellae, reticulum on top of the tectum.

Syncolpate: Two or more colpi fused at their ends.

Tectum: As tectate but forming the outer layer of the sexine: Tectate-to have.

Tetrad: Four pollen grains joined as a group.

Glossary of palynology related terms continued.

Tetrapantocolpate: Scattered over the surface in no apparent arrangement are four colpi.

Tetrazonocolpate: Four colpi arranged in the equatorial zone.

Tetrazonocolporate: Four colpi arranged in the equatorial zone, with each colpi having a porus in its centre.

Tetrazonoporate: Four pori arranged in the equatorial zone.

Trizonocolpate: Three colpi arranged in the equatorial zone.

Trizonocolporate: Three colpi arranged in the equatorial zone, with each colpi having a porus in its centre.

Trizonoporate: Three pori arranged in the equatorial zone.

Unifloral: Originating from one species of plant, often relating to the content on honey derived from the nectar of one plant.

Verrucae: Nodules or wart-like elements, broader than height: Verrucate-to have.

Vestibulum: A small chamber, in the vicinity of the porus, where the sexine and nexine have split: Vestibulate-to have.

Zonoaperture: Either pori or colpi running in a band around the equator.

Zonoaporate: Pori running in a band around the equator.

Zonocolpate: Colpi running in a band around the equator.

Zonocolporate: Colpi running in a band around the equator, with each colpus having a porus in its centre.

Zoogamous: Plants that produce moderate amounts of pollen and depend on pollinators (animals, birds, insects and beetles) for pollination.

Literature cited.

Ackerman, J., D., Williams, N., H., 1981. Pollen morphology of the CHLORAEINAE (ORCHIDACEAE: DIURIDAE) and related subtribes. *American Journal of Botany*, 68 (10): 1392-1402.

Arrone, G., De Micro, V., Scalar, M., 2004. Multivariate analysis of pollen spectra to identify geographical origin of honey, 132-133[online]. *In* Dodo, L. P. von deer Hoe, W. Ed. *First European Conference of Aphidology,* September 2004 Udine. Available from: http://web.uniud.it/eurbee/Proceedings/ quality%20control%20residues.pdf [Accessed 10 2007].

Australian Database Resources, 2007. *Botanical Databases* [online]. Available from: http://www.anbg.gov.au/cpbr/databases/index.html [Accessed: August 3 2007].

Blackmore, S., Heath, G.L.A., 1984. The northwest European pollen flora, 30: *Beberidaceae. Rev. Palaeobotany and Palynology*. 42: 7-21.

Burga, C.A., Hussendörfer, E., 2001: Vegetation history of *Abies alba* Mill. (silver fir) in Switzerland – pollen analytical and genetic surveys related to aspects of vegetation history of *Picea abies* (L). H. Karsten (Norway spruce). *Vegetation History and Archaeobotany.* 10: 151–59.

Bryant, V.M. Jr., Mildenhall, D. C. 2007. *Forensic palynology: a new way to catch crooks* [online]. Available from: http://www.crimeandclues.com/pollen.htm [Accessed: August 11 2007].

Bryant, Jr., V. M., Jones, J. G., Mildenhall, D. C. 1990. Forensic palynology in the United States of America. *Palynology* 14, 193-208.

Canadian Association of Palynologists, 2007. *World wide web sites for Palynologists* [online]. Available from: http://www.scirpus.ca/cap/links/websites.htm [Accessed: August 3 2007].

Carine, M. A., Scotland, R. W., 2000. 68 taxa and 32 characters: resolving species relationships using morphological data. In: M. M. Harley, C. M. Morton and S. Blackmore (editors). *Pollen and Spores: Morphology and biology,* pp. 187-203. Royal Botanic Gardens, Kew.

Clarke, G.C.S., 1977. The Northwest European pollen flora, 7: Guttiferae. *Rev. Palaeobotany Palynology* 21: 125-142.

Comps, B., Gömöry, D., Letouzey, J., Thiébaut, J., Petit, R.J., 2001: Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics* 157: 389–97.

Literature cited continued.

Coutts, J. 1937. *Everyday Gardening*. London: Ward, Lock and Co.

Culhane, K.J., Blackmore, S., 1998. The Northwest European pollen Flora, 41: Malvaceae. *Rev. Palaeobotany Palynology* 57, 45-74.

Cwynar, L.C., Spear, R.W., 1991. Reversion of forest to tundra in the central Yukon. *Ecology*. 72: 202-212.

Damián, E., Cernadas, E., Formella, A., Pilar De Sa-Otero, M. 2006. Pollen classification of three types of plants of the family Urticaceae [online]. Available from: http://trevinca.ei.uvigo.es/~formella/inv/aaa/formella-2 [Accessed August 10 2007].

Davis, M.B., Shaw, R.G., 2001: Range shifts and adaptive responses to quaternary climate change. *Science* 292: 673–79.

Delph, L.F., Johannsson, M. H., Stephenson, A.G., 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology*. 78 (6) 1632-1639.

Dettmann, M. E., Jarzen, D. M., 2000. Pollen of extant *Wollemia* (Wollemi pine) and comparisons with pollen of other extant and fossil Araucariaceae. In: M. M. Harley, C. M. Morton and S. Blackmore (editors). *Pollen and Spores: Morphology and biology*, pp. 187-203. Royal Botanic Gardens, Kew.

Dixon, K. M., Kell, S. P., Barrett, R. L., Cribb, P. J., 2003. *Orchid conservation*. Natural History Publishing: Koata Kinabalu.

Dodson, J. R., 1978. A vegetation history from north-east Nelson, New Zealand [online]. In *New Zealand Journal of Botany*. 16: 371-378. Available from: http://www.rsnz.org/publish/nzjb/1978/43.php [Accessed August 15 2007].

Elliot, M.B., 1999. Modern pollen-vegetation relationships in Northland, New Zealand. In *New Zealand Journal of Botany*. 37: 131-148.

Erdtman, G., Berglund, B.E. Praglowski, J., 1961. *An introduction to Scandinavian Pollen Flora. I.* Almqvist and Wicksell, Stockholm.

Erdtman, G., Praglowski, J., Nilsson, S. 1963. *An introduction to Scandinavian Pollen Flora. II.* Almqvist and Wicksell, Stockholm.

Literature cited continued.

Faegri, K., Iversen, J., 1989. *Textbook of pollen analysis*. Chichester: John Wiley & Sons.

Flenley, J., R., 1968. The problem of pollen recognition. In *Problems in picture interpretation*, Clowes MB, Penny JB (eds.). CSIRO: Canberra; 141-145.

Garrard, I., Streeter, D., 1983. *The wild flowers of the British Isles.* London: Macmillan London Limited.

Guinet, P., 1986. Geographic patterns of the main pollen characters in the genus *Acacia (Leguminosae*) with particular reference to subgenus Phyllodineae. *In Pollen and Spores: Form and Function,* eds. S. Blackmore & I. K. Ferguson. London: Academic Press.

Herber, B.E., 2001. Pollen morphology of the Theymelaeaceae in relation to its taxonomy. *Plant Systematics and Evolution*. 232: 107-121.

Heywood, V. H., Brummitt, R. K., Culham, A., Seberg, O., 2007. *Flowering plant families of the world.* London: The Brown Reference Group plc.

Horrocks, M., Walsh, K. A. J., 1998. Forensic palynology: assessing the value of evidence. *Review of Palaeobotany and palynology*. 103: 69-74.

Huntley, B., Cramer, W., Morgan, A.V., Prentice, H.C., Allen, J.R.M., 1997: Predicting the response of terrestrial biota to future environmental changes. In Huntley, B., Cramer, W., Morgan, A.V., Prentice, H.C. and Allen, J.R.M., editors, *Past and future rapid environmental changes: the spatial and evolutionary responses of terrestrial biota*. NATO ASI Series I, Vol. 47, Global environmental change. Berlin: Springer-Verlag. 487–504.

Jato, V., Aira, M. J., Iglésias, M. I., ., Alcázar, P., Cervigón, P., Fernández, D., Recio, M., Sbai, L., 1999. Aeropalynology of Birch (*Betula sp.)* in Spain [online]. In Polen, 10: 37-47. Available from: http://lap.uab.cat/aerobiologia/general/pdf/polen10/betula.pdf [Accessed: August 14 2007].

Jones, W., G. Hill, K. D., Allen, J. M., 1995. *Wollemia nobilis*, a new living Australian genus and species in the Araucariaceae. *Telopea* 6: 173-176

Joosten, H., de Klerk, P., 2002. What's in a name? Some thoughts on pollen classification, and nomenclature in Quaternary palynolgy. *Review of Palaeobotany & Palynology*. 122: 29-45.

Literature cited continued.

Lacourse, T., Gajewski, K., 2000. Late quaternary vegetation history of Sulphur Lake, south west Yukon Territory, Canada [online]. In *Artic.* 53: 27-35. Available from: http://pubs.aina.ucalgary.ca/arctic/ Arctic53-1-27.pdf [Accessed August 16 2007.

Lammers-Helps, H., 2007. The importance of pollen. *Landscape Trades,* 29 (5) 13-14.

Lampert, D., Buglass, S., Xu, J., Tan, L., Kieliszewski, M. 2007. Origin of land plants: is *Coleochaete* their closest living relative? The writing is on the wall. *Physiologia Plantarum.* 130, 4: 130.

Langford, M., 1988. *Some applications of digital image processing for automation in palynolgy*. PhD thesis, University of Hull.

Linnaei, C. 1754 (1960 reprint). *Genera plantarum* (with introduction by W.T. Stearn) . Codicote: Wheldon and Wesley Ltd.

Macintyre, B., 2007. Wake up! The bees are on their knees. *The Times*, August 10, p.15.

Mildenhall, D. C., Wiltshire, P.E.J., Bryant, V. M., 2006. Forensic palynology: Why do it and how it works. *Forensic Science International.* 163(3):163-172.

Milkovska, S., M., Bislimovska, J. K., Matevski, V., Risteska-Kuc, S., Miniv, J., 2006. Birch (Betula sp.)
pollen in the atmosphere of Skopje [online]. In *Facta Universites, Medicine and Biology* 13, 1: 32-35. Available from:
http://facta.junis.ni.ac.yu/facta/mab/mab200601/mab200601-07nn.pdf
[Accessed: August 14 2007].

Missouri Botanical Gardens, 2007. *MO databases* [online]. Available from: http://www.mobot.org/ MOBOT/Research/alldb.shtml [Accessed: August 3 2007].

Mcglone, M.S., Moar, N.T., 1998. Dryland Holocene vegetation history, central Otago and the Mackenzie basin, South Island, New Zealand. *In New Zealand Journal of Botany*. 36: 91-111.

Moar, N. T., 1985. Pollen analysis of New Zealand honey. *New Zealand Journal of Agricultural Research*. 28: 39-70.

Montali, E., Mercuri, A. A., Grandi, G.T., Accorsi, C. A., 2006. Towards a “crime pollen calendar”-Pollen analysis on corpses throughout the year. *Forensic science international.* 163: 211-223.

Literature cited continued.

Moore, P. D., Webb, J.A., 1978. *An illustrated guide to pollen analysis*. London: Hodder and Stoughton.

Moore, P., D., Webb, J., A., Collinson, M, E., 1991. *Pollen analysis.* Oxford: Blackwell Scientific Publications.

Nakagawa, T., de Beaulieu, J-L., Kitagawa, H., 2000. *Pollen-derived history of timber exploitation from the Roman period onwards in the Romanche valley, central French Alps.* Available from:http://www.springerlink.com/content/x560v78484g7t065/ [Accessed: August 2 2007].

Nilsson, S., Praglowski, J., Nilsosson, L., 1997. *Atlas of airborne pollen grains and spores in Northern Europe*. Örebro: Ljungföretagan.

PEW, 2007. Pew initiative on food and biotechnology [online]. Available from: http://pewagbiotech.org/ [Accessed August 15 2007].

Parnell, J., (jparnell@tcd.ie), 12 February 2007. *RE: Pollen samples of endemic Irish species.* E-Mail to. M. Pascoe (mpascoe@fanshawec.ca).

Philips, R., 1977. *Wild flowers of Britain.* London: Ward Lock Limited.

Ray, D.E., 2004. GMO crops-Who is responsible for the consequences of pollen drift? In *Mid America Farmer Grower*. 21: 17 [online]. Available from : http://apacweb.ag.utk.edu/weekpdf/194.pdf [Accessed August 15 2007].

Rogers, C. A., 1996. Modelling and forecasting airborne allergenic Ambrosia pollen concentrations at Toronto [online]. Thesis (PhD). University of Toronto. Available from: http://www.collectionscanada.ca/obj/s4/f2/dsk3/ftp04/NQ27804.pdf [Accessed: August 14 2007].

Rösch, M. 1999. Evaluation of honey residues from Iron Age hill-top sites in south western Germany: Implications for local and regional land use and vegetation dynamics [online]. In *Vegetation history and Archaeobotany*. 8: 105-112. Available from:http://www.springerlink.com/content/x12119u733055446/ [Accessed August 19 2007].

Rousseau, D., Schevin, P., Duzer, D., Jolly, D., Cambon, G., 2004. Long Distance Pollen Transport to the Arctic: a Useful Proxy to Calibrate Atmospheric Circulation? [online]. *American Geophysical Union, Fall Meeting 2004*, abstract #PP43A-0590 Available from:

Literature cited continued.

http://adsabs.harvard.edu/abs/2004AGUFMPP43A0590R [Accessed: August 5 2007].

Royal Botanic Gardens, Kew. 2000. *Studying plant diversity-plant classification*, Kew information sheet BI [online]. Available from www.rbgkew.org/ksheets/pdfs/blclassification.pdf [Accessed: August 8 2006].

Royal Botanic Gardens, Kew. 2007. *ePIC. The Electronic Plant Information Centre* [online]. Available from: http://www.kew.org/epic/index.html [Accessed: August 3 2007].

Salas, M. R., 1983. Long-distance pollen transport over the southern Tasman Sea: evidence from Macuarie island [online]. *In New Zealand Journal of Botany,* Vol. 21: 285-292. Available from: www.rsnz.org/publish/nzjb/1983/39.pdf [Accessed: August 15 2007].

Seppä, H., Bennett, K., D., 2003. Quaternary pollen analysis: recent progress in paleoecology and paleoclimatology. *Progress in physical geography.* Available from: http:// www.sagepub.com/ journalsReprints.na [Accessed: August 1 2007].

Skvarla, J., Rowley, J. R., Hollowell, V.C., Chissoe, W.F. 2003. Annulus-pore relationship in Gramineae (Poaceae) pollen: The pore margin of Pariana [online]. In *American journal of botany*. 90(6): 924-930. Available from:http://www.amjbot.org/cgi/reprint/90/6/924 [Accessed August 20 2007].

Soria, A.C., González, M., de Lorenzo, C., Martíez-castro, I., Sanz, J., 2003. Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composistion data [online]. *Food Chemistry*. 85: 121-130. Available from: http:// www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T6R- 49WMWV4-5&_user=128860&_coverDate=03%2F31%2F2004&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000010638&_version=1&_urlVersion=0&_userid=128860&md5=bb2a98b23374a41216229c6386293dee [Accessed: August 10 2007].

Stace, C., 1997. *New Flora of the British Isles*. Cambridge: Cambridge University Press.

Suyama, Y., Lascoux, M., Bennett, K., D., 2005. Ancient DNA from pollen: a genetic record of population history in Scots pine. *Molecular Ecology*. 14: 2873-2882.

Treloar, W., J., Taylor, G., E., Flenley, J., R., 2004. Towards automation of Palynology 1: analysis of pollen shape and ornamentation using simple geometric measures, derived from scanning electron microscope images [online]. *Journal of Quaternary Science.* Available from: www.intersceince.wiley.com [Accessed: July 12 2007].

Literature cited continued.

Tsou, C-H., Tseng, I-J., Lin, F-L., Hong, H-Y., 1997. Aeropalynological investigation in Taichung, Taiwan, 1993-1995 [online]. In *Botanical Bulletin of Academic Science* 38: 57-62. Available from: http://ejournal.sinica.edu.tw/bbas/content/1997/1/bot381-09.pdf [Accessed: August 14 2007].

University of British Columbia, 2007. *Vascular Plant Collection* [online]. Available from: http://www.botany.ubc.ca/herbarium/vascular/index.html [Accessed: August 3 2007].

Vincens, A., Lézine, A.M., Buchet, G., Lewden, D., Le Thomas, A., 2006. African pollen database inventory of tree and shrub pollen types. *Review of Palaeobotany & Palynology*. 145: 135-145.

Widmer, A., Lexer, C., 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends in Ecology & Evolution* 16: 267–69.

Wikipedia, the online encyclopaedia. 2007. *Database* [online]. Available from: http://en.wikipedia.org/wiki/Database [Accessed: August 3 2007].