Lucy Cranwell Lecture 1997

What is pollen for? - The rise and fall of the male gametophyte in conifers

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Introduction

It seems appropriate to discuss pollen in a lecture honoring Lucy Cranwell's achievements in the field of palynology. She was a pioneer in the field of paleoecology, using pollen in recent deposits as an indicator of the past composition and distribution of vegetation (Cranwell and Von Post 1936), a discipline that has expanded considerably in recent decades. This is an indirect use of pollen ("pollen analysis"). Palynologists also study pollen morphology in a systematic or evolutionary context, in this instance concentrating on its considerable structural diversity, which has allowed pollen to be identified by paleoecologists. Pollen is similarly an important indicator fossil in stratigraphic analysis. All these applications are made possible by the resistant nature of its wall, the sporoderm, determined by the complex polymer, sporopollenin.

In this lecture I want to concentrate on an aspect of pollen that relates directly to its purpose in the life cycle of the seed plant, i.e., the transfer of the male gametes or sperm from one plant to another, strictly from the microsporangium or pollen sac to the vicinity of the undeveloped seed, the ovule. This process of pollen transfer or pollination is the field of interest for the pollination biologist and is extensively studied because successful seed set in both wild and cultivated plants is normally dependent on it. Most attention has been devoted to flowering plants in relation to their animal pollinators. Today, I want to consider wind-pollination and conifers, a subject at first seemingly of little interest but I will demonstrate otherwise by considering the structure of conifer pollen in relation to its ultimate function of sperm transfer. A surprising variety of "pollen capture" mechanisms exist if one correlates pollen morphology with cone structure at the time of pollen reception. One can then, at least in principle, demonstrate the adaptive significance of pollen features, especially of the sporoderm. This "functional" approach to pollen morphology may seem somewhat pretentious when applied to something as small as a pollen grain, but I will demonstrate otherwise. I will start by asking a very simple functional question and show how the matter becomes complex when put in a comparative context. The subject is one that has been approachable using the New Zealand flora and much of the information has been derived by recent visits to this country. It deals exclusively with the conifers, which are, of course, of major commercial significance in the economy of New Zealand. The subject involves a detailed consideration of the coniferous cone, not at maturity, but at the time of pollination, when the cones are small and seemingly insignificant.

Pollen and saccate pollen

One of the most distinctive of pollen types found among extant seed plants is that described as saccate (Fig.1), in which the pollen grain wall has one or more, but usually two, sac-like extensions of the outer sporoderm, variously referred to as "wings" or "bladders." Since a good example of this pollen type is found in *Pinus*, one may suggest that it is perhaps the most common pollen type in New Zealand and, indirectly, the most commercially significant. Of course, *Pinus* is not native to New Zealand and paleoecologists of the future may come to puzzle over its sudden appearance in the New Zealand flora!

However, saccate pollen occurs in all native species of the family Podocarpaceae (Cranwell 1940). Apart from most Podocarpaceae and Pinaceae, saccate pollen is otherwise found only in a number of extinct groups of gymnosperms, including the lineage that is presumed to have given rise to modern conifers (Florin 1954). It is not found in angiosperms, many of which are wind-pollinated; why this is so can probably be answered, once functional attributes have been recognized.

Despite their diminutive size, pollen grains, especially of the saccate type, are quite complex little multicellular organisms. They represent the highly reduced male gametophyte of seed plants, distinguished from the

Spores of homosporous plants by the fact that they are partly developed at the time of dispersal, and normally including a number of cells, of which two are significant, one the tube cell or nucleus controlling pollen tube development on germination, the other, the generative or body cell, being that which directly produces the two male gametes. Pollen also differs from the spores of lower vascular plants in the method of germination. In conifers the number of cells involved in pollen maturation varies in a systematically significant way, but these details do not concern us. Rather, we must concentrate on the sporoderm, in which distinctive structural features reside. As in most pollen grains this consists of the highly resistant outer exine, made of sporopollenin, and an inner cellulosic intine, which is easily digested and essentially provides the wall of the pollen tube.

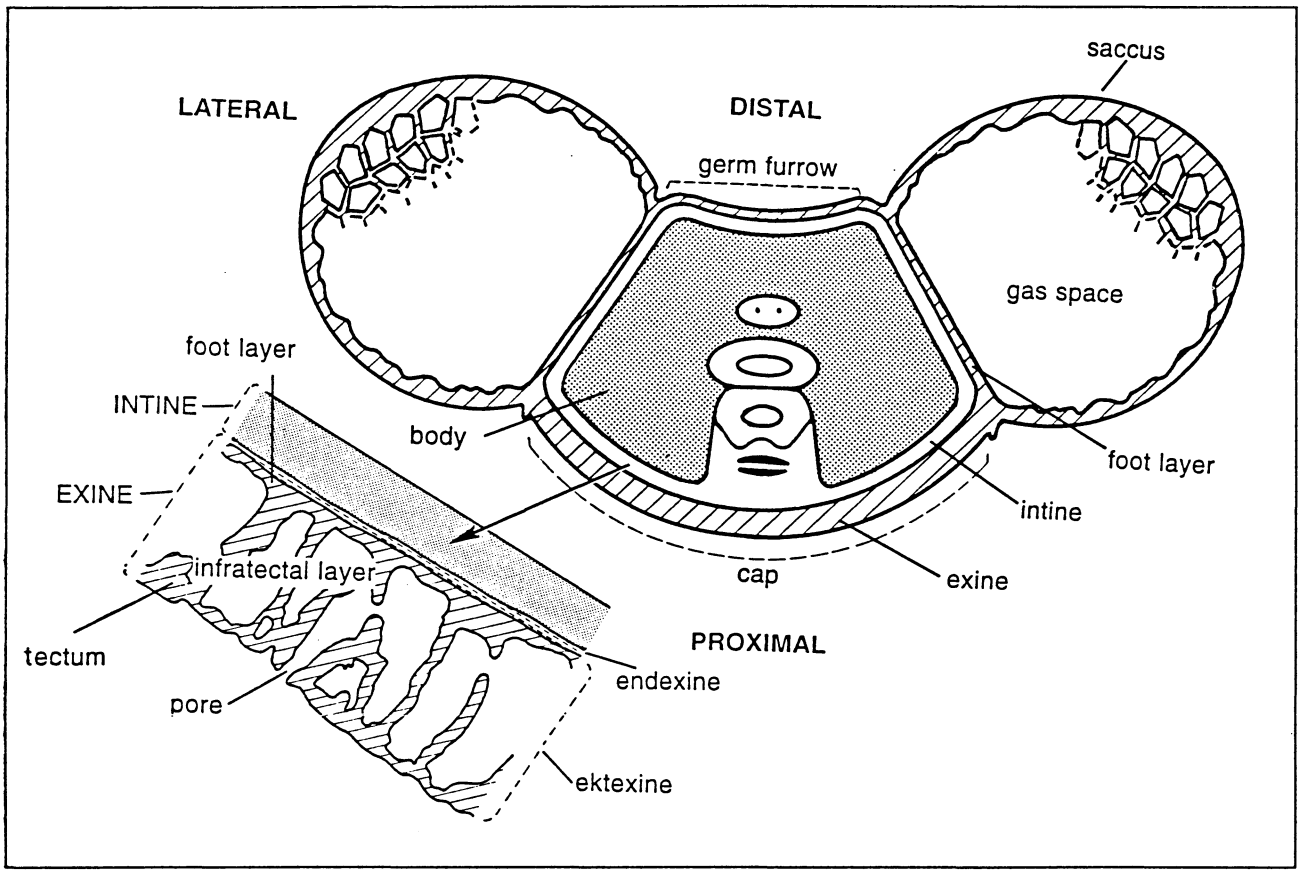


Fig.1. A pinaceous saccate pollen grain (from Tomlinson 1994 : fig.2).

In saccate pollen the exine has a honeycomb or alveolate texture and the sacci are simply inflated, exaggerated extensions of this texture, occupying precise lateral positions. As a result the "body" of the cell, i.e., its cellular component is distinguished from the non-cellular "wings," or wall component. This configuration is determined early in development and is dependent on the organization of the pollen tetrad, the four-celled result of meiosis. It is noteworthy that the pollen wall completes its maturation while the body is uninucleate (Kurmann 1989); the sporoderm is therefore the result of spore and not gametophyte development. The saccus itself is rigid, the rigidity being provided by the inner reticulation of the exine, forming a miniature geodesic dome. So we offer a mechanical explanation for the structure of the saccus, which must maintain its rigidity at all phases of pollen activity, whereas the body is quite flexible about the groove (sulcus) between the saccus pair, this flexibility being determined by the state of hydration of the cellulosic intine and cell contents and the thin exine of the sulcus. The sulcus itself is the site of pollen tube emergence, once the grain germinates on a suitable substrate. This site is distal (away from the center of the original pollen tetrad), unlike the proximal germination site of homosporous plants (toward the center of the pollen tetrad).

Of major significance is the porous nature of the saccus wall, which is otherwise fairly smooth on its outer surface. These minute perforations allow gas exchange and account for the fact that sacci at the time of pollen release are gas-filled (presumably by air). This can be seen in pollen mounted in water, since air-filled sacci

appear black because of refraction. The gas is not easily displaced in water, because of capillarity and the small external diameter of the saccus pores. The gas contents may change by diffusion in air, but persist indefinitely in wet pollen; they are normally displaced rapidly in alcoholic fixatives.

The function of sacci

Several explanations have been offered to account for the existence of sacci. Since none are mutually exclusive and all may be appropriate, it seems difficult to determine how natural selection has worked to produce these structures. The generally accepted adaptive function is that sacci facilitate the dispersal of pollen in **air**, an explanation that has the sanction of physics. Sacci reduce the density of the grains, not by reducing its mass (they may actually increase it) but by increasing the volume of the grain appreciably. Stoke's Law, which describes the fall of a small particle in an ambient fluid, tells us that the velocity of particle fall is proportional to the difference in density between particle and fluid; reduce the density of the particle and one reduces the rate of fall. Most biologists (and all text-books!) have accepted this incontrovertible aerodynamic explanation. It is based on the assumption that a lighter saccate grain will be carried further by wind currents because it remains air-borne longer, i.e., its dispersal capability is increased. There is empirical evidence to support this explanation, but it is still an assumption that increased dispersibility itself is of adaptive benefit. I will show that this adaptive explanation is wrong because it results from the failure to consider saccate pollen in relation to other kinds of conifer pollen and in the context of its total life span, since it deals only with pollen dispersal. Pollen **capture** is the key to what I consider the real function of sacci, pollen capture being defined as the process whereby pollen reaches its ultimate destination, which in most gymnosperms is the pollen chamber of the immature seed or ovule.

The taxonomic distribution of saccate pollen also raises doubts about this dispersal explanation. Its absence from many gymnospermous groups, most of which are wind-pollinated, and from all of the many groups of wind-pollinated angiosperms has to be explained. Stoke's Law itself can be further invoked, since it also shows that the most direct way to increase dispersibility is to reduce particle diameter, since the rate of fall of a small particle increases with the square of its radius. Consequently, a small decrease in radius produces a large decrease in rate of descent. Curiously, the range of pollen diameters (diameter of the pollen grain body in saccate pollen) of all conifers is such that saccate pollen has not only the largest diameter (some Pinaceae) but also the smallest (some Podocarpaceae). In fact, podocarp pollen is consistently smaller than pinaceous pollen. Furthermore, the range of size of sacci in different taxa that possess them is considerable and why this should be so is unexplained. A paradox for the dispersal theory is that larger grains of Pinaceae tend to have a smaller saccus volume (Tomlinson 1994).

The most telling evidence, however, comes from the observation that some members of the Pinaceae and Podocarpaceae, families otherwise characterized by saccate pollen, are without sacci or may show non-functional vestiges. The special features of pollen capture in these unusual members provides the clue. But first we must put pollen capture in the general context of seed plant reproduction in order to provide a necessary background to the explanation.

Gymnosperm and Angiosperm reproduction

Taken in their strict biological sense, the words "gymnosperm" (meaning naked-seeded) and "angiosperm" (meaning enclosed-seeded) have been misnomers ever since they were created and they fail to recognize the astuteness of the Scottish botanist Robert Brown who is indirectly responsible for them. He was first to point out that a fundamental difference between the two major groups of seed plants, the conifers and cycads, on the one hand, the flowering plants, on the other, lies not in whether the **seeds** are exposed or enclosed, but **at the time of pollination**, in the former group the **ovules** are exposed and in the latter group the **ovules** are enclosed. The significant biological difference is that in conifers and similar taxa, for the most part, the ovule is the site of pollen reception, whereas in flowering plants, pollen is received by a stigma, an extension of the carpel, a structure which encloses the ovules at pollination. The basic difference is therefore between gymno-ovuly and angio-ovuly, but we have been mislead by "gymnosperm" and "angiosperm," terms not coined by Brown himself. Most gymnosperms are biologically angiosperms while angio-ovulate plants are often gymnospermous at the time of seed release.

Gymno-ovuly and angio-ovuly therefore relate to the process of pollen capture, with the fundamental observation that the stigma of flowering plant carpels is the site of a pollen recognition system that is probably the most distinctive evolutionary innovation of angiosperms. Looking backward: - from where does this mechanism originate? Can we find its precursor in any group of gymno-ovulate plants? In fact, some

gymnosperms have progressed markedly in the direction of angio-ovuly and surprisingly, in view of their remote lineage, the conifers themselves offer partial evidence. To understand this we must turn temporarily from pollen to ovule.

The pollination drop. Pollination in gymnosperms seems difficult because the target for pollen reception, the micropyle of the ovule, is very small and pollen is undirected in its dispersal. Wind pollination works because pollen can be produced in high quantities and has a guaranteed vector. Nevertheless the ultimate site of germination for pollen is the pollen chamber of the ovule, seemingly inaccessible directly. It has been known since 1841 that pollen capture in most extant gymnosperms is greatly facilitated by the existence of a pollination drop, a secretion of the nucellus of the ovule, which is extruded through the micropyle and becomes the target of pollen reception. It is a drop of water with included amino acids, sugars and other substances, normally in very dilute quantities. Once pollen is acquired it is drawn into the pollen chamber by retraction of the drop. This simple process, however, has one obvious drawback in that, since it is a physical process, it cannot seemingly discriminate between pollen types and, most significantly, cannot avoid receiving other particles, notably the spores of pathogens or parasites. This makes the system very vulnerable to predation. However, it is almost universal in gymnosperms - how it can be made more effective is the next part of the story.

Pollen capture in Podocarpaceae

The Podocarpaceae is a large and diverse conifer family, but is least studied. They have a tropical and southern distribution. New Zealand is one major center of diversity with seven genera and 14 species, and provides excellent opportunity for their study. *Phyllocladus* can be treated as a separate family, with three out of its five species endemic to New Zealand and showing considerable morphological diversity. In many ways podocarps do not conform to a northern model of conifers, especially in their reproductive biology. The seed producing organs are reduced with the cone frequently including a single ovule, very unlike the multi-ovulate cones of Pinaceae. Seeds are typically animal-dispersed and associated with fleshy attractive structures at maturity. A characteristic component is the epimatium, which is the ovule-supporting structure of the seed complex and believed to be the homologue of the ovuliferous scale that characterizes the same complex in some other families, especially Pinaceae. However, the epimatium does not play a consistent role in seed dispersal but seems of more significance in pollen capture.

Whether or not podocarps possessed a pollination drop was initially answered by Joseph Doyle (1945), who examined cultivated specimens in Irish gardens and observed the drop in two species. Subsequently their presence was demonstrated in New Zealand taxa (Tomlinson *et al.* 1991) and from the configuration of the cone is probably present in all genera of podocarps with the significant exception of the South American genus *Saxegothaea*. In most podocarps the ovule becomes morphologically inverted by a developmental process mediated by the epimatium (Tomlinson 1992), i.e., a function can be ascribed to this structure. At pollen reception this inverse orientation is sustained by the erect position of the cone axis, an orientation of great significance in pollen capture. Usually the ovule is almost completely enclosed by the epimatium, but the gymno-ovulate condition is maintained because the micropyle is always exposed, protruding slightly from the epimatial envelope. A significant feature is that when the drop is extruded through the micropylar orifice it makes contact with an adjacent non-waxy and wettable surface, in contrast to adjacent waxy, non-wettable surfaces. The drop is thus extended to limits of non-wettable surfaces. The actual morphology and layout of the highly reduced cone at the time of pollination is quite variable and follows generic lines, but the principle mechanism seems constant.

Observation of pollination in a diversity of taxa demonstrates a mechanism of "pollen scavenging," which is dependent on the physical properties of saccate pollen, but in **water**, not air. In principle, the pollination drop has become extended both in space and time. To understand the significance of these observations we must have a general observation of how the different kinds of conifer pollen behave in water. Briefly, pollen may either sink or swim.

Conifer pollen and water

We have seen that an essential feature of pollination in conifers, is the pollen capture process mediated by the pollination drop, i.e. pollen ultimately contacts water. If one carries out the simple experiment of adding dry conifer pollen to a drop of water, two sharply contrasted categories can be demonstrated. Saccate pollen of Pinaceae and Podocarpaceae floats and rises to the highest point of the meniscus, without entering the body of the water drop. This buoyancy is clearly the result of its diminished density, a consequence of the gas-filled sacci, which function essentially as water wings. This bubble-like property can be seen in nature anywhere where such pollen is shed in quantity onto a water surface, and the physics is again explained by Stokes' Law.

In contrast, pollen of all members of the families Cephalotaxaceae, Cupressaceae, Sciadopityaceae, Taxaceae and Taxodiaceae sinks in water. It enters a pollination drop also and sinks. Almost immediately a remarkable transformation occurs because the pollen swells and bursts, rupturing the exine, which is shed. Presumably this is the result of the swelling of the cellulosic intine by imbibition, causing its volume to increase enormously, well beyond the confines of the rigid exine. Splitting of the exine is always in the region of a narrow distal pore, obscure in most taxa, but represented by a short papilla in Taxodiaceae. The significance of this exine shedding is not known and requires further study.

There is still a miscellaneous assemblage of unrelated genera in which pollen sinks in water but does not immediately burst. These taxa are significant in understanding the evolution of pollen capture mechanisms, because with one exception they all lack a secreted pollination drop, **and** sacci. Noteworthy are *Larix, Pseudotsuga* and *Tsuga* of Pinaceae, which are the only members of the family without sacci, as well as *Saxegothaea* of Podocarpaceae, whose pollen is non-saccate and it also lacks a pollination drop. *Phyllocladus* is yet another category; its ovules produce a pollination drop, but its pollen sinks in water (without bursting) and the pollen possesses sacci, which are small and best described as vestigial, since they do not provide buoyancy for pollen either in water or in the pollination drop (Tomlinson *et al.* 1997).

Clearly, the situation is complex, but there is a correlation that can be grasped easily - where pollen with well developed sacci occurs in conifers, it is always correlated with a cone structure in which ovules are inverted, in both Pinaceae and Podocarpaceae. The latter family provides the best explanation for this correlation, because ovules (or at least their pollination drops) are exposed so that the process of pollen capture can be observed directly and the significance of "pollen scavenging" can be appreciated.

Pollen scavenging

The distinctive feature of pollen capture in most Podocarpaceae is that pollen need not land directly on the pollination drop, but can be picked up belatedly from the wettable cone area by the emerging drop once it is secreted. The pollination drop is, in essence, extended both in space and time. In space because the drop is no longer spherical and its surface area is larger for a given volume, in time because pollen can arrive over an extended period before drop secretion. Pollen simply has to fall within scavenging range of the subsequent drop. Pollen capture is further facilitated by repeated secretion of a drop by the same ovule over several successive evenings, disappearing each time during the day, presumably by evaporation. Pollen scavenging seems an appropriate term for this behavior.

Perhaps the most distinctive feature of the mechanism is its dependence on the bubble-like qualities of saccate pollen, once it has been scavenged by the secreted drop. Such pollen immediately rises up the surface meniscus of the drop, usually to the vicinity of the micropyle, its journey into the pollen chamber being dependent on the ultimate retraction of the drop, its diminished surface carrying the pollen with it as the drop finally recedes into the micropyle. The biological significance of this process is that any foreign particles that chance to land on the wettable receptive surface (in New Zealand these are often carbon particles from diesel exhausts!) are excluded from the micropyle because they do not have bubble-like properties. We may thus see the glimmerings of a pollen selection process, since only saccate pollen works in the system. However, the process is purely physical and does not discriminate between saccate pollen of different species. In fact, the mechanism may not be directed at foreign pollen at all, but is conceivably a process that screens out the spores and eggs of potential pathogens and parasites which, lacking any flotation mechanism, are unable to reach the micropyle in the inverted drop and become stranded as it recedes.

The overall result is, however, a satisfactory explanation for the function of saccate pollen and produces a rational scenario for pollen capture in conifers generally, since it brings together a suite of morphological characters that are clearly interdependent because they all contribute to the same objective - a guarantee that pollen will reach the pollen chamber in adequate quantity. The picture is made particularly clear if we put the observations in an evolutionary context, since the specialized examples within the Pinaceae and Podocarpaceae that lack saccate pollen, may be seen as highly derived types, their modified pollen being the result of loss of the pollination drop. This is strong evidence for the very strict selective control operating on the pollen capture mechanism. Once disrupted, the assemblage of interdependent features is rapidly lost and replaced by a new set of structures. This was the approach adopted by Doyle (1945) in his classical paper, an approach much elaborated by more recent comparative work. It is not possible to go into these details in a limited space, but an overview is presented in Tomlinson and Takaso (1998).

Conclusions

One can pursue these observations in a comparative context by examining conifers in which the pollen never enters the pollen chamber, but germinate outside the ovule so that the pollen tube itself assumes the responsibility of gamete transfer over a relatively extended distance. This occurs in Araucariaceae, in *Tsuga* (Pinaceae) and *Saxegothaea* (Podocarpaceae). The latter genera provide examples of anomalous non-saccate pollen types in the two families otherwise characterized by pollen with sacci. This is a process of "extended siphonogamy" that points towards the angiosperm condition in which pollen tube growth through the style of the gynoecium is necessary because of the angio-ovulate condition. Members of the Araucariaceae approach the condition quite closely; they are virtually angio-ovulate because the pollen does not need to germinate near the ovule (Owens *et al.* 1995). Clearly this is a parallelism in the trend toward the reproductive condition in flowering plants. Wind-pollinated flowering plants have no use for sacci - they are not involved in a pollen delivery process dependent on the free water of a pollination drop, water is detrimental to pollen function in all but hydrophilous angiosperms.

These observations, set out very briefly, demonstrate the value of a broad comparative approach in studying reproductive mechanisms in plants. Concentration on a limited aspect in a limited range of taxa can never produce basic information that is necessary for biotechnological application. I hope this message is not lost on funding agencies in New Zealand.

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"Manuka" and *"kanuka"*

Rhys Gardner

The ancestry of these names for our two *Leptospermum* species, *L. scoparium* and *L. ericioides* (*Kunzea ericioides* to some) does not seem to have been discussed before. The genus is an Australian one, not generally present in the Pacific islands (but see below), and so the Maori, on encountering here in New Zealand, would have had to coin a name for it, or transfer a name from some plant in the country that they had left behind.

Apparently, they did the latter. There is a widespread myrtaceous shrub, *Decaspermum fruticosum*, common at higher altitudes in the islands, and quite similar to the leptospermums in its silky sweet-smelling, smallish leaves and its abundant white blossom. This plant, and closely related species, occur in the Western Pacific and