

Pollenkitt – its composition, forms and functions

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Abstract

Two types of sticky pollen coat material exist in angiosperms, both produced by the anther tapetum. Pollenkitt is the most common adhesive material present around pollen grains of almost all angiosperms pollinated by animals, whereas tryphine seems to be restricted only to Brassicaceae. Tapetal cell protoplasts have different patterns of development according to the products formed during their development and degeneration. If tryphine is formed, the tapetal cell protoplasts lose their individuality at the microspore stage. If pollenkitt is formed, their contents degenerate at later stages. Cell content is totally reabsorbed, when ripe pollen is not surrounded by any gluing material. Current knowledge of pollenkitt formation, deposition on pollen grains and chemical composition are reviewed and discussed. Methods for detecting this viscous fluid are also presented. The many functions of pollenkitt, deduced from personal observations and the literature, act in the period between anther opening and pollen hydration on the stigma; they are: (1) to hold pollen in the anther until dispersal; (2) to enable secondary pollen presentation; (3) to facilitate pollen dispersal; (4) to protect pollen from water loss; (5) to protect pollen from ultra-violet radiation; (6) to maintain sporophytic proteins responsible for pollen–stigma recognition inside exine cavities; (7) to protect pollen protoplasts from fungi and bacteria; (8) to keep together pollen grains during transport; (9) to protect pollen from hydrolysis and exocellular enzymes; (10) to render pollen attractive to animals; (11) to render pollen visible to animal eyes; (12) to hide pollen from animal eyes; (13) to avoid predation of pollen through smell; (14) to enable adhesion to insect bodies; (15) to enable pollen packaging by bees and to form corbicules; (16) to provide a digestible reward for pollinators; (17) to enable pollen clumps to reach the stigma; (18) to allow self-pollination; (19) to facilitate adhesion to the stigma; (20) to facilitate pollen rehydration. Depending on the developmental program of the species, these functions may act during pollen presentation, in relation to pollinators, during pollen dispersal and when pollen reaches the stigma.

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Introduction

Female reproductive organs of angiosperms, unlike those of gymnosperms, contain from one to tens of thousands ovules per ovary. It is probably because of the variable number of ovules per ovary that different

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types of pollen dispersing units are present in angiosperms (Pacini and Franchi, 1999); pollen dispersal units may be composed of one to about 100,000 grains (Pacini, 2000). There is a positive correlation between the number of ovules per ovary and the number of grains per pollen dispersal unit (Pacini and Franchi, 1998). An extreme case is represented by massulate orchids (Pacini and Hesse, 2002, also for review).

Pollen of anemophilous species, whether gymno- or angiosperm, is dispersed as single grains with few exceptions, and angiosperms with this pollination syndrome generally have only one ovule per ovary (e.g., Poaceae, Moraceae). On the contrary, the zoophilous species examined thus far generally have more than one ovule/ovary and pollen grains surrounded by different kinds of gluing material i.e. different devices to enable transport “en masse” (Hesse, 1981b; Pacini and Franchi, 1998). The only exception is the case of massulate orchids (Pacini and Hesse, 2002). There are four types of anther derived pollen-gluing or pollen connecting materials: (a) pollenkitt; (b) tryphine; (c) elastoviscin, a fluid with high viscosity in orchids and Asclepiadaceae, but interpreted as a pollenkitt analogue (Dannenbaum and Schill, 1991; Schill and Wolter, 1986); and d. thread-like structures connecting individual pollen grains or pollen tetrads, namely sporopollenin viscin threads, thin ropes continuous with exine or intermingled with grains produced by the tapetum, or anther mucilage derived for example from connective cells and lacking sporopollenin (Hesse et al., 2000; Pacini, 1997). All these agents differ in composition, origin, development, and in part also in function. Besides common walls, exine and intine may also enable transport dispersing unit *en mass* of pollen, as in all species having tetrads as pollen dispersing units, or multiples of tetrads such as massulae, polyads and different kinds of pollinia (Pacini and Franchi, 1999; Pacini and Hesse, 2002). We focus on pollenkitt and mention tryphine when necessary, but do not deal with other agents.

Pollenkitt was recently discovered very well preserved in fossil flower buds on and between fossil pollen grains. Amorphous, highly electron-opaque substances (pollenkitt) and orbicules both very similar to those of recent *Tilia*, were observed on and between pollen grains of fossil flower buds of *Craigia bronnii* (Tilioideae, Malvaceae). Staining and sectioning of this amorphous substance demonstrated that it was indeed pollenkitt (Zetter et al., 2002, also for review).

The systematic occurrence and meaning of pollenkitt in plant reproduction was studied by Dobson (1989, 1994), who also proposed a method to collect and analyse pollenkitt. She also determined its chemical composition in certain angiosperm species (Dobson, 1988).

The aim of this paper is to define differences between pollenkitt and tryphine, to describe the methods used for

its detection, to summarize recent knowledge on pollenkitt and to suggest directions for further research. The authors have already written on this subject, especially from a cytological point of view, but here the topic is considered in a broad sense. Data without cited references are from personal observations.

Pollenkitt and tryphine

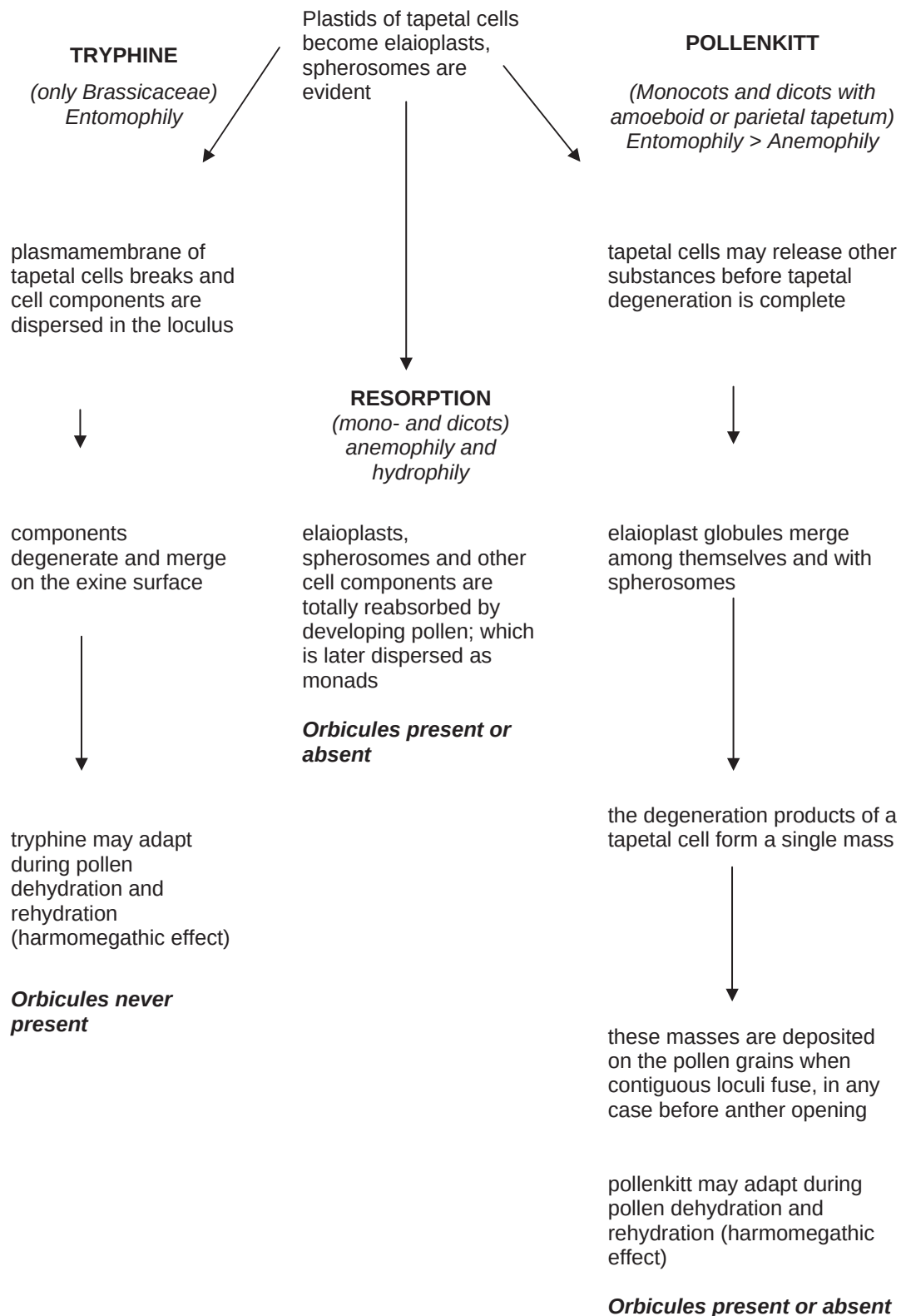
Role and fate of the tapetum

Tapetal cells have nutritive functions, degenerating at different stages of development with different results. Their content is reabsorbed or gives rise to different kinds of substances (Table 1) (Pacini, 1997). Tapetal cell plastids always differentiate from proplastids to elaioplasts during microspore stage, but subsequently differentiation varies according to tapetum fate and the substances produced (Clément and Pacini, 2001).

If tapetal plasmamembranes disappear during microspore stage, as occurs in Brassicaceae (Piffanelli et al., 1998), the cytoplasmic contents merge around the grains and degenerate into tryphine. This is *extra situ* degeneration because tapetal degeneration only occurs after loss of tapetal cell individuality. Pollenkitt is formed when tapetal cell plasmamembranes rupture after complete degeneration of tapetal protoplasts. This is *in situ* degeneration (Pacini and Franchi, 1992). The tapetal cytoplasm may disappear without giving rise to any fatty substance and is therefore reabsorbed by the pollen grains, as for example in *Lolium* (Pacini, 1997; Pacini et al., 1992). Table 1 summarizes all the steps of tapetum development, considering modes of degeneration, resorption and the presence or absence of orbicules (see also diagrams in Piffanelli et al., 1998).

Who discovered and named pollenkitt? A short history

It was Knoll (1930), who termed pollenkitt “alle jene Substanzen, welche eine Verbindung der Pollenkörner zu Klumpen bewirken Ohne Rücksicht darauf, ob solche Stoffe bei der Anthese flüssig bleiben oder bald erstarren, oder ob sie beim Austreten aus dem Antherenfach bereits fest sind” (all substances which connect single pollen grains to pollen clumps irrespectively of whether the substances remain fluid during anthesis or become hard, or are non-fluids from the beginning of anthesis onwards). Knoll evidently included not only oily/fatty fluids, but also the viscous pulps of *Cypripedium*, and even the viscin threads of *Oenothera*. He was not aware that his various “Pollenkitt” substances differed in ontogenesis, chemistry, structure, form and function.

Table 1. Differences between pollenkit and tryphine and the case of absence of fluids around pollen

The first step is the same irrespective of subsequent development.

However, Knoll had some forerunners in discovering and naming pollen coatings. Troll (1928) had already coined the term “Pollenklebstoff” (“pollen adhesive”) for sticky pollen surface substances, but the term was not successful. Pohl (1930) described pollen-“Klebstoffe” as being of variable colour and viscosity in an astonishingly “modern” manner, but without knowledge of Knoll. He called the lipid substances on the pollen surface of anemophilous plants “Kittstoffreste” (remnants of glues), and this term was in part accepted by the scientific community. However, “Pollenkitt” was and is the generally accepted *terminus technicus*.¹

To our knowledge the term tryphine was first used by Rowley and Erdtman (1967, p. 564, figure legend 3) and subsequently by Echlin and Godwin (1968, p. 167), in both cases without explanation. The etymology is the Greek tryphos, that means fragment, and Greek trypheros, that means soft (Hesse, 1978a). Tryphine formation was first described by Dickinson and Lewis (1973) in *Raphanus*. They also coined this term to distinguish the specific Brassicaceae features from pollenkitt known since Knoll's papers. It has only been studied and described for Brassicaceae (a precise definition is given by Dickinson et al., 2000), but is probably also present in other groups (Pacini, 1997).

Pollenkitt and tryphine, irrespective of their mode of formation, composition and so on, are similar in many respects because both are formed by fusion of tapetal elaiosomes and spherosomes (Platt et al., 1998). Nevertheless, pollenkitt and tryphine differ in the following aspects (see also Dickinson et al., 2000): (a) time of deposition on pollen surface (Pacini, 1997; Pacini and Franchi, 1993); (b) pollenkitt may coexist with orbicules, but tryphine has never been observed together with orbicules. Orbicules are also present in species whose tapetum is totally reabsorbed by developing pollen and whose pollen is devoid of any glue enabling en masse transport (Table 1).

Pollenkitt is hydrophobic because it is mainly composed by lipids, while tryphine is a mixture of hydrophilic and hydrophobic substances (Dickinson and Lewis, 1973). Tapetal cells produce pollenkitt, irrespective of tapetum type (secretory or amoeboid) and maintain their individuality until degeneration; vice

versa tapetal cells producing tryphine are always of the secretory type (Pacini, 1997).

In the case of tryphine the tapetal plasmamembrane ruptures at late microspore stage when organelles can still be recognized. In the case of pollenkitt, this only occurs when all the tapetal components have degenerated and intermingled, namely during the final stage of pollen maturation (Pacini and Franchi, 1993). Tryphine ripens after the tapetal plasmamembrane has disappeared; pollenkitt ripens in situ and the plasmamembrane only disappears when pollenkitt has already formed and before its deposition on the grains. Tryphine is certainly responsible for pollen adhesion to the stigma by virtue of a species specific interaction mediated by lipophilic molecules in the pollen exine (Zinkl et al., 1999). In pollenkitt, this kind of molecule has not been described but the function exists.

At least up to a certain stage the pattern of tapetum development is essentially the same whether pollenkitt or tryphine forms. Elaioplasts and spherosomes form first but later the pattern of development depends on whether pollenkitt or tryphine is produced, or if tapetal content is totally reabsorbed as in strictly anemophilous plants (Table 1). Reabsorption has been described in *Lolium perenne* (Pacini et al., 1992). Tryphine and the functions of pollen coats in general are extensively reviewed by Dickinson et al. (2000).

Despite these differences, pollenkitt and tryphine share some functions and this may be why some authors do not distinguish them, or call pollenkitt tryphine and vice versa, or use the generic term pollencoat (Hernandez-Pinzon et al., 1999; Piffanelli et al., 1998). These authors published a survey on tryphine biogenesis and its physico-chemical characterization.

Presence or absence of pollenkitt

Pollenkitt is produced as a tapetum derivative which has been found in almost all zoophilous angiosperms studied so far i.e. with entomophilous and related pollination syndromes, but not in gymnosperms (for details and reviews see Hesse 1978a–d, 1979a–e, 1980a–c, 1981a, b, 1984). Pollenkitt as pollen binding agent is typical of almost all entomophilous plants with monads and tetrads (Hesse, 1993); however, the distribution of pollenkitt on pollen surfaces is highly variable. The characters of the so-called Pollenverkitung (for this perfectly apt German word, a rough translation is “the variable degree of pollen stickiness”), namely amount, consistency and distribution of pollenkitt in relation to exine ultrastructure has been investigated in closely related genera with anemo- and entomophilous species, for example, *Plantago*, *Fraxinus*, *Acer*, *Carex*, *Sanguisorba*, and *Artemisia* (Hesse, 1979c).

¹A curiosity should not be overlooked: Long before Knoll already Kerner had observed the pollenkitt, but he interpreted it first as a slimy polysaccharidic substance and called it “Bassorin” (Kerner von Marilaun, 1873). In his famous “Pflanzenleben” (several editions) he referred to oily coatings, neither using the term “Bassorin” any more nor using “pollenkitt” or similar terms. Botanical histochemistry was not yet developed. Bassorin, also called Bassora-Gummi, is a residual of gums or slimes (polysaccharides!) mainly from stems of *Astragalus* sp., for technical use as sticky, highly viscous liquid, and surely no lipid, hence no real pollenkitt precursor term. Kerner misinterpreted this Bassorin as deriving from the cell walls (!) of pollen mother cells, or as plasmatic residual from microspore division. He does not mention tapetal cells, for any reason.

It is absent from the pollen surface of strictly and primary anemophilous taxa in the Fagaceae (except *Castanea*), Urticaceae, Chenopodiaceae, (most) Poaceae, but not in Cyperaceae (see the strictly anemophilous *Carex acutifolia* and *C. vulpina* versus the entomophilous *Carex baldensis* with a lot of sticky, active pollenkitt, Hesse, 1980b). Vice versa in some secondary anemophilous members of otherwise entomophilous families such as the Ranunculaceae (*Thalictrum*), Oleaceae (*Olea*, *Fraxinus*), Euphorbiaceae (*Mercurialis* and *Ricinus*) and Asteraceae (*Artemisia* and *Ambrosia*), pollenkitt is still present, at least in patches on the pollen surface (Pacini and Franchi, 1998, for details see also Hesse, 1979a, b, and for conspectus 1981a, b). An excellent example for transformation within a single genus, with several more or less entomophilous and anemophilous species, respectively, is *Acer*, where the respective flower syndrome becomes less entomophilous and more anemophilous, and simultaneously pollenkitt amount, density and homogeneity decreases (Hesse, 1979d).

There is always the same strategy: sticky pollen is typical of many entomophilous taxa, it has a homogeneous, electron opaque appearance and is mostly deposited on the exine. In contrast pollen typical of anemophilous taxa usually has few uneven less electron opaque pollenkitt not on the exine, but usually between the columellae and inside exine cavities. It is noteworthy that in many families, a transformation series from entomo- to anemophily exists. This means that the flower can be successfully pollinated by insects and (simultaneously or later) by wind. In these amphiphilous taxa, which allow pollination by insects and wind, the

pollenkitt shows intermediate characters of structure and distribution, for example in *Fagopyrum* (Hesse, 1979b), *Galanthus* (Hesse, 1981b) and *Castanea* (Hesse, 1978d). Fig. 1 shows strategies to place pollenkitt with its different appearance on exine or in the exine cavities.

From these numerous examples it can be noted that de-activation of large quantities of pollenkitt follows several strategies: (1) by filling only exine cavities, while the exine surface is completely devoid of pollenkitt, as in *Aquilegia* (Hesse, 1979a) and in *Teucrium* (Hesse, 2003), (2) by avoiding release of pollenkitt lumps by their permanent inclusion in a tapetal membrane (an excellent example is *Populus*), and (3) less electron-density, less even distribution, hence less stickiness in taxa with entomophilous and anemophilous species, e.g. in *Artemisia* and *Acer*.

Several examples demonstrate the complexity of this strategy: in the Solanaceae pollenkitt is present in *Petunia*, which has exposed pollen, but absent in *Lycopersicum peruvianum* where anther dehiscence is poricidal. In fact, this kind of anther opening is not linked to the absence of pollenkitt because, for instance, it is present in the poricidal Ericaceae.

Unlike this relict presence on the pollen surface, its absence in strictly anemophilous species cannot be interpreted as a way to avoid the loss of high energetic substances such as lipids because the pattern of development of the tapetum is the same, at least up to a certain stage (Table 1). Contrary to the previous examples, *Castanea sativa* is amphiphilous (Daumann, 1972), and its pollen is actively collected by bees and other insects (Fægri and Van der Pijl, 1979). Pollenkitt is present in large quantities hidden in exine cavities

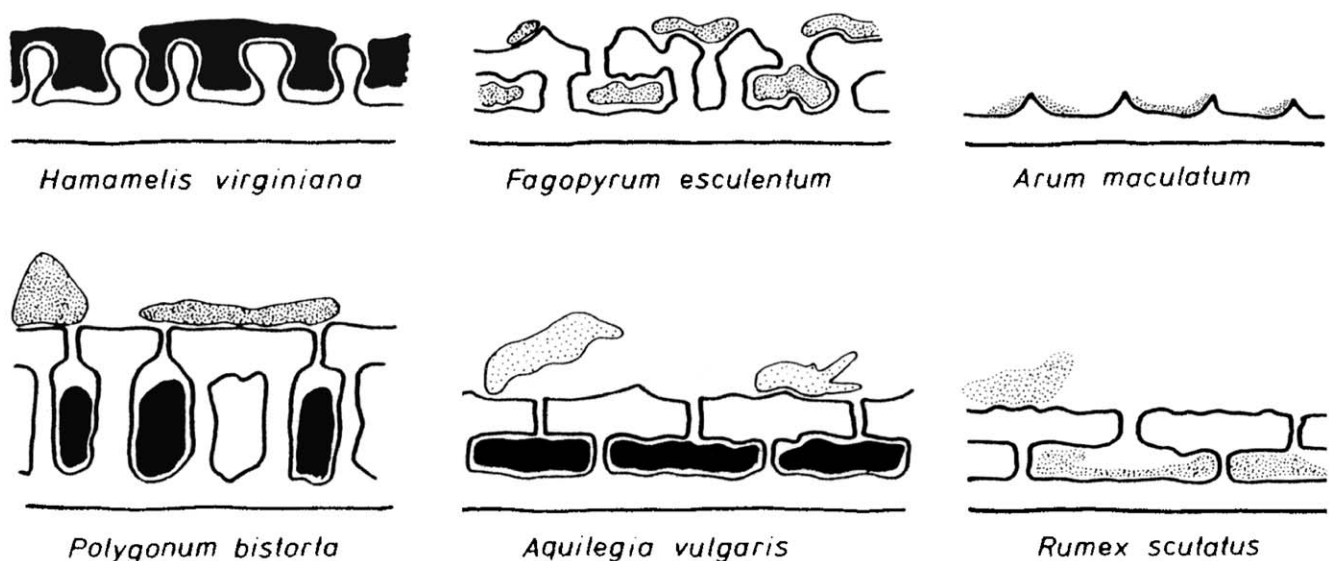


Fig. 1. Distribution and consistency of pollenkitt in angiosperms with amphiphilous pollination ecology: black deposits indicate electron-dense, homogeneous, very sticky pollenkitt, stippling indicates heterogeneous electron-transparent and less sticky pollenkitt. Faint dots without enveloping lines indicate less viscous, very few and nearly unsticky pollenkitt.

(Hesse, 1978d). The ecological meaning of the absence of pollenkitt or other sticky materials is that pollen grains may leave the anther as soon as it opens (Pacini and Hesse, 2004).

In the epi- or hypo-hydrophilous marine monocotyledons (the so-called seagrasses with surface or submarine pollination, Ackerman, 2000, also for review) pollenkitt is not absent, as demonstrated optically and cytochemically for the spherical pollen of *Thalassia* (Pettitt, 1976, 1980), while in other genera with filiform pollen TEM has shown a slimy or gelatinous coat consisting mostly of polysaccharides plus some proteins and lipids (Pettitt, 1976, 1980; Pettitt et al., 1984). Hence, a mucilaginous fluid composed of polysaccharides, proteins and lipids is present around dispersing pollen grains in at least some underwater-pollinated taxa. With the stepwise disappearance of pollenkitt, the exine is also progressively reduced and may be absent as demonstrated by Cooper et al. (2000) in some representative members of the Callitricaceae, which tend towards submergence (Martinsson, 1993); the same occurs in submerged monocots, the seagrasses with their filiform pollen, being the most extreme adaptation (Blackmore et al., 1987; Pettitt et al., 1984).

Ontogenesis of pollenkitt

The starting point of pollenkitt formation varies. Pollenkitt is formed from prophase I onwards, at the end of meiosis, after meiosis, during tetrad stage, shortly before break-up of tetrads, or after tetrad stage (Hesse, 1978b). Judging from studies using different TEM staining methods, it seems that pollenkitt is mainly a blend of two main components: elaiosomes and spherosomes. The former are the product of a distinct type of plastid with lipid reserves, the elaioplasts (Clément and Pacini, 2001; Pacini and Casadoro, 1981; Reznickova and Dickinson, 1982; Santos et al., 2003); the latter are the result of accumulation of smooth ER lipids in cistern dilations (Santos et al., 2003; Ubera-Jimenez et al., 1996). Other parts of the tapetum cytoplasm may also be involved in pollenkitt formation: during tapetal cytoplasm disappearance other cell components may contribute to spherosome-borne material.

At least during microspore stage the tapetum is engaged in exocytotic processes, and other substances may be released into the loculus and absorbed by pollen grains or deposited on them (Pacini and Juniper, 1979): the tapetum shows a different secretory nutritional activity according to the stage of pollen development. Finally, pollenkitt is deposited on the pollen as a homogeneous coating or as a non-homogeneous layer of variable electron density, especially in not strictly entomophilous taxa.

In species in which development of the tapetum has been studied ultrastructurally, plastids, initially at the stage of proplastid divide and accumulate plastoglobules to become elaioplasts (Ciampolini et al., 1993; Pacini et al., 1992). Later smooth ER gives rise to spherosomes. In *Ilex paraguayensis* the opposite occurs, elaioplasts with unsaturated lipids form first and later plastoglobules (Santos et al., 2003). In some cases there is a transitional phase of chromoplasts (Clément and Pacini, 2001) or amyloplasts (Ubera-Jimenez et al., 1996); however, it is noteworthy that in *Rosmarinus*, plastids do not contribute to pollenkitt formation, only spherosomes (Ubera-Jimenez et al., 1996).

Few papers give a detailed description of the final dissolution of tapetal cells, i.e. the merging of elaioplasts into spherosomes. In some cases, this only seems to occur when the tapetal plasmamembranes are lost (Lesniewska and Charzynska, 2000).

Tapetum consists of fragile cells, presumably because they totally or partially lose their inner tangential and radial walls for quick exocytosis (Pacini, 1994). Tapetal integrity is easily damaged during dissection before fixation, especially if long large anthers are divided into small pieces. The pollen is suspended in the locular fluid and if the anther is sectioned or damaged or compressed, the tapetal plasmamembrane may be ruptured, releasing its contents. Even the molarity of the fixative can cause artefacts in tapetum structure. Artefacts arising from damages may be interpreted as degeneration. This difficulty also arises from the fact that the plasmamembrane is not always easily discernible and it is sometimes difficult to state whether or not it is present.

Since tapetal cells die to nourish the developing pollen they have features typical of programmed cell death, i.e. apoptosis (Pacini et al., 1999; Wu and Cheung, 2000).

Mode and time of deposition on the pollen surface

The question of the wholesale transfer and regular deposition of coatings on pollen walls, and in some species in pollen walls, is complex (Dickinson et al., 2000). However, some points have been established. Deposition mode has been described for some genera and species. Weber (1996) showed that *Geranium robertianum* tapetum first releases coating vesicles and later real pollenkitt, which completely envelops the grains. In *Smyrniium* (Weber, 1991) and *Alisma* (Hesse, 1980b) small droplets act as a “transport form”, whereas large lumps (e.g. in *Tilia*) are released by the degenerating tapetum. Deposition timing has only been described for a few species (Hesse, 1978b), but it seems to be stimulated by the sudden increase in volume of the

cavity containing pollen by fusion of contiguous loculi (Keijzer, 1987a, b; Nepi and Pacini, 1993; Pacini, 2000).

Clément et al. (1988) described pollenkitt formation in *Lilium* in detail. Some droplets are extruded directly by plastid stroma through the plastid envelope while others, unsaturated lipid globules, are presumably derived from the interaction of smooth ER and plastids.

When any type of tapetum has completely degenerated, i.e. when cytoplasmic components are no longer evident, TEM may reveal different aspects: a homogeneous mass as in olive, cherry, *Cichorium* (Pacini and Casadoro, 1981; Pacini and Keijzer, 1989; Pacini et al., 1986), or non-homogeneous, with electron opaque and electron translucent areas, as in *G. robertianum* (Weber, 1996). When deposited on the grains, it appears as homogeneous masses inside the pollen ornamentation. It may be a continuous homogeneous electron opaque mass as in *Rosmarinus officinalis* (Uberta-Jimenez et al., 1996), or it may consist of electron opaque and electron translucent masses or foamy bubbles, as in *G. robertianum* (Weber, 1996).

An underrated example (Hesse, 2003) of the exine reservoir function may be the presence of various lipids, proteins and other compounds, plus unstable substances on the surface and inside the exine. Only in optimally preserved pollen of the apparently strictly entomophilous *Teucrium montanum* (Lamiaceae), may it be seen that the remarkable amount of pollenkitt (the electron-opaque substance) is almost completely absorbed in exine cavities. The real pollen surface (tectum) lacks pollenkitt entirely. Hence, the pollen of this species is not really sticky, which is perhaps related to its pollination biology (thought to be an obligate self-pollinated species).

The different modes observed in the final stages of tapetum degeneration and pollen coating may also be linked to the following processes: the moment when tapetal plasmamembranes break or disappear, fusion of contiguous loculi, locular fluid resorption, pollen dehydration and anther opening. All these phases, especially the last three, seem very specific, differing from species to species, and deeply influenced by environmental parameters such as: temperature, relative humidity and light intensity (Bianchini and Pacini, 1996; Lisci et al., 1994; Pacini, 1996, 2000).

Appearance under LM, SEM and TEM

The appearance of pollenkitt in ripe pollen by LM depends on the miscibility of the mounting medium with water. If miscible, pollenkitt may form spheres on the pollen surface, because it is hydrophobic and probably because the increase in volume at rehydration causing pollenkitt to extrude from the exine cavities. During

rehydration pollenkitt droplets even emerge from exine cavities in the complex double tectate-columellate ectexine of Asteraceae. Pollen rehydration modifies pollen shape and increases its volume, causing rearrangement of pollenkitt on the pollen surface.

If the mounting medium is immersion oil or another apolar fluid, the pollen does not rehydrate and pollenkitt dissolves. This is particularly evident when pollenkitt is dark in colour: deep red, violet or brown as in some lilies, tulips and *Petunia*, or blue-black as in some poppies (*Papaver*). Under the light microscope one can see that pollenkitt has been removed and dissolved by currents in the mounting medium.

If pollen is observed by LM without any mounting medium, i.e. directly on the slide, and without a coverslip, pollenkitt is visible inside the exine cavities or coating the pollen surface totally in a thin layer. Small patches are only seen where pollen grains adhere together.

SEM visualization of pollenkitt likewise depends on the method used. After alcohol fixation, pollenkitt tends to form strands, as in the entomophilous *Bryonia dioica* (Hesse, 1981b). After aldehyde fixation it is mostly evident. In *Cucurbita pepo* and *Lavatera arborea* where it is abundant, it obscures some minor exine ornamentations that become evident only after its removal with lipid solvents (Nepi and Pacini, 1993, 1999). An effective method to clean pollen surfaces of pollenkitt is the so-called DMP-CPD-technique (Halbritter, 1998); however, removal may fail if pollenkitt is extremely viscous.

TEM visualization depends largely on sample preparation. Because of its lipidic composition, pollenkitt can only be preserved if the material is fixed in osmium tetroxide or other fixatives that stabilize lipids. Pollenkitt is often dissolved during fixation and embedding.

Classical palynologists used to clean pollen before the observation by the acetolysis technique, which removes all structures not resistant to this strong solvent, except exine (Hesse and Waha, 1989). Using non-aqueous fixation methods, the pollen coating appears as an evenly thin coating seen by TEM, especially in Brassicaceae (Dickinson et al., 2000, also for review of chemical composition and function).

Methods for pollenkitt identification

Here we report some methods to reveal pollenkitt on pollen collected from anthers. The pollen is deposited on a slide and a droplet of water and a coverslip are added. The pollen quickly rehydrates and pollenkitt appears as birefringent droplets on the exine surface. These drops eventually merge and grow in size. In this way it is also possible to determine the real colour, because in the anther the colour is the result of interference with that of

pollen. A similar method enables slow rehydration, because of glycerine in the mounting medium and involves mixing pollen grains with Carlberla fluid (Ogden et al., 1974). After 6–24 h the exine turns pink and pollenkitt drops appear on its surface. The advantage of this method is that slides can be observed several hours later.

By virtue of its lipid composition, pollenkitt stains with all Sudan dyes and all other dyes for lipids. The problem is that it is necessary to observe the slide immediately after the dye is added, i.e. within 2–10 min, depending on the amount of pollenkitt and size of the pollen, because exine stains too, but later than pollenkitt. If the slide is observed too late it is difficult to distinguish the two, especially if the quantity of pollenkitt covering the pollen is small. On the other hand, if the quantity is large and emerging from the exine cavities in drops, observation can wait 15–30 min. Heslop-Harrison and Heslop-Harrison (1985) stained *Eucalyptus* pollenkitt with Scarlet R in ethanol with good results.

Another good method when pollenkitt is abundant is to dust pollen on a slide just wiped with chloroform. The pollen is then removed by gently blowing and is observed directly or after staining with dyes for lipids; pollenkitt droplets of different size adhere to the slide.

A method to check for pollenkitt in small quantities is to prepare two aliquots of pollen, one of which is washed with a mixture of chloroform/carbon disulphide (1:1) for a few seconds and allowed to dry. The other is untreated. The treated and untreated pollen is then observed by SEM (Pacini and Vosa, 1979). If the images are similar it means that pollenkitt is absent; if they differ and washed pollen shows better ornamentation, it means that pollenkitt is present.

The acetolysis method destroys all component of pollen except of the exine (Hesse and Waha, 1989), whereas treatment of pollen with 2% osmium tetroxide at 40° for 6 days destroys the exine, leaving the pollenkitt at least in *Trachymene pilosa* (Audran, 1981).

A task for further research is to check the number of pollen grains per clump adhering to insect bodies. The insect must be prepared without the use of substances such as chloroform and carbon disulphide, which remove lipids. The whole insect or some dissected parts can be viewed directly with a high power stereomicroscope, after sputtering by SEM or in water by LM.

Chemical composition

It seems unlikely that pollenkitt composition is the same in all species, because of its different consistency, colour, etc. Little research has been done on this topic.

Dobson (1988) developed a method to separate pollenkitt by double extraction, first extracting pollen with benzene for 20 s and then chloroform: methanol (2:1) for 23–28 h. The rationale was that the first quick extraction removed the external lipids, namely pollenkitt and the second internal and any external lipids not completely removed by the first extraction. Inner and outer lipid components can then be analysed by thin layer chromatography (TLC) or other analytical methods. Comparison of the results of both extractions provides information about internal (cytoplasmic) and external (pollenkitt) lipid composition (Piffanelli et al., 1998).

Pollenkitt is a hydrophobic mixture of materials, composed mainly of saturated and unsaturated lipids (Dobson, 1988), carotenoids (Hesse, 1993; Lunau, 1995), flavonoids (Wiermann and Gubatz, 1992), proteins and carbohydrates (Parkinson and Pacini, 1995; Santos et al., 2003; Weber, 1996, also for review). Differences in pollenkitt composition have been reported in the heterantherous species *Lagerstroemia indica* which has two pollen forms produced by pollinating and feeding anthers (Nepi et al., 2003). Only the pollinating pollen contains neutral lipids, a peculiar partition factor.

According to an isolated observation by Pohl (1932), the pollenkitt of *Philodendron* is crumbly and somewhat slimy, not lipidic or oily like all other known pollenkitt.

The physico-chemical properties of pollenkitt depend on the substances in the mixture and their proportions. Substances responsible for hiding pollen from animal eyes are understandably absent on pollen actively collected by animals. Molecules responsible for pollen flavour and smell, are probably species specific.

We do not know if pollenkitt composition is stable or whether it changes during pollen exposure and pollen transport by animals, as occurs for carbohydrate reserves (Vesprini et al., 2002). Changes in colour and consistency have been noticed, e.g. in *Cyclamen* (Pohl, 1930), and not only in entomophilous species, where this change could be to discourage animals from visiting wilted flowers when anthesis is already over. This change is known in *Mercurialis annua* (anemophilous) where pollen is only exposed for 1 day and by afternoon, the initially yellow pollen is blue green (Lisci et al., 1994 and our unpublished data). On the other hand, in *Erica arborea*, which is first entomophilous and then anemophilous, a change in viscosity occurs when the nectary ceases to produce nectar and pollen is dispersed by wind (Franchi and Pacini, 1996).

The many functions of pollenkitt

The pollenkitt starts to play its role as soon as anther opens, continues during pollen exposure and dispersal,

and presumably ceases when pollen rehydrate on the stigma (Table 2). Twenty proposed functions in the literature are listed and discussed below:

1. *To hold pollen in the anther until dispersal*: This occurs mainly in entomophilous flowers, where pollen remains firmly attached to the exposed anther even when the air is turbulent, and in upside down anthers such as *Passiflora* and vertical ones such as *Acanthus mollis*. In some Liliaceae pollen may be lost when wind speed is high probably because it is not firmly attached to the anther. This enables simultaneous dispersal of pollen by insect and wind (Dafni, 1986). In some species stamens move when touched by insects, the result of this movement, sometimes sudden, is intended as a way to avoid self pollination (Proctor et al., 1996) and brushes pollen on the body of the dispersing animal. In some *Opuntia* species, this movement reveals the nectary, previously hidden by clustered stamens (Schlindwein and Wittmann, 1997). Despite these movements, pollenkitt prevents loss of pollen from anthers not touched by animals.
2. *To enable secondary pollen presentation*: Some families or members of angiosperm families do not present pollen for dispersal on the anther but in other flower parts. This means that as soon as the

anther opens, pollen is stuck to other floral parts mainly by virtue of pollenkitt or other devices (Erbar and Leins, 1995; Pacini and Hesse, 2004), and is later removed by pollinating agents. Presentation outside the anther has different apparent reasons. In the Asteraceae and related families, the flowers are tiny, with long corollas and densely packed in the inflorescence. Pollen is stuck to the style in Asteraceae (Pacini, 1996) and to the stylar head in *Petromarula* sp. (Campanulaceae) (Igersheim, 1993). In *Acrotriche* (Epacridaceae) the flowers are conspicuous, the corolla is full of nectar and pollen is presented on the petals (McConchie et al., 1986). In many Araceae, such as *Zantedeschia* and *Anthurium*, male and female flowers are tightly packed but separated in different parts of the inflorescence (Mayo et al., 1997). Their pollen, mixed with a highly viscous pollenkitt, is extruded from the anther by a piston mechanism (Yeo, 1993) in coiled threads or noodles, up to 3–4 mm long, subsequently loaded by pollinators.

3. *To facilitate pollen dispersal*: Pollen grains are packed inside the developing anther, but in many cases when it opens and during presentation they form a kind of sponge, i.e. there is much space between grains and the external part is fringy with grains forming threads (Fig. 2). This change in

Table 2. Pollenkitt functions and the stage when they act

Pollenkitt functions	During pollen presentation	During pollen dispersal	During pollinator interactions	On stigma
1. To hold pollen in the anther until dispersal	X			
2. To enable secondary pollen presentation	X			
3. To facilitate pollen dispersal	X			
4. To protect pollen from the waterloss	X	X		
5. To protect pollen from the UV radiation	X	X	X	
6. To conserve sporophytic proteins inside exine cavities	X	X		
7. To protect the pollen protoplasts from fungi and bacteria especially during presentation	X	X	X	X
8. To keep pollen grains together during transport		X	X	
9. To protect pollen from hydrolysis and exocellular enzymes	X	X		
10. To render pollen attractive to animals	X			
11. To render pollen visible to animal eyes	X			
12. To hide pollen from animal eyes	X			
13. To avoid predation of pollen through smell	X			
14. To enable adhesion to insects bodies			X	
15. To enable pollen packaging by bees and form corbicules			X	
16. To provide a digestible reward for pollinators			X	
17. To enable pollen clumps to reach the stigma				X
18. To allow self-fertilization	X	X		X
19. To facilitate adhesion to the stigma				X
20. To facilitate pollen rehydration				X

Some only act at a certain stage, others at different stages.

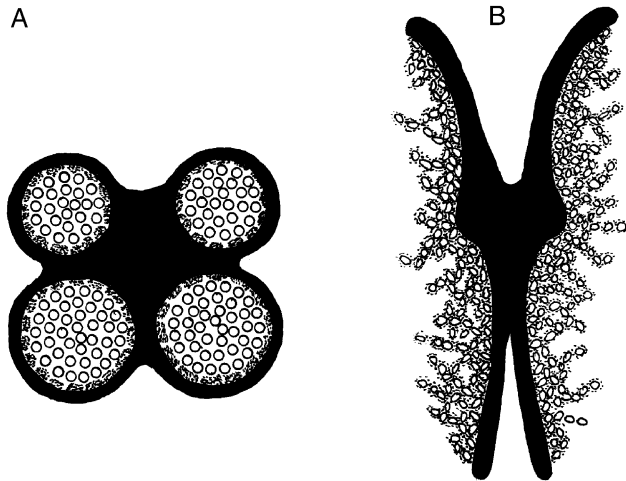


Fig. 2. Semidiagrammatic drawings showing two transverse sections of the anther at different stages of development in a species with partially dehydrated pollen – (A) before anther opening and locular fluid disappearance and (B) during pollen exposure: (A) Tapetal content has already been converted to pollen kitt and occurs as spheres (dotted) lining the locular cavity. It will cover the pollen grains when contiguous loculi fuse. Pollen grains are still spherical and fully hydrated. (B) Pollen, now partially dehydrated and oval in shape is coated in pollen kitt (dots) and exposed for dispersal. Pollen forms a sponge with threads on the surface. This is made possible by pollen kitt, which sticks the grains to each other and to the anther. This disposition allows removal by pollinators and wind. Image B is idealised but derived from observations of living anthers with a high power light microscope. Such an image is not possible by SEM because pollen grains “jump” during observations due to electrostatic charging.

pollen arrangement, before and after anther opening, is due to anther wall movements during opening and the physico-chemical properties of pollen kitt. The composition and viscosity of pollen kitt and the size and ornamentalions of the pollen grains, determine whether the sponge has big or small cavities. This loose disposition of pollen allows gradual release of grains. The best way to observe real pollen disposition on the anther is with a high powered stereo microscope. Observation by SEM is not useful for this purpose because many grains are lost due to the vacuum.

4. *To protect pollen from water loss:* It seems unlikely that some species would have protection against water loss, while others, such as those with viscin threads, hidden anthers, or without any pollen glue material, would not. The function of pollen kitt therefore is only to hinder or slow down water loss and its action is often weak. This seems the case of *C. pepo* where pollen kitt is so abundant as to obscure ornamentalions but pollen viability decreases quickly because of water loss and viability decreases at the same rate during the short pollen

presentation (Nepi and Pacini, 1993). However, this decrease in pollen viability seems to be linked to the quantity and quality of carbohydrate reserves that influence water retention, as well as to water content at opening (Nepi et al., 2001; Speranza et al., 1997). When pollen kitt is lacking and water content is high, as in Poaceae, the pollen can be expected to be very vulnerable and viability to be lost in a few hours. However, in this family pollen flight is short, especially in annuals (even a fraction of a second) because plants grow close together (Franchi et al., 1997).

5. *To protect pollen from UV radiation:* While understandable as a function in a general way, it was said to prevent damage to vegetative cells and sperm nuclei or the generative nucleus (Stanley and Linskens, 1974); this is only a hypothesis.
6. *To maintain sporophytic proteins responsible for pollen stigma-recognition inside exine cavities:* Pollen kitt sometimes contains substances involved in pollen–stigma recognition (Heslop-Harrison, 1975; Reznickova and Dickinson, 1982). This was demonstrated in Brassicaceae pollen which is coated with tryphine (Piffanelli et al., 1998) and not with pollen kitt. The oily component of pollen kitt conserves/protects these recognition proteins during presentation and transport.
7. *To protect the pollen protoplast from fungi and bacteria, especially during presentation:* The air is full of spores and some could fall, germinate and penetrate pollen, especially when it waits several days in the exposed anther before dispersal. The growth of these spores would be accelerated by high relative humidity (Bassani et al., 1994; Pacini et al., 1997). Fungal hyphae start to form on pollen of the palm *Chamaerops humilis* kept in a closed micro-environment for 5 days at 98% relative humidity (Bassani et al. 1994). When the pollen was kept at lower RH (55%) mould growth was delayed and at 20% RH it did not grow. Under certain climatic conditions, pollen kitt could act as a barrier against fungal and bacterial invasions. This hypothesis has yet to be verified, but seems likely when pollen presentations is long as in some tropical plants.
8. *To keep pollen grains together during transport:* Pollen kitt is absent when pollen grains are dispersed individually, but present when they are dispersed in clumps. These clumps are not stable but may break up during transport. This was demonstrated in experiments with *M. annua*, a secondary anemophilous angiosperm. Pollen dispersed in the air at different wind speeds, was collected by gravitation on slides coated with high viscosity silicon at various distances from the pollen source (Lisci et al., 1996). The average number of grains per clump varied with distance from the source being higher near the

source and lower far from it. We do not know if it is possible to generalize this observation to other anemophilous species with pollenkitt or if there is even a loss of pollen from the bodies of flying insects.

9. *To protect pollen from hydrolysis and extracellular enzymes:* Parallel to protect glycoalkaloid components by accumulated sporopollenin from hydrolysis and extracellular enzymes (Rowley et al., 1999) pollenkitt may function like sporopollenin sealing most of the pollen surface, at least in entomophilous taxa.
10. *To render pollen attractive to consuming animals:* Pollenkitt may have different perfumes odors and certain different substances needed by pollinators (Dobson and Bergström, 2000). Pollen of *Rosa rugosa* has a perfumed pollenkitt (Dobson, 1994, also for review).
11. *To render pollen visible to animal eyes:* Lunau (1995) published a detailed and exhaustive study on the spectrum of pollen colours according to visibility for humans and pollinating animals. Pollen colour depends on pollenkitt and if pollenkitt is removed pollen is invariably yellow (Nepi et al., 2003).
12. *To hide pollen from animal eyes:* In many plants, flower visitors collect nectar but not pollen, even when the anthers are evident and exposed outside the flower. The reason for this may be that pollen is not evident to animal eyes due to some compounds in pollenkitt. This seems the case in *L. indica*, which has flowers with two sets of anthers, one inside and an outer encircling set. Both produce viable pollen, but the inner set of anthers produces pollen with little pollenkitt. The pollen of the inner anthers is feed pollen and that of the outer set is fertile pollen. The grains of the two sets differ in colour, but both are yellow when pollenkitt is removed (Pacini and Bellani, 1986). This hiding mechanism may even occur at the end of the blooming period; if the pollen is no longer visible pollinators are presumably discouraged from visiting the flower.
13. *To avoid predation of pollen through smell:* Dobson and Bergström (2000) found α -methyl alcohols and ketones in the pollenkitt of some anemophilous species and *Filipendula* and hypothesized that this was to avoid predation of pollen by discouraging the insect through smell. These compounds also seem toxic for some insects (Dobson and Bergström, 2000).
14. *To enable adhesion to insect bodies:* Pollenkitt was necessary in early angiosperms to stick pollen to pollinator bodies. The first insects involved in this mutual activity were Diptera and Coleoptera (Thien, 1980 and references therein); the latter have little or no body hairs to trap pollen. Researches have shown that pollen is electrostatically attracted by the insect even if it does not touch the anther. The pollen adheres to the body by electrostatic forces between insect and the flower (Vaknin et al., 2000). We do not know whether or to what extent pollenkitt is involved in this electrostatic mechanism.
15. *To enable pollen packaging by bees and to form corbicules:* Pollen of species visited by Apoidea which collect pollen to store as food always has pollenkitt (Pacini, 2000). When other pollen sources are not available these insects also collect pollen of *Zea mays* and other grasses or anemophilous species devoid of pollenkitt (Wille and Wille, 1983). To form corbicules they must however use pollen of species with pollenkitt to pack pollen devoid of it. An alternative mechanism is to regurgitate a sticky fluid composed mainly of sugars thus using a pollenkitt substitute (Klungness and Peng, 1983). Pollenkitt fills the spaces between grains so that the mass becomes more coherent and compact. For a given viscosity, the larger the diameter of pollen, the more pollenkitt is necessary to stick it together. This is probably why large pollen grains have a thick layer of pollenkitt, sometimes even covering ornamentations, and adhere to pollinators as individual grains (Nepi and Pacini, 1993). Not only the physicochemical properties of the pollen grains but also their geometrical shape influence the possibility of forming compact corbiculae. Indeed if pollen is tetrahedral, as in many Myrtaceae, it can be closely packed with little space between grains, in respect to ovoid or spherical pollen grains.
16. *To provide a digestible reward for pollinators:* Unlike the other substances in pollen grains protected by relatively impermeable walls, pollenkitt is immediately dissolved by digestive enzymes after ingestion (Franchi et al., 1997). Pollenkitt could therefore be one of the first substances absorbed by the digestive tract of pollen consumers (Nepi et al., 2000).
17. *To enable pollen clumps to reach the stigma:* Pollenkitt is responsible for the formation of pollen clumps before transport. When pollen of *M. annua* reaches the stigma surface the clumps are already broken up, probably due to impact with the stigma (Lisci et al., 1996). Break up of clumps also reduces competition among grains because they are scattered over the stigma. This does not seem to be the case for entomophilous pollination where pollen grains adhere to the stigma in clumps (Nepi and Pacini, 1999).
18. *To allow self-pollination:* Wang et al. (2004) described the programmed, non-incidental, transport of pollen in a film of pollenkitt from the anther to the concave stigma in *Caulokaempferia coenobialis* (Zingiberaceae); this seems to be a case of obliged self-fertilization.
19. *To facilitate adhesion to the stigma:* Angiosperm pollen may or may not have pollenkitt, and likewise

Table 3. Some examples of the presence and/or absence of pollenkitt and stigma exudate in angiosperms

	Pollenkitt	
	Present	Absent
<i>Stigma exudate</i>		
Present (wet stigma)	<i>Olea europaea</i> <i>Prunus</i> sp. <i>Petunia</i> sp. <i>Lilium</i> sp. <i>Helleborus</i> sp.	<i>Lycopersicum peruvianum</i> All massulate orchids
Absent (dry stigma)	<i>Cucurbita pepo</i> ^a <i>Mercurialis annua</i> <i>Ricinus communis</i> Asteraceae Malvaceae ^a	Chenopodiaceae ^a Corylaceae ^a Some Plantaginaceae ^a <i>Humulus lupulus</i> ^a <i>Parietaria</i> sp. ^a Most Fagaceae Spinacia sp. ^{a,b} Most Poaceae ^a

Data on stigma exudates from Heslop-Harrison and Shivanna (1977) and personal observations.

^aThese species emit pollen tubes a few minutes after pollen adhesion to the stigma because they are partially hydrated at shedding. In the other species listed pollen tube formation lasts for more than 1 h because the pollen is partially dehydrated (Franchi et al., 2002).

^bOur unpublished observations.

stigma exudates may or may not be present (Table 3). Stigma exudates also have a main lipid component (Lush et al., 1998). Table 3 gives examples of presence/absence of coating substances on stigma and pollen.

20. *To facilitate pollen rehydration:* Despite its lipid composition, pollenkitt does not totally seal the pollen surface because pollen still rehydrates, even if dead (Pacini, 1990) or when some grains of a clump do not touch the stigma papillae. This was demonstrated elegantly in some Solanaceae by in vitro pollination of dry and wet stigma exudates and different lipids, with and without water (Lush et al., 2000). Pollen only germinated if water was intermingled with stigma exudates or lipids. This suggests a role of pollenkitt so far overlooked; if the exine is greatly reduced, its role is taken over by other wall layers, namely endexine and intine. The role of thick endexine or intine in many inaperturate pollen may be taken over by abundant pollenkitt. Even common tricolpate pollen in dry state may behave like inaperturate pollen and does not fold. A great amount of pollenkitt prevents folding of pollen with thin walls, as in some Passifloraceae (*Adenia*, Halbritter, 2000a, and *Passiflora racemosa*, Halbritter, 2000b). Because lipid coatings are impermeable like waxes, pollenkitt is in this respect like leaf cuticle. For the complex diversity of harmomegathic folding and the role of exine strata or other components see Halbritter and Hesse (2004, and references therein).

The functions listed above are not always present in a given species and may not act simultaneously (Table 2).

In pollen of anemophilous species such as *Ricinus*, pollen is released ballistically as soon as the anther opens (Bianchini and Pacini, 1996) and at least in this case, a major widespread function, namely to keep pollen on the anther until dispersal, is absent. Nevertheless pollenkitt is absent in the anemophilous *Parietaria* which launches its pollen by a sudden movement of the filament (Pacini and Hesse, 2004). Other functions as that of number 19 (to facilitate adhesion to the stigma surface) become predominant when the stigma is devoid of stigma exudates (see examples in Table 3).

Some of the properties and functions of pollenkitt are due to its sticky nature (1, 2, 3, 8, 14, 15, 17, 18, 19), others depend on its components with their physico-chemical properties.

The presence and functions of pollenkitt can be correlated with pollen presentation and exposure, as well as pollen water content. If pollen is dispersed with a high water content, as in many grasses, and without pollenkitt, being released as soon as the anther opens, its flight is commonly short (a few seconds) because a stigma of an individual of the same species is in the immediate surroundings (a few centimetres or decimetres). In the case of palms, which are anemophilous, and have pollen with a low water content, and are often dioecious, pollenkitt is present and abundant, probably to protect pollen during its long exposure and transport (Pacini, 2000).

Further studies

Little research has been done on pollen clumping and the number of grains per clump (Lisci et al., 1996; Nepi

and Pacini, 1993), in entomophilous, secondary anemophilous (for example *Olea europaea*) or hypo-hydrophilous species. It would be interesting to know if this number is constant, and if so, why. There are very few records of the number of pollen grains per clump deposited on the animal bodies. In the case of *C. pepo*, which has large pollen, up to 200 µm in diameter, clumping is rare and single grains are mostly observed.

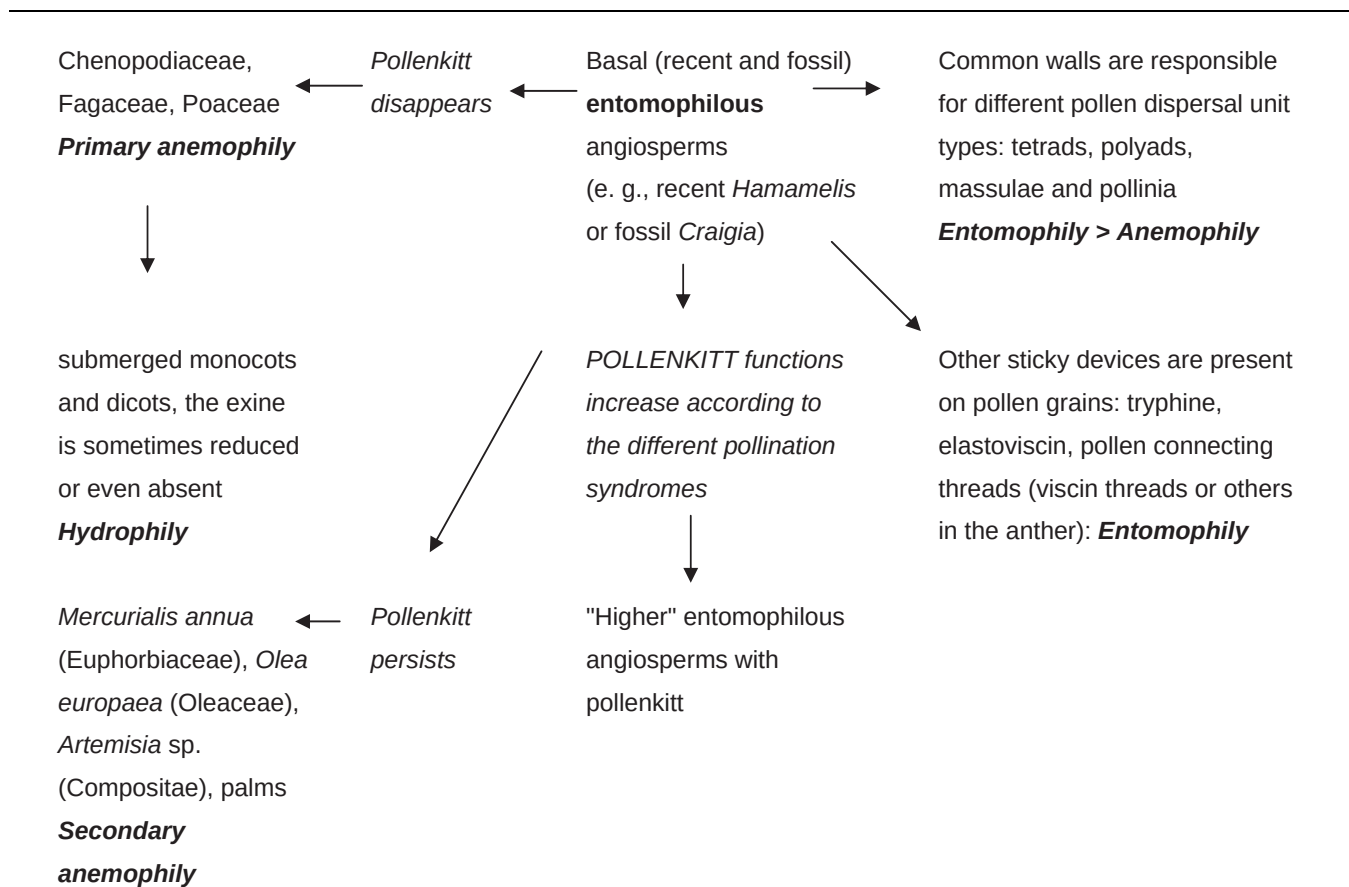
Has all the pollen of an anther the same amount of pollenkitt? If so, is there a recognition substance in pollenkitt and on the pollen surface? Does the pollenkitt naturally cover the whole pollen surface or is it absent from some sites, for instance, the pores, when they protrude as in some partially hydrated grains (Franchi et al., 2002; Nepi et al., 2001)? Investigations by SEM suggest that it is regularly distributed in some cases as in *C. pepo* (Nepi and Pacini, 1993, Fig. 4a) where it covers protruding pores. However, in many examples, it does not seem to be regularly distributed, especially when pollen is ovoid with furrows (being partially dehydrated) and reticulate exine. Pollenkitt is a viscous fluid and can move/flow during pollen presentation or handling (Wang et al., 2004). Is there an efficient mechanism

governing a uniform distribution of pollenkitt? This question arises especially for large anthers crowded with grains. If pollenkitt is not regularly distributed the destiny of pollen may be affected through male competition. Only pollen grains with sufficient pollenkitt will succeed in sticking firmly to the pollinator body and later to the stigma. Does a remnant of pollenkitt remains in the anther? Is all pollenkitt deposited on pollen or does some persist in the anther?

Differences in pollenkitt colour and composition in heterantherous species have only been demonstrated in *L. indica*, a species having flowers with two types of pollen: feed pollen and real pollen (Nepi et al., 2003). Differences in composition can also be expected in heterantheric and tristylous species where pollen grains have different colours, such as *Lythrum*, and probably also in the heterostylous species *Primula* sp. and *Eichhornia crassipes*. Differences in spectral reflection of pollen have been reported in heterantherous and heterostylous species such as *Lythrum salicaria* (Lunau, 1995 and references therein).

Are composition and viscosity the same or similar in species of the same family such as *Anthurium* and *Arum*

Table 4. Flow diagram showing a possible path of evolution of substances responsible for formation of pollen dispersing units (PDU) and pollination syndrome in angiosperms



Pollenkitt is the most common way to disperse pollen clumps.

(Araceae), or different because of different pollen presentation mechanisms? In the first case pollen retained by pollenkitt is extruded from the anther (see above), but in the second it falls to the base of the spathe whence it is collected by trapped pollinators: sapromyophilous syndrome (Fægri and Van der Pijl, 1979). Is the composition the same or very similar in anemophilous species belonging to a mainly entomophilous family?

Is there a recognition system between pollenkitt deposited from the outside and substances present in exine cavities or even in the cytoplasm? Since pollenkitt is a viscous fluid, it could be interesting to know whether viscosity varies widely or little from species to species and whether this physical parameter is affected by changes in temperature and relative humidity around the flower. We could also expect to find different pollenkitt viscosity according to pollen grain size and packaging mode.

If pollenkitt and/or stigma exudates enable the passage of water for pollen rehydration, as demonstrated by Lush et al. (1998), how does rehydration occur if both of these lipid structures are absent, as in grasses? Grasses and other species without pollenkitt and stigma exudates (Table 3) are partially hydrated at dispersal and germinate quickly.

Conclusions

Pollenkitt is certainly the commonest device responsible for dispersal of pollen in clumps. Early angiosperms are widely considered to have been entomophilous (Friis and Pedersen, 1996). Table 4 illustrates a hypothetical pathway of pollenkitt evolution in angiosperms.

The main functions of pollenkitt have to do with pollen adhesion: pollen to pollen, pollen to anther, pollen to animal bodies/hairs, pollen to stigma and to facilitate pollen dispersal irrespective of syndrome. In biological systems, sticky fluids are not normally composed of lipids but rather proteins or carbohydrates. Why is this high-energy fluid used as glue? A reason may be because pollen is often dispersed in an arrested developmental state, usually by means of partial dehydration (Franchi et al., 2002; Fotitt and Cohn, 2001). Coating pollen with a semipermeable fluid is a way to reduce water loss and uptake, especially during long presentation and dispersal. The presence of lipids outside the pollen protoplast could also serve as reserves during pollen rehydration and activation on the stigma. This hypothesis seems to be corroborated by the fact that pollenkitt is absent in the case of underwater pollination (Blackmore et al., 1987) and certain viscous carbohydrates have taken over some of the functions of pollenkitt (Ackerman, 1995, 2000; Pettitt, 1980).

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