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CONTRASTED POLLEN CAPTURE MECHANISMS IN PHYLLOCLADACEAE AND CERTAIN PODOCARPACEAE (CONIFERALES)[1]

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Comparative study shows that *Phyllocladus* and representative Podocarpaceae differ in the mechanism by which pollen is introduced into the pollen chamber and onto the apex of the nucellus ("pollen capture"). Both types involve a pollination drop, but only in Podocarpaceae is it consistently inverted and in contact with adjacent surfaces. *Phyllocladus* has functionally nonsaccate pollen (although a vestigial saccus has been claimed); its pollen is wettable and sinks in water. Podocarpaceae (except *Saxegothaea*) have saccate pollen, which is nonwettable and floats on water. In *Phyllocladus* the pollination drop receives the pollen directly and presence of pollen stimulates complete drop withdrawal, which may be a metabolic process. Once pollinated, an ovule does not resecrete a pollination drop. In Podocarpaceae the drop usually receives the pollen indirectly via pollen scavenging and saccate pollen is preferentially captured. The retraction of the drop appears to be the result of evaporation and is presumably nonmetabolic. Drop secretion can be repeated in the presence of pollen. A major consequence of these contrasted mechanisms is that in *Phyllocladus* the entire contents of the pollination drop are ingested, whereas in Podocarpaceae only that part of the drop that includes saccate pollen is ingested. Because of differences in repeatability of the secretion process, Podocarpaceae are likely to capture more pollen. In neither mechanism does the process favor "own" pollen, but in Podocarpaceae all but saccate pollen is excluded. We thus have further evidence for differences in pollen capture mechanisms in conifers with a pollination drop, and differences in the behavior of the pollination drop itself.

Key words: conifers; *Phyllocladus*; Podocarpaceae; pollen capture; pollination drop.

In conifers it has recently been emphasized that there is an almost complete correlation between pollen morphology and ovule structure, leading to the concept of functional morphology of pollen (Tomlinson, 1994). This extends the initial suggestion by Doyle (1945) that saccate pollen is a hydrodynamic and not an aerodynamic feature of gymnosperms, although Doyle's ideas were generated without detailed knowledge of the Podocarpaceae. Subsequent understanding of this family lends support to Doyle's character correlation, but suggests an additional hydrodynamic mechanism in terms of a process of selection for saccate pollen as well as pollen orientation on the nucellus (Tomlinson, Braggins, and Rattenbury, 1991; Tomlinson, 1994). In this earlier work the status of *Phyllocladus* (Phyllocladaceae sensu Page, 1990) was uncertain. We now show that *Phyllocladus* is distinctive and that it differs in important details of drop secretion and pollen capture, compared with selected Podocarpaceae. Pollen capture has been used in several senses; here we imply the final stage of pollination in which the pollen enters the pollen chamber of the ovule, having been transported by wind to the vicinity of the ovule.

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In most gymnosperms pollen capture is effected by a drop of fluid (pollination drop) secreted by the ovule and exuded through the micropyle. Pollen may either land directly on the pollination drop, or be "scavenged" by extension of the drop in space and time (Tomlinson, Braggins, and Rattenbury, 1991). Functional aspects of pollen relate to its behavior in water and seem largely determined by wall structure, especially in the distinction between saccate and nonsaccate pollen. Variation in ovular structure and the distribution of waxy surfaces may have considerable influence on the pollen capture mechanism.

In a few conifers the drop mechanism is absent and this is correlated with major changes in pollen and ovule structure (Table 3 of Discussion). The overall picture suggests that the complex of characters involved in pollen capture is under tight selective control. Consequently study of the process may be informative of evolutionary changes within gymnosperms, at least those that appear to increase the efficiency of reproduction.

In the earlier overview (Tomlinson, 1994) the difference between saccate and nonsaccate pollen was emphasized. Saccate pollen shows an incomplete relation to systematics because although it is restricted to modern Podocarpaceae and Pinaceae, not all genera possess it. A closer correlation is shown with the pollination drop and ovule orientation, which is always inverted in those taxa that have saccate pollen. Saccate pollen was described as "nonwettable" because it is bubble-like, i.e., buoyant in water, whereas nonsaccate pollen was described as "wett-

TABLE 1. Taxa investigated and location.

Taxon	Locality (New Zealand)	Pollination month
Phyllocladaceae		
Phyllocladus glaucus	Auckland U. Campus	Sep–Oct
Phyllocladus trichomanoides	Whangaparaoa	Sep–Oct
Podocarpaceae		
Dacrycarpus dacrydioides	Manurewa	Oct
Lagarostrobos colensoi[a]	Auckland U. Campus[b]	Sep–Oct
Podocarpus totara	Manurewa	Oct
Prumnopitys ferruginea	Waitakere	Oct–Nov
Prumnopitys taxifolia	Manurewa	Oct–Nov

[a] Or *Manoao colensoi* (Molloy, 1995).

[b] ♀ only; cultivated outside its natural range and with abnormally early cone development; in its natural range pollination occurs in November–December.

able" because it sinks in water. Nonsaccate pollen may be subdivided further according to whether the pollen swells and bursts in water (most remaining families) or sinks without bursting (e.g., Araucariaceae). However, Araucariaceae and certain Pinaceae and Podocarpaceae lack a pollination drop so that water is not involved in pollen capture. In the absence of direct observation the situation in *Phyllocladus* was uncertain. It had a pollination drop, which was not inverted and its pollen has, at most, reduced and possibly vestigial sacci (Ueno, 1960a, b). We here show that pollen of *Phyllocladus* is wettable. This further strengthens the correlation between ovule morphology and pollen type since *Phyllocladus* shows none of the ovular features that characterize genera with fully developed sacci.

Further observations and experiments also demonstrate quite appreciable differences in the pollination drop secretion mechanism when *Phyllocladus* is compared with Podocarpaceae, and show the important functional role of structural variants. The overall picture that is emerging is that within conifers the mechanisms of pollen capture are quite varied and that structural features and physiological processes that may appear unconnected are, in fact, functionally correlated. The elaboration of this character complex in different groups of conifers may explain their persistence and diversification in evolutionary time.

MATERIALS AND METHODS

Both Phyllocladaceae and Podocarpaceae provide ideal subjects for direct examination of pollen capture because ovules, or at least their micropyles, are exposed and can be viewed without dissection. Observations were made in September–November 1995 in Auckland, New Zealand. Seven taxa were available because they shed pollen in this period (Table 1).

The pollination period is more extensive throughout the total range of each taxon; the dates listed refer only to the trees investigated. Trees are not always strictly dioecious and observations on *Phyllocladus trichomanoides* were made on an individual with both male and female cones. Only *Podocarpus* was available as a large population with abundant individuals of both sex ensuring natural pollination. For experimental purposes, most observations were made on shoots taken from trees before pollination could occur (i.e., before pollen shedding). A number of nonnative taxa supplied "foreign" pollen as mentioned in the Results.

Pollination drops—Observation of pollination drops was made on cut shoots or single cones enclosed in jars with added water. Single cones of Podocarpaceae, or in *Phyllocladus* entire fertile phylloclades were enclosed in Eppendorf centrifuge tubes with a few drops of added water. Under these conditions mature ovules can provide pollination drops overnight. These will persist if they remain in the humid atmosphere of the closed container.

Simulation of pollination—Pollen may be added to cones by flicking a camel-hair brush dusted with pollen near to the pollination drop. However, this is not very precise and the amount of added pollen is not controlled. For greater consistency pollen was added to a pollination drop by touching it with the point of a stainless steel dissecting needle coated with pollen. A control was provided by touching drops with a clean needle. The extent of pollen capture can be determined by prying off the apex of the ovule with a dissecting needle under the stereomicroscope to expose the nucellus without disturbing it.

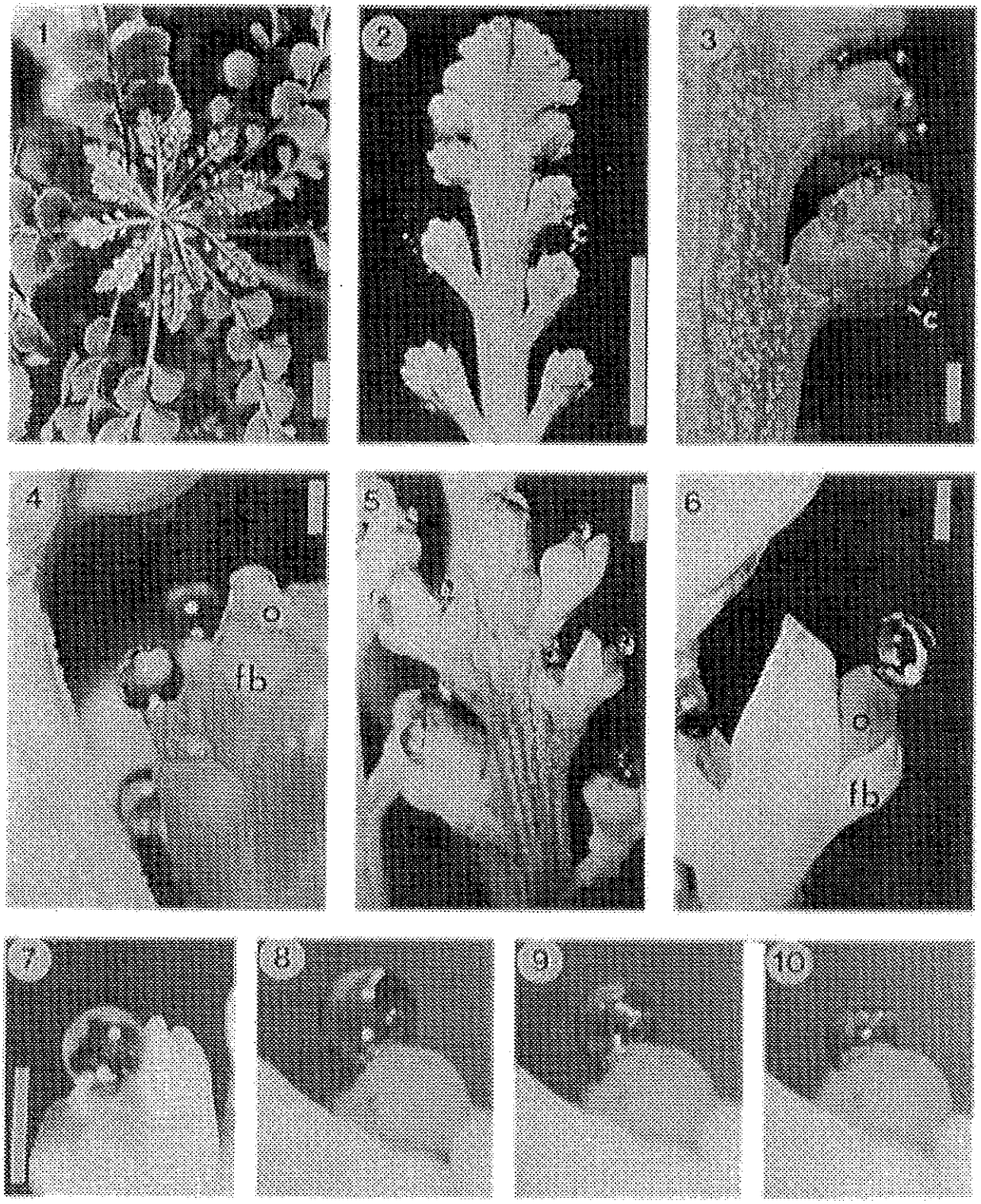
Removal of pollination drops—Cones were either dipped in water or sprayed with drops from a Pasteur pipette or 5 mL hypodermic syringe. This procedure was adopted so that pollination drops could be removed for repeated observation of their generation.

Stereomicroscopy—Observations were made by means of a stereomicroscope at up to 50× magnification. At the higher magnification it is possible to observe the behavior of individual grains. Cones of Podocarpaceae were observed in natural (i.e., vertical) orientation by mounting the microscope with the optical axis horizontal. Illumination was provided by a standard microscope spot-light, which generated some heat. The cone can thus be supported easily at a suitable height, focussing being effected by moving the specimen rather than the microscope lens. Some photographic documentation (Figs. 3–14) was made using a Wild M-5 stereomicroscope with automatic camera, but such images convey little of the dynamic processes involved.

Further manipulative procedures are described under the Results section.

RESULTS

Phyllocladus—Cones are borne laterally and basally on the flattened shoot complex (phylloclade) that characterizes this genus (Figs. 1, 2). Developmental details are provided in Tomlinson, Takaso, and Rattenbury (1989). Phylloclades are borne in whorls at the base of each new shoot increment in the axil of a basal scale leaf, and they expand and function well before the extension of the distal portion of the shoot (Fig. 1). In *P. glaucus* cones are arranged quite regularly on the basal margin of the phylloclade, usually in a distichous series of up to 12. The distal portion of the fertile complex is represented by its planated terminus (Fig. 2). Sometimes cone arrangement is polystichous and the phylloclade approaches radial symmetry. Individual cones themselves have a spiral arrangement of bracts with up to eight ovules per cone (Fig. 3), each fertile bract subtending and partly enclosing its single axillary ovule (Fig. 4). *Phyllocladus trichomanoides* is less regular, the complex is lax with fewer cones (6–8) per phylloclade, and the individual cones have an irregularly decussate arrangement of bracts, which are quite fleshy (Figs. 5, 6). Most commonly in the material examined there were only one or two ovules per cone (Fig. 6). In both species distal cones may be transitional to the flattened terminal segment. Individual ovules are somewhat flattened laterally, especially in *P. trichomanoides*, and the micropylar orifice is el-



Figs. 1–10. *Phyllocladus.* Cones and cone complex. 1–4. *P. glaucus.* 5–10. *P. trichomanoides.* 1. Expanding bud with whorl of fertile phylloclades above vegetative phylloclades of previous season. 2. Distal portion of fertile phylloclade. 3. Single sessile cone from distal portion of a fertile phylloclade, all ovules with a pollination drop. 4. Detail of single ovule with pollination drop. 5. Part of fertile phylloclade of *P. trichomanoides,* each cone with 2 ovules. 6. Single cone with two ovules, that to the right with a well-developed pollination drop, that to the left showing the pigmented mouth of the micropyle. 7. Pollination drop with included pollen magnified by the lens-like action of the sphere. 8–10. Sequence showing drop retraction after pollination. 8. Unpollinated drop. 9. Same drop with added pollen after 5 min. 10. Same drop after 11 min, the loss

liptical with the lip two-lobed. The whole reproductive complex is covered in a waxy deposit except for the margin of the micropylar orifice, which is often distinguished by its red pigmentation. The pollen capture mechanism in both species is the same and they can be described collectively, although most observations were made on *P. glaucus* because of the larger number of ovules per complex.

Pollination drops are produced abundantly under the conditions of the experiment and were extensively available on a specimen of *P. trichomanoides* close to the field site. Drops are usually 200–250 μm in diameter but exceptionally may be 500 μm (Figs. 4–7) and do not normally touch adjacent surfaces, but larger drops may do so depending on the location of the ovule in relation to adjacent structures (e.g., large drop in upper left of Figs. 11–14). Such drops, especially those retained for several days, may become difficult to detach by touching them with a dissecting needle. Drops in contact with the micropyle alone are readily removed. Each drop retains its position on the micropyle because only the outer rim is wettable, the rest of the ovule surface is waxy and nonwettable.

That the drop retains its position by surface tension is demonstrated by adding a surfactant like dishwashing liquid to the drop by means of a fine needle coated with the detergent. Under these conditions the drop collapses and spreads down the ovule. Untreated drops can be shaken from the ovules or washed off with water as described in the Methods (simulated rain). Subsequently a new drop will reappear overnight and the process of drop removal and reappearance can be repeated, to a maximum of 2 wk in some samples, but only if pollen is excluded. Under our viewing conditions virgin pollination drops evaporate very slowly and can persist for over an hour so that they serve as ideal controls in manipulative experiments (Figs. 11–14). Pollination drops otherwise persist in closed containers for several days, but this does not occur in field conditions. Persistent drops become increasingly viscous, as suggested by stretching their surface with a needle.

Effect of pollen—Phyllocladus pollen has at best, vestigial sacci and is wettable, so that it enters a drop of water and usually sinks (Fig. 7). It does not burst in water and shed the exine, unlike the wettable grains of Cephalotaxaceae, Cupressaceae, Sciadopityaceae, Taxaceae, and Taxodiaceae (Tomlinson, 1994). Addition of pollen in some quantity (10–20 grains) to a pollination drop causes the drop to be resorbed, as compared with adjacent untreated drops. Drop retraction is rapid (over a range of 4–20 min in 20 samples), with the majority resorbed in <10 min. A sample sequence is shown for one drop in Figs. 8–10. Control, unpollinated drops show no change in the same period. Figures 11–14 show a single sequence over a period of 14 min, with no change in adjacent unpollinated drops. Since an immediate change in size of a sphere is difficult to detect, the rapidity of the response to added pollen (within 2–3 min) is best shown by misshapen drops contacting an adjacent surface in which a shape change is obvious. Rate of drop resorption is related to its size and possibly the amount of pollen added. It is important to emphasize that drops are not objects of standard size so that more precise numerical statements would be misleading.

Pollen behavior in drops corresponds to that in free water. Most pollen enters the drop and falls through the drop to its lower surface. Pollen within the drop is very obvious because of the magnification provided by the lens-like property of the drop itself (e.g., Figs. 7, 12, 13). Some pollen may remain on the surface of the drop and occasionally falls to the lowest point of the drop along its surface. Pollen within the drop does not normally enter the micropyle directly, unless the drop is erect with the micropyle at its lowest point. Pollen is thus normally carried into the micropyle with the surface meniscus as the drop retracts.

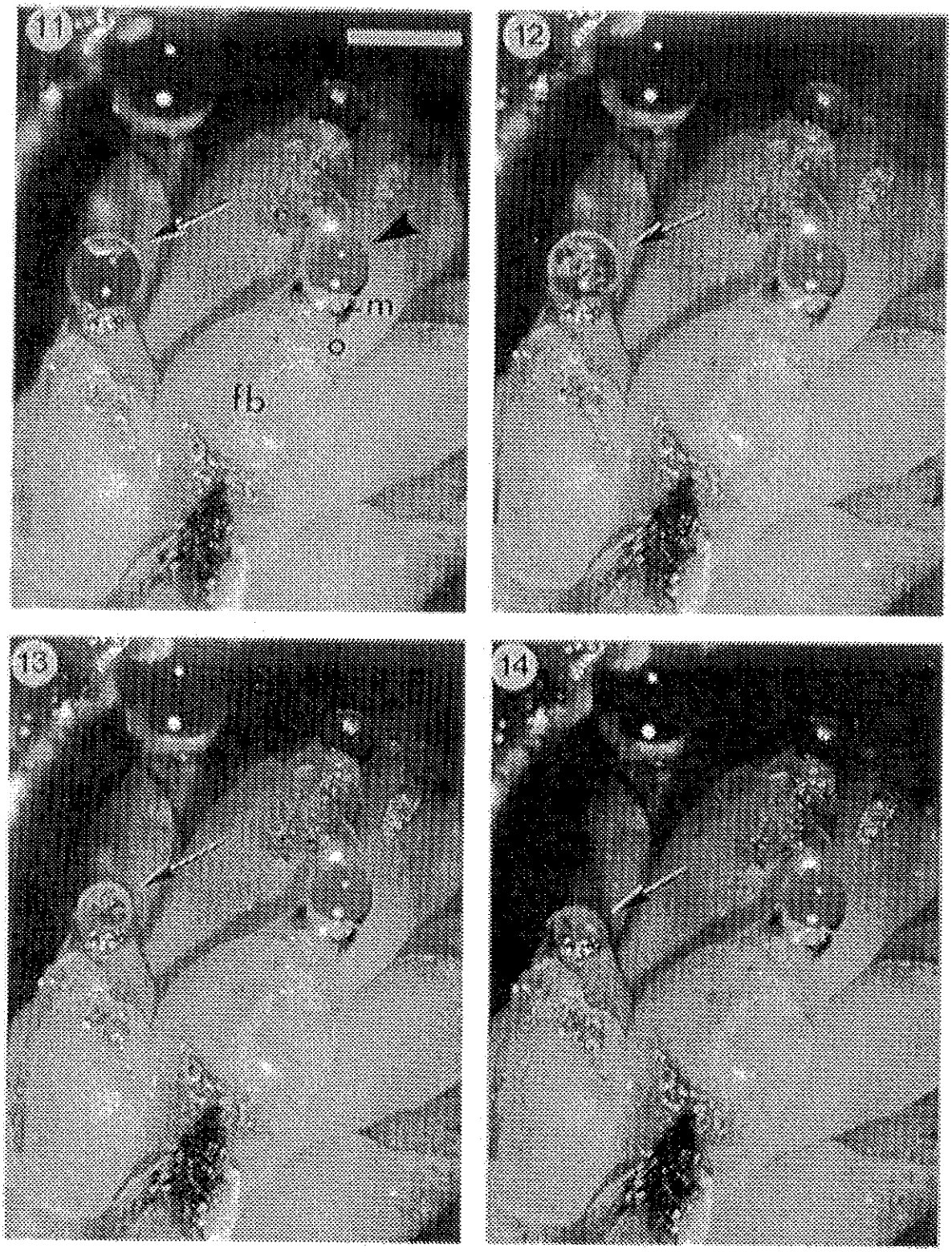
Pollen is the sole stimulus for drop withdrawal, but the mechanism does not discriminate between different kinds of pollen. It can be initiated by foreign pollen of various kinds, among those tested were different species of the same genus (*P. glaucus* on *P. trichomanoides* and vice versa), saccate pollen (e.g., of *Podocarpus*), and wettable pollen of other species (e.g., *Cephalotaxus harringtonia, Cupressus macrocarpa*). That drop withdrawal stimulated by pollen deposition is not due to evaporation was verified by randomly dusting pollen onto several phylloclades with cones that were abundantly provided with pollination drops. The phylloclades were re-enclosed so that they remained within the humid atmosphere of the container. Drops that received pollen all retracted. Any drops that did not immediately retract could be shown by direct observation to lack pollen. For controls, phylloclades in closed containers with cones that were not pollinated retained their pollination drops, in the manner described earlier.

Mechanical stimulation does not provoke drop withdrawal, as can be demonstrated by touching a drop repeatedly with a dissecting needle. A further experiment was to add glass Balloti spheres (diameter ≈75 μm) to drops. These become incorporated into the drop, and usually sink to its lowest point, like pollen, but this did not induce drop retraction. When pollen is subsequently added to such a drop, the drop retracts in the usual way, although the glass spheres are too large to pass down the micropyle. This experiment demonstrates that inorganic material does not stimulate drop withdrawal.

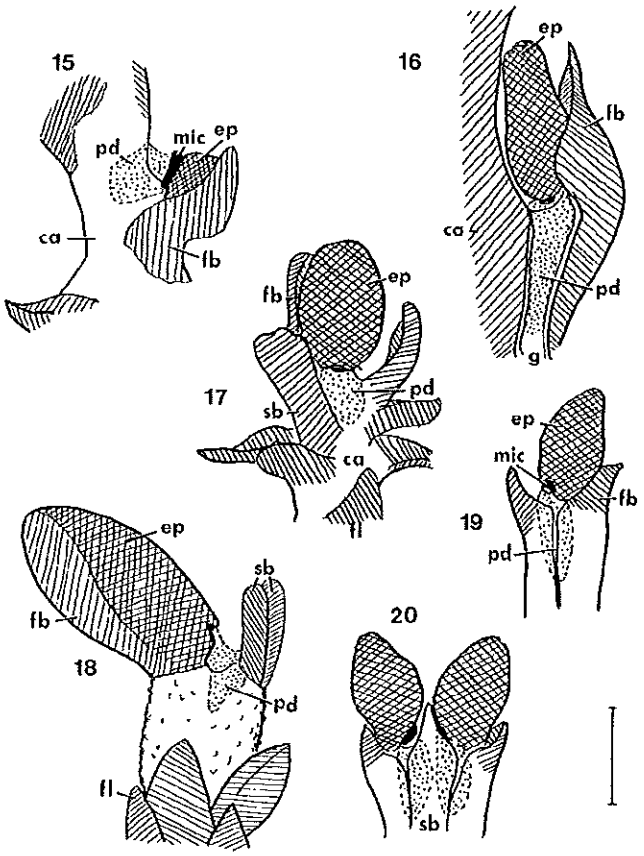
Podocarpaceae—Ovulate cone morphology—This has been described in a comparative developmental context by Tomlinson (1992). The examples studied here (Table 1) all have an epimatium whose growth results in an inverted ovule. Cones are always erect so that the pollination drop is inverted. We are not concerned here with the range of variation in number of ovules per cone or the position of cones on the vegetative shoot system (cf.

←

of the spherical shape is characteristic of drops with several included grains. Scale bars: 1 = 2 cm; 2 = 1 cm; 3 = 1 mm; 4 = 0.25 mm, 5 = 1 mm; 6 = 0.25 mm; 7–10 = 0.5 mm. c = ovulate cone; fb = fertile bract; o = ovule.



Figs. 11–14. *Phyllocladus glaucus*. Time course of drop absorption after pollination. Experimental pollination drop to left (arrow), unpollinated control drop to right (dart). **11.** Virgin drop at time zero. **12.** Drop with pollen added and visible within drop, time + 2 min. **13.** Drop at time + 8 min. **14.** Drop at time + 14 min, the meniscus now almost totally receded and contents of drop within micropyle. Note that all unpollinated drops do not change size. Uppermost large drop (out of focus) represents maximum drop size, it is misshapen by contact with the surface of adjacent cone. Scale bar = 0.5 mm for 11–14. fb = fertile bract; o = ovule; m = micropylar rim; in this cone the micropylar rim is only slightly pigmented.



Figs. 15–20. Podocarpaceae. Details of ovulate complex at time of pollination drop; all drawn from color transparencies (cf. Tomlinson, 1991). 15 and 16 show one complex from a cone; 17–20 show a whole cone. All diagrams show the structure in natural orientation. **15.** *Lagarostrobos colensoi.* **16.** *Prumnopitys taxifolia.* **17.** *Prumnopitys ferruginea.* **18.** *Dacrycarpus dacrydioides.* **19.** *Podocarpus totara* with a single ovule. **20.** *Podocarpus totara* with a pair of ovules and a sterile bract. Pollination drop is shown dotted with dashed outline; nonwettable waxy surfaces are hatched or cross-hatched; wettable surfaces are unshaded; micropyle, if visible is black; cross-hatching indicates outer surface of epimatium. Scale bar = 1 mm for 15–20. Abbreviations: ca = cone axis; ep = epimatium; fb = fertile bract; fl = foliage leaf; mic = micropyle; pd = pollination drop; sb = sterile bract.

Tomlinson, 1992) but with the organization of the individual units. Important features include the distribution of waxy (nonwettable) and nonwaxy (wettable) surfaces and the extent to which the micropyle is enclosed by the epimatium (Tomlinson, Braggins, and Rattenbury, 1991). Wettable surfaces determine the shape of the pollination drop and the extent of its ability to scavenge pollen. Figures 15–20 show these units in the five taxa studied at the time of pollination drop extrusion, i.e., at the time of pollen reception. Details for each taxon follow, representing a considerable range of possibilities based on the same mechanism. Photographic illustration of these cones at the time of drop secretion are provided in Tomlinson (1991) and Tomlinson, Braggins, and Rattenbury, (1991). Photographic documentation of drop withdrawal is uninformative because control and experimental cones behave alike.

Lagarostrobos colensoi (Fig. 15)—Each of the (2–5) ovulate complexes consists of an epimatium inserted on the adaxial surface of the fertile bract, the epimatium enclosing the ovule, which at pollination is largely represented by the protruding trumpet-shaped integument. The micropyle is thus directed horizontally or obliquely downward and faces the cone axis. Wettable, nonwaxy surfaces include the adaxial surface of the fertile bract base, the inner mouth of the micropyle, and the cone axis, the rest of the cone being waxy and nonwettable, together with the subtending foliage leaves. During drop secretion the fluid sits on the adaxial surface of the bract, but it may continue onto the cone axis, sometimes fusing with similar extended drops from adjacent ovules. The upper portion of the cone includes several sterile bracts, which are also waxy. Because of the erect orientation of the cone, the upper meniscus of the drop commonly stands above the micropylar orifice so that captured pollen cannot immediately enter the micropyle. The extent of the wettable surface is such that the pollination drop may descend to the base of the cone axis, being restricted by the waxy foliage leaves. Scope for pollen scavenging is thus extensive.

Prumnopitys taxifolia (Fig. 16)—Cones have extended axes with up to 12 spirally arranged complexes separated by long (2–5 mm) internodes. At pollination time in late October to early November distal internodes elongate, separating the fertile bracts. Each unit consists of a subtending bract with an attached epimatium, the ovule directed basally with its micropyle more or less obscured. Access to the micropyle is provided by two lateral grooves with raised margins that extend axially downward for about two internodes. The groove is nonwaxy and wettable, but remaining parts of cone axis, bracts, and epimatium are waxy and nonwettable. Pollination drops are secreted into the groove down which they extend in such a way as to scavenge existing pollen. The micropyle is only visible laterally because the erect complex of epimatium and bract is quite closely appressed to the cone axis.

Prumnopitys ferruginea (Fig. 17)—Extended cones at the time of pollination have numerous scale leaves and terminate in a series of more expanded sterile colored bracts and one (sometimes two) fertile complexes. Sometimes there are additional reduced sterile bracts opposed to the ovulate complex. Wettable, nonwaxy surfaces include the cone axis adjacent to the ovule and the bract bases. All remaining surfaces of the cone are waxy and nonwettable. The mouth of the micropyle is almost totally concealed by the epimatium. The exuded pollination drop is strictly limited to the wettable portion of the cone, below the ovule.

Dacrycarpus dacrydioides (Fig. 18)—Cones consist of a single subterminal complex, with the fertile bract fused to the epimatium (fb in Fig. 18). The wettable surface is provided by the surface of the lower part of the (1–3) sterile bracts and the outer surface of the mouth of the micropyle, which is otherwise hidden by the lobed apex of the epimatium. This wettable surface is papillate and continuous with the short cone axis as far as the sub-

tending scaly foliage leaves. All other parts of the cone are densely waxy so that water is restricted to a precise region continuous with the micropylar orifice. This area defines the shape of the exuded pollination drop. It is restricted to a narrow column by the short waxy tips of the sterile bracts.

Podocarpus totara (Figs. 19, 20)—Each lateral cone consists of two bracts with an extended base and reduced terminal appendage. One (Fig. 19) or both (Fig. 20) bracts may subtend an ovulate complex. There may be additional vestigial reduced bracts, usually at right angles to the enlarged basal pair (sb in Fig. 20). The apex of the fertile bract or bracts is largely free of the epimatium, which itself encloses the inverted ovule except for the protruding funnel-shaped micropyle. The base of the fused basal pair of bracts forms a smaller structure with a medium groove. Its outer surface is wettable (nonwaxy). Otherwise wax covers the apex of all bracts, the outer surface of the epimatium, and the exposed outer surface of the integument, making them nonwettable. The exuded pollination drop descends the wettable surface, in extreme cases totally bathing the swollen bract bases. Drop withdrawal proceeds from below upwards, but water is retained longest in the vertical groove between the two bracts. This can provide a preferred and also late pathway for upward migration of pollen during drop withdrawal. Orientation of the whole cone into an erect position is provided by the naked peduncle, which can rotate the cone through $> 90°$, as necessary during initial cone exposure.

Aspects of drop function—Since pollen capture is intrinsically the same in all taxa they may be described collectively. Drops are secreted at night, in the usual way, and can accumulate and persist if cones are enclosed in a humid atmosphere. Addition of pollen to drops under these conditions does not cause drop withdrawal, which is the converse of the situation in *Phyllocladus*. Drops or cones placed in dry air and specifically under the conditions of observation with heat from a microscope lamp retract rapidly (within 3–4 min in most cases). Retraction is equally rapid whether pollen is present or not and appears to be a process of evaporation. Control unpollinated and pollinated drops can be observed simultaneously in cones with more than one ovule, or by arranging two different cones side by side so that they can be observed simultaneously. Observations are again qualitative because of the varying size of drops, their varying configurations, and the difficulty of handling pairs of cones. The response of drops to drying air is, however, totally consistent, only the rate of withdrawal varies.

Under simulated conditions saccate pollen added to drops floats upwards within the surface meniscus as far as the mouth of the micropyle where it accumulates. Only under special circumstances does it enter the micropyle directly (P. B. Tomlinson, unpublished data). Where the mouth of the micropyle is concealed the first grains to reach the orifice disappear from view and may enter the micropyle. However, most pollen is drawn into the micropyle by the receding part of the meniscus as the drop evaporates. That the drop is lost by evaporation rather than direct resorption is most clearly seen as it recedes. Only the upper part of the drop maintains contact with the micropyle; the remainder is left behind, but continues to evaporate. Sometimes the meniscus breaks abruptly and visibly, dividing the drop into upper and lower portions. The rejected lower portion, however, continues to dry out at the same rate as the whole drop. This is particularly obvious in *Dacrycarpus* where the drop is usually large and extended. Drying of the lower portion is strikingly visible because of the papillose surface of the lower cone.

The selection process whereby saccate pollen is exclusively captured was most clearly demonstrated by adding mixtures of saccate (own or foreign) pollen and nonsaccate (*Phyllocladus*) pollen to a drop prior to its withdrawal. Initially there is clear divergence related to the different structural abilities of the two types of pollen. Saccate pollen rises and nonsaccate pollen sinks. In no instance where this procedure was adopted did any nonsaccate pollen enter the pollen chamber, as revealed by subsequent dissection. The contrast between the two pollen types in a pollination drop is shown dramatically by blowing gently on the surface of the drop, preferably with puffs of air from a small syringe. Saccate pollen "sails" about the surface of the drop because it is held within the surface meniscus, nonsaccate pollen within the drop does not "sail." The mobility of saccate pollen in the surface of artificial drops of water is also convincingly demonstrated by blowing on the surface. The selection mechanism does not distinguish between the saccate pollen of different species, as can be demonstrated by following the ingestion of foreign saccate pollen, which behaves in the same way as "own" pollen. The whole mechanism is only effective so long as pollen floats to the vicinity of the micropyle; frequently the configuration of the meniscus of the drop is such that large portions of the added pollen become stranded away from the micropyle. This lack of efficiency may be compensated, in part, by the repeatability of drop secretion.

Drop replacement—Existing pollination drops can be removed from ovules either by allowing them to dry out or by rinsing the cones in water (simulated rain) and allowing them to dry out. When re-enclosed, the drop can be resecreted during the following night. This process may be repeated four or five times before the ability to secrete drops is lost. The presence of captured pollen does not inhibit later drop resecretion. This is demonstrated by allowing ovules that have been previously pollinated to dry out and then resecrete drops in closed containers. Under these conditions although captured pollen may be flushed out, it can be recaptured, while there is the possibility of capturing further pollen that escaped the first scavenging event. This accounts for the relatively large amounts of pollen (up to 50 grains) that can be found in the pollen chamber and micropyle of naturally pollinated trees as observed in *Podocarpus totara*. This feature of pollen capture will be reported in detail elsewhere.

The conclusions from these observations in comparison with *Phyllocladus* are presented in Table 2 and summarized in the Discussion.

TABLE 2. Comparison of pollen capture mechanism in Phyllocladaceae and Podocarpaceae.

Feature	Phyllocladaceae	Podocarpaceae
Ovule		
1. Cone orientation	Random	Erect
2. Wax distribution (nonwettable surface)	All parts except micropylar rim	Leaving a wettable surface continuous with micropyle
Pollen		
3. Form	Nonsaccate (or sacci vestigial)	Saccate
4. Hydrodynamics	Wettable, sinks in water, accumulates at lowest point of drop	Nonwettable, floats in surface meniscus, rising to its highest point of drop
Pollination drop		
5. Shape	Free, spherical	Attached to adjacent surfaces, irregularly shaped
6. Detachability	Easily detached	Not easily detached
7. Longevity in dry air	Relatively long-lived (evaporating slowly)	Short-lived (evaporating quickly)
8. Orientation	Random	± Inverted
9. Repeated secretion	Stops with pollen capture	Continues after pollen capture
Pollen capture		
10. Source of pollen	Direct from air (must fall onto drop)	Indirect, mainly by pollen scavenging
Drop Retraction		
11. Stimulus	Requires pollen	Does not require pollen
12. Mechanism	Metabolic?	Physical (evaporation)
Selection mechanics		
13. Drop function	All drop ingested	Only proximal (upper) part of drop ingested
14. Rejection	Nothing discarded	Rejects all but saccate pollen
Pollen chamber (Nucellus)		
15. Amount acquired	Few grains	Numerous grains
16. Location	Exclusively on nucellus, not clumped	Clumped on nucellus and packed in micropyle

DISCUSSION

Contrasted capture mechanisms—These sets of observations demonstrate basic differences in the mechanism by which pollen is introduced into the pollen chamber in *Phyllocladus* as compared with representative Podocarpaceae. Both taxa are ideal for the study of this aspect of pollination because they have pollination drops that are exposed and readily visible (unlike, e.g., Pinaceae where ovules are well concealed at the time of pollination). Furthermore the ready production of pollination drops in vitro allows direct observation and simple manipulation of the process. It might be objected that the study would be more informative if carried out on attached cones in vivo. The mechanical difficulties of doing this are considerable, but not impossible, as in Brown and Bridgewater (1987). However, the processes are largely physical and the fact that comparative study was done on plants under identical conditions, with the demonstration of clear-cut differences, shows that the mechanisms observed are not likely to be artifacts. The value of these kinds of observations is that comparative data have been produced in a relatively short period of time, providing a guide for future in vivo studies. The observations show that in a suite of ovular, pollen, and functional characters *Phyllocladus* and Podocarpaceae differ (Table 2).

Pollen capture in terms of ovule structure and orientation, pollen structure, mechanism of pollination drop secretion and the mechanism of drop withdrawal are contrasted in the taxa investigated. In the absence of a larger sample size for Podocarpaceae it might be dangerous to generalize about the whole family, but the features of ovule orientation, pollen structure and presence of a pollination drop, in so far as they are known, strongly suggest that the features here described are likely to occur in other members of the family that possess saccate pollen (Tomlinson, 1991, 1992, 1994; Tomlinson, Braggins, and Rattenbury, 1991).

Apart from the obvious difference in pollen morphology, which can be translated into a difference in hydrodynamic response, and the differences in ovule structure and orientation, these observations draw attention to the method of secretion and function of the pollination drop in the two types. In *Phyllocladus* the pollination drop may be described as "robust" in the sense that once secreted it tends to persist because it evaporates only slowly. In Podocarpaceae the drop is less robust because it evaporates quickly. This difference can be translated into a difference in the method of drop absorption. In *Phyllocladus* it seems to be a metabolic process in the sense that it is triggered by the generalized presence of pollen. The actual stimulus is obscure, but the response is rapid and further experimental work should elucidate the mechanism, which must relate to a generalized feature of conifer pollen. In Podocarpaceae pollen is not a necessary stimulus for drop withdrawal, which seems to result simply from drop evaporation. Ingestion of pollen is then totally physical and involves the retraction of the upper part of the drop meniscus in relation to the surface retention of saccate pollen.

These observations add to the existing information, which suggests that there are different kinds of pollination drop secretion in conifers. Information has been summarized by Chesnoy (1993). Differences occur in the extent of cell breakdown at the apex of the nucellus. In

TABLE 3. Ovule mechanisms in selected conifers.

1. Pollination drop absent
   A. Pollen germinating inside pollen chamber, pollen engulfed by integumentary ingrowth e.g., *Larix, Pseudotsuga.*
   B. Pollen germinating outside pollen chamber, gametes delivered to nucellus by extended siphonogamy, e.g., Araucariaceae, *Tsuga.*
2. Pollination drop present
   C. Pollen saccate, floating on water, ovule inverted; rejection of nonsaccate pollen
      a. Pollen capture by drop resorption, stimulated by presence of pollen; drop production ceases with pollen capture, e.g., *Picea.*
      b. Pollen capture by drop evaporation, not stimulated by presence of pollen; drop production continues after pollen capture, breakdown of nucellar cells during drop secretion, e.g., some Podocarpaceae.
   D. Pollen nonsaccate, or with a vestigial saccus, sinking in water, ovule without preferred orientation; no rejection of pollen drop contents.
      c. Pollen bursting in water and strictly nonsaccate, e.g., Cephalotaxaceae, Cupressaceae, Taxaceae, Taxodiaceae, Sciadopityaceae.
      d. Pollen not bursting in water, possibly with a vestigial saccus; drop resorption stimulated by presence of pollen, drop production ceases after pollination, no breakdown of nucellar cells during drop secretion, e.g., Phyllocladaceae.

some conifers the cells break down in their entirety as can be seen in longitudinal sections of the ovule at the appropriate stage. *Cephalotaxus* is an example. *Podocarpus* seems to be similar (P. B. Tomlinson, unpublished data). The net result of this in terms of surface configuration is that the apex of the nucellus develops a shallow depression with the visible remains of cell debris. In *Taxus,* as illustrated by Chesnoy (1993), the cell contents of apical nucellar cells are lost, but there is no breakdown of cell walls. This condition may correspond to those taxa in which there is no externally visible breakdown of the nucellar cells, as reported by Takaso and Owens (1996) for *Sequoiadendron.* Preliminary observations suggest that *Phyllocladus* is of this type. In all examples it appears that there is loss of the cuticle in the apical portion of the nucellus; this would seem to be a requirement for the pollination drop to be exuded and re-absorbed, but the phenomenon has been little recorded (cf. Fujii, 1903). The apical discontinuity of the nucellar cuticle can be demonstrated by digesting the ovule with 20% chromic acid (P. B. Tomlinson, unpublished data).

Chesnoy (1993) also records differences in the composition of the sugar and amino acid content of the pollination drop. We do not know if these differences are correlated with differences in drop secretion mechanism necessary for pollen germination, or in the environment.

Doyle and O'Leary (1935) seem to have been the first to observe that pollen is a stimulus for withdrawal of the drop. This experiment was done under controlled conditions by observing the contrasted behavior of a pair of ovules, both of which had a pollination drop, on a single ovuliferous scale of *Pinus.* Only added pollen caused drop withdrawal. The approach corresponds to the experimental method adopted here, but with the difference that only *Phyllocladus* shows a response to pollen. Drop withdrawal under the stimulus of added pollen has been recorded or implied in a number of subsequent studies, although it is usually only mentioned incidental to other features of pollination biology. Apart from *Pinus* (e.g., Doyle and O'Leary, 1935; McWilliam, 1958) it has been mentioned, for example, in *Callitris* (Baird, 1953), *Chamaecyparis* (Owens, Simpson, and Molder, 1980), *Cryptomeria* (Yokoyama, 1975), *Thuja* (Owens and Molder, 1980) but has not been observed by us in *Taxus* (P. B. Tomlinson, Braggins, and Rattenbury, unpublished observations). However, Lill and Sweet (1977) did not confirm the mechanism in their study of *Pinus radiata* in New Zealand. Similarly most authors suggest that the pollination drop can be secreted repeatedly in the absence of pollen although there is little information about the point established for *Phyllocladus*, that pollination prevents resecretion of the pollination drop. The situation in *Picea* and *Pinus,* which have a kind of "pollen scavenging" is paradoxical. Here pollen first lands on the integumentary extensions and is subsequently picked up by the pollination drop. On this basis, the stimulus for drop secretion and drop withdrawal would appear to operate simultaneously. Runions, Catalano, and Owens (1995) describe pollination drop secretion from all ovules in *Picea,* whether or not they had already received pollen. Furthermore, Runions and Owens (1996) have emphasized that in *Picea* pollen scavenging similar to that in Podocarpaceae can exist, especially if the pollination drop is enlarged by rain water. These authors emphasize the possible significance of rain in facilitating pollination.

Podocarpaceae, however, are distinguished by the absence of any response by a drop to pollen, and the ability of a drop to be resecreted in the presence of pollen. This seems a very effective mechanism, since pollen capture is dependent on physical processes (pollen flotation and evaporation) without the additional biological mechanisms of drop withdrawal. The general conclusion is that there are clear differences in the pollen capture mechanism in conifers as revealed by this comparative study amplifying the work of earlier authors (e.g., Doyle, 1945). It may be significant that *Phyllocladus* and Podocarpaceae differ in the structure of the nucellus at the time of pollen capture (P. B. Tomlinson, unpublished data). We thus may have structural evidence for the contrasted functional processes. The overall result in terms of pollen capture is that Podocarpaceae have the ability to select from the contents of the pollination drop and to repeat the process of pollen capture, whereas *Phyllocladus* absorbs all the contents of the pollination drop in an indiscriminate manner, but does not repeat the process. The effect of this in terms of pollen capture, as seen by direct examination of naturally pollinated ovules, is under investigation.

We are still a long way from a complete understanding of the mechanisms of pollen capture in conifers, but it is clear that they are very diverse and our results add to this diversity. Until the process is examined in detail in a wide variety of taxa it will be difficult to understand the biological implications for the reproductive behavior of

gymnosperms as a whole. Our present understanding has been summarized in Table 3 where the extent to which different structural and functional features correlate with mechanisms of pollen capture is stated. Addition of the newer information about the Phyllocladaceae suggests that where a pollination drop is present, at least four contrasted processes may be involved (Table 3).

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