

# Why does some pollen lack apertures? A review of inaperturate pollen in eudicots

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Apertures are key characters of pollen grains with systematic importance in angiosperms. They function as sites for pollen tube exit, water uptake, transfer of recognition substances and accommodation of volume changes. Not all pollen has apertures; inaperturate pollen (lacking obvious apertures) characterizes many angiosperm groups, especially in early divergent angiosperms and monocots, but also eudicots. In order to expand our knowledge of the systematic distribution, possible functional significance and development of inaperturate pollen in angiosperms, this review focuses on inaperturate and cryptoaperturate (with hidden apertures) pollen in the large eudicot clade, which comprises about 75% of present-day angiosperm species. It includes new TEM observations of inaperturate pollen from four exemplar taxa selected from different parts of the eudicot phylogeny. Two categories of inaperturate (including cryptoaperturate) pollen occur in eudicots. (1) Sterile attractant or feeding pollen associated with functional dioecy has evolved iteratively at least six times in conjunction with complex breeding systems in the core eudicots. (2) Fertile pollen has evolved numerous times independently throughout eudicots, though generally in a relatively small number of individual taxa. Notable exceptions are the petaliferous crotonoid Euphorbiaceae *s.s.*, in which fertile inaperturate pollen occurs in *c.* 1500 species, and two subfamilies of Apocynaceae *s.l.* (Secamonoideae and Asclepiadoideae) with *c.* 2500 species with fertile inaperturate pollen in pollinia. Fertile inaperturate pollen is sometimes (but not always) associated with an aquatic habit, parasitism, insectivory, heterostyly, anemophily or pollinia. Most fertile inaperturate pollen has a thin exine, or the exine is largely restricted to isolated components (muri, protuberances, subunits) separated by thinner areas which probably function as apertures. In cryptoaperturate pollen, the aperture is covered by continuous exine which probably has a protective function, similar to an operculum. Developmentally, inaperturate pollen is not associated with any particular tetrad type or meiotic spindle orientation (unlike some apertures) due to the absence of a colp shield of endoplasmic reticulum or other organelles and hence is independent of microsporogenesis type. The lack of a colp shield during the tetrad stage of development permits complete deposition of first primexine and then exine around each microspore, possibly mediated by the action of the DEX1 protein. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 155, 29–48.

**ADDITIONAL KEYWORDS:** aperture function – cryptoaperturate – evolution – exine – functional dioecy – pollen development – primexine – systematics – tetrads.

## INTRODUCTION

Apertures are distinctive and often systematically significant features of pollen grains. They function as exits for the pollen tube in germination, as sites for the uptake of water, in the accommodation of volume changes (harmomegathy) and as sites for the transfer

of recognition substances (Blackmore & Crane, 1998; Edlund, Swanson & Preuss, 2004). They affect the environmental vulnerability to desiccation, fungal invasion and mechanical stress of pollen grains (Edlund *et al.*, 2004). However, inaperturate pollen, in which an obvious aperture is lacking, occurs in some groups. It is relatively common in early divergent angiosperms including the ANITA grade, magnoliids and monocots, which are otherwise normally characterized by monosulcate (or monosulcate-derived)

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pollen (Furness & Rudall, 1999a, 2000; Sampson, 2000; Furness, Rudall & Sampson, 2002b). This absence of apertures is probably related to the relatively thin exine that characterizes pollen of these early divergent lineages; endexine is sparse or absent compared with eudicots which have well-developed, acetolysis-resistant endexine and tricolpate (or tricolpate-derived) pollen (Zavada, 1983; Rudall & Furness, 1997; Blackmore & Crane, 1998; Furness, Magallón & Rudall, 2007). Virtually every major monocot group includes some taxa with inaperturate pollen, indicating that this character has evolved several times. In many monocots the presence of inaperturate pollen is correlated with aquatic or moist habitats, e.g. in Alismatales (aquatics: Furness & Rudall, 1999a, 2000) and Zingiberales (moist rain forests: Kress, 1986). Another possible correlation is with the mycoheterotrophic habit, e.g. in Triuridaceae (Furness & Rudall, 2000; Furness, Rudall & Eastman, 2002a).

Furness & Rudall (1999a, 2000) described two categories of inaperturate pollen in monocots: (1) functionally monoaperturate pollen, in which there is a localized thickening of the intine for pollen tube germination, although the exine is uniform, e.g. *Pentstemon* (Stemonaceae: van der Ham, 1991); (2) omniaperturate pollen (*sensu* Thanikaimoni, 1978), in which the intine is uniformly thick and frequently channelled, the pollen tube may germinate from anywhere on the pollen wall and the exine is often highly reduced, e.g. *Canna* (Cannaceae: Skvarla & Rowley, 1970; Kress & Stone, 1982). This type of pollen is sometimes referred to as 'exineless'. A third category occurs only in eudicots: (3) cryptoaperturate (*sensu* Thanikaimoni, 1978; Punt *et al.*, 1994), in which there is an endoaperture (an aperture in the endexine) that is not apparent in surface view, because there is no ectoaperture (aperture in the ectexine). This last type has been documented in some *Phyllanthus* species (Phyllanthaceae: Meewis & Punt, 1983; Punt, 1986, 1987).

The occurrence of inaperturate pollen both in basal angiosperms and in monocots, in which the pollen is normally monosulcate (or monosulcate derived), and in eudicots in which the pollen is normally tricolpate (or tricolpate derived), is an interesting example of convergent evolution. Multiple pores distributed globally over the pollen surface (pantoporate or polyporate apertures) have a similar taxonomic distribution pattern, and a few other aperture types, such as spiral apertures, also occur in both monocots and eudicots (Furness, 1985; Blackmore & Crane, 1998). Here I review the categories of inaperturate pollen and their distribution in eudicots in a systematic context using information from the literature and observations. Data on inaperturate eudicot pollen are

often based on acetolysed pollen, and examination of the structure of fresh pollen, including the intine (which may provide clues to how these grains germinate), using transmission electron microscopy (TEM), is relatively rare. Here I examine sections of fresh pollen or microspores from four exemplar taxa chosen from different parts of the eudicot phylogeny. These four species all possess inaperturate pollen, though they differ in other aspects of their pollen morphology, such as surface sculpturing. I also examine aspects of pollen development, particularly early exine deposition in the tetrad stage of one species, to investigate possible reasons for the lack of apertures. One process that has been described to account for the development of pollen apertures is the formation of an organelle shield, typically endoplasmic reticulum (the colp shield), which blocks the formation of primexine and deposition of sporopollenin at the aperture sites (Heslop-Harrison, 1963; Sheldon & Dickinson, 1983, 1986). Previous developmental studies of inaperturate pollen in eudicots are relatively rare, although some have been carried out on *Callitriche* (Plantaginaceae) by Osborn, El-Ghazaly & Cooper (2001).

## MATERIAL AND METHODS

### TAXONOMIC BACKGROUND

Eudicots include c. 75% of extant angiosperm species and encompass a wide range of morphological diversity, especially in the two largest subclades, Rosidae and Asteridae. These comprise the 'core' eudicots, together with a putatively basal clade, Gunnerales, and several small clades (APG, 1998; APG II, 2003; Soltis *et al.*, 2003). In addition to the core eudicots, there is a basal grade consisting of a few lineages that are relatively species-poor. These lineages are termed early divergent eudicots (e.g. Furness *et al.*, 2007).

### PLANT MATERIAL

Pollen was examined from four exemplar taxa from three orders from different parts of the eudicot phylogeny (Table 1). Each of these has inaperturate pollen (Table 1) though the pollen morphology is very different in other aspects. Plant material was obtained from the living collections of the Royal Botanic Gardens, Kew (HK followed by accession number): *Nepenthes ventricosa* Blanco  $\times$  *inermis* Danser (Caryophyllales: Nepenthaceae) HK: 1990-1685; *Jatropha pohliana* aff. var. *subglaber* Müll. Arg. (Malpighiales: Euphorbiaceae s.s.) HK: 1980-282; *Populus balsamifera* L. (Malpighiales: Salicaceae) HK: 1985-8617; *Matthiola incana* (L.) R.Br. (Brassicales: Brassicaceae) HK: 1967-3305.

**Table 1.** Occurrence of inaperturate (including cryptoaperturate) pollen in eudicots following classification in APG II (2003); placement of Rafflesiaceae according to Barkman *et al.* (2004), Davis & Wurdack (2004)

Family	Taxa with inaperturate pollen	References
<b>EUDICOTS</b>		
Ranunculales:		
Berberidaceae	<i>Ranzania</i> (or six-pantocolpate)	Kosenko (1980), Nowicke & Skvarla (1981)
Menispermaceae	<i>Disciphania</i> , <i>Tiliacora</i>	Erdtman (1952), Thanikaimoni (1968), Ferguson (1975)
Ranunculaceae	<i>Souliea vaginata</i>	Erdtman (1952), Wang <i>et al.</i> (1993)
<b>CORE EUDICOTS</b>		
Dilleniaceae (unplaced to order)	Some <i>Tetracera</i> (female), also cryptoaperturate	Kubitzki & Baretta-Kuipers (1969), Dickison <i>et al.</i> (1982), Anderson & Symon (1989)
Caryophyllales:		
Nepenthaceae	<i>Nepenthes</i>	Erdtman (1952), Ueno (1981), Takahashi & Sohma (1982), this paper, Fig. 1A–F
Santalales:		
Loranthaceae	<i>Atkinsonia</i>	Feuer & Kuijt (1980)
<b>ROSIDS</b>		
Vitaceae (unplaced to order)	<i>Vitis riparia</i> (female), <i>V. vinifera</i> (female)	Kevan, Longair & Gasawski (1985)
Geraniales:		
Geraniaceae	<i>Balbisia</i> , <i>Wendtia</i>	Erdtman (1952), Bortenschlager (1967)
<b>EUROSIDS I</b>		
Malpighiales:		
Euphorbiaceae	c. 1500 species of petaliferous crotonoids including <i>Baliospermum</i> , <i>Croton</i> , <i>Jatropha</i> , <i>Joannesia</i> , <i>Neoboutonia</i> , <i>Tritaxis</i>	Erdtman (1952), Punt (1962, 1987), Nowicke (1994), Wurdack <i>et al.</i> (2005), this paper, Figs 2A–F, 3A–F
Linaceae	<i>Anisadenia</i> , <i>Reinwardtia</i> , <i>Tirpitzia</i>	Erdtman (1952), Sugawara <i>et al.</i> (2002)
Malpighiaceae	<i>Digoniopterys</i> (female), <i>Microsteira</i> (female), <i>Rhynchophora</i> (female)	Lobreau-Callen (1983, 1984), Anderson (2001)
Phyllanthaceae	Some <i>Phyllanthus</i> cryptoaperturate	Meewis & Punt (1983), Punt (1986, 1987)
Rafflesiaceae	<i>Rafflesia</i> , <i>Rhizanthus</i> , <i>Sapria</i>	Erdtman (1952), Blarer <i>et al.</i> (2004)
Salicaceae	<i>Populus</i>	Erdtman (1952), Risch (1960), Hesse (1979), this paper, Fig. 4A–D
<b>EUROSIDS II</b>		
Brassicales:		
Brassicaceae	Some <i>Matthiola</i>	Erdtman (1952), Rollins & Banerjee (1979), this paper, Fig. 5A–F
<b>ASTERIDS</b>		
Cornales:		
Hydrostachyaceae	<i>Hydrostachys</i>	Erdtman (1952), Straka (1988)
Ericales:		
Actinidiaceae	<i>Saurauia veraguensis</i> (female)	Haber & Bawa (1984), Anderson & Symon (1989)
<b>EUASTERIDS I</b>		
Icacinaceae (unplaced to order)	<i>Chlamydocarya</i> cryptoaperturate?, <i>Pyrenacantha</i> cryptoaperturate?, <i>Stachyanthus</i> cryptoaperturate?	Erdtman (1952), Lobreau-Callen (1972, 1973)
Gentianales:		
Apocynaceae	Secamonoideae (c. 170 species), e.g. <i>Secamone</i> ; Asclepiadoideae (c. 2300 species), e.g. <i>Asclepias</i> , <i>Ceropegia</i> , <i>Cynanchum</i> , <i>Matelea</i> , <i>Stapelia</i>	Verhoeven & Venter (2001), Vinkier & Smets (2002), Verhoeven <i>et al.</i> (2003)

Table 1. Continued

Family	Taxa with inaperturate pollen	References
Rubiaceae	<i>Cephaelis</i> , <i>Hymenocoleus</i> , <i>Palicourea</i> , some <i>Psychotria</i> , some <i>Rudgea</i>	Erdtman (1952), Baker (1956), Robbrecht (1975), Jung-Mendacoli & Melham (1995)
Lamiales:		
Martyniaceae	<i>Craniolaria?</i>	Erdtman (1952), Bretting & Nilsson (1988)
Plantaginaceae	Some <i>Callitriche</i>	Erdtman (1952), Osborn & Philbrick (1994), Cooper <i>et al.</i> (2000), Osborn <i>et al.</i> (2001)
Solanales:		
Solanaceae	<i>Mandragora</i> cryptoaperturate, some <i>Solanum</i> (female)	Erdtman (1952), Diez & Ferguson (1984), Anderson & Symon (1989), Zavada & Anderson (1997), Knapp <i>et al.</i> (1998), Zavada <i>et al.</i> (2000)
EUASTERIDS II		
Apiales:		
Araliaceae	<i>Polyscias pancheri</i> (female)	Anderson & Symon (1989), Schlessman <i>et al.</i> (1990)
TAXA OF UNCERTAIN POSITION		
Apodanthaceae	<i>Berlinianche</i>	Blarer <i>et al.</i> (2004)

## METHODS

Anthers were dissected from buds of various sizes and placed in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, deaerated under vacuum and stored in the glutaraldehyde at 4 °C. For examination using light microscopy (LM) and TEM the anthers were removed from the glutaraldehyde, washed in phosphate buffer and fixed in 1% osmium tetroxide for 2 h at room temperature. They were washed again in phosphate buffer and dehydrated through a graded ethanol series. Anthers were embedded in medium-grade LR White Resin in gelatin capsules. Sectioning was carried out using a Reichert Ultracut. Semithin (c. 1 µm) sections were cut using a dry glass knife, stained with toluidine blue and mounted in DPX. They were examined using a Leitz DMRB optical microscope and photographed using an Olympus DP70 digital camera. Ultrathin (gold) sections were cut using a Diatome diamond knife and stained with uranyl acetate followed by lead citrate in an LKB Ultrastainer. Sections were examined using a JEOL JEM 1210 TEM at 80 kV.

## RESULTS

## NEPENTHES

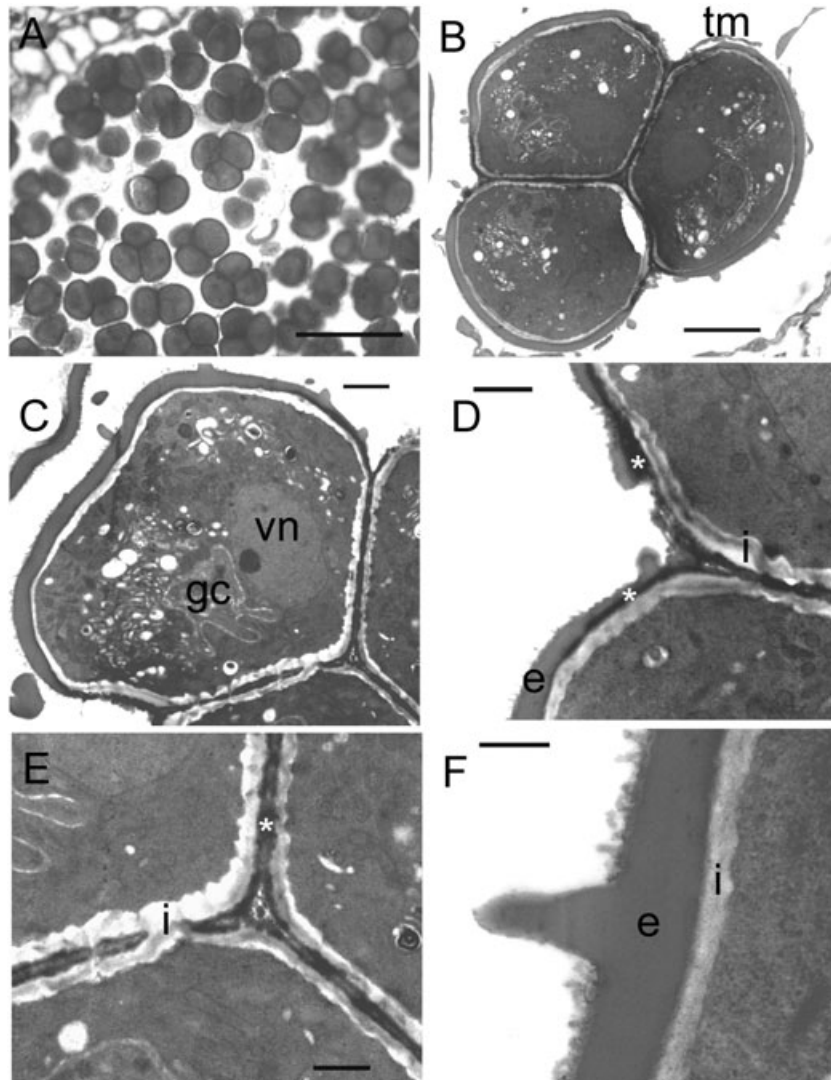
Pollen of *Nepenthes ventricosa* × *inermis* (Fig. 1A–F) is shed as thin-walled tetrahedral tetrads (Fig. 1A–C). The exine consists of a homogeneous layer which is probably ectexine with scattered spines on top (Fig. 1B, C, F). Some tapetal material appears to be deposited onto the exine (Fig. 1B). Beneath the ect-

exine is a thin intine (Fig. 1F). The ectexine becomes thinner close to the junction of the grains where a darker, more lamellated layer is visible which continues between the grains (Fig. 1D). This dark layer is possibly endexine. The grains are joined by fused endexines although there are some small gaps (Fig. 1E). There are also some intine connections between the grains (Fig. 1E).

## JATROPHA

Microsporocytes in *Jatropha pohliana* aff. var. *subglaber* (Figs 2A–F, 3A–F) undergo simultaneous cytokinesis, in which the two nuclei produced by the first meiotic division are not separated by a callose wall (Fig. 2A). There is a thin callose wall surrounding each microsporocyte (Fig. 2A). After the second meiotic division tetrahedral tetrads are formed which are enclosed by thick callose walls (Fig. 2B, C). The anther locule is surrounded by secretory tapetal cells (Fig. 2B). Each microspore of a tetrad is completely surrounded by a primexine layer which is deposited between the plasma membrane and the callose wall (Fig. 2D–F). Numerous vesicles occur adjacent to the primexine which are visible all round each microspore (Fig. 2D–F). A colpal shield of endoplasmic reticulum or other organelles is absent. A lamella runs through the centre of the primexine, dividing it into two layers (Fig. 2E, F). Electron-dense sporopollenin elements condense out of the primexine matrix (Fig. 2F). By the vacuolate microspore stage the callose has all dissolved and the microspores have separated (Fig. 3A). Each has a large vacuole, the cytoplasm is

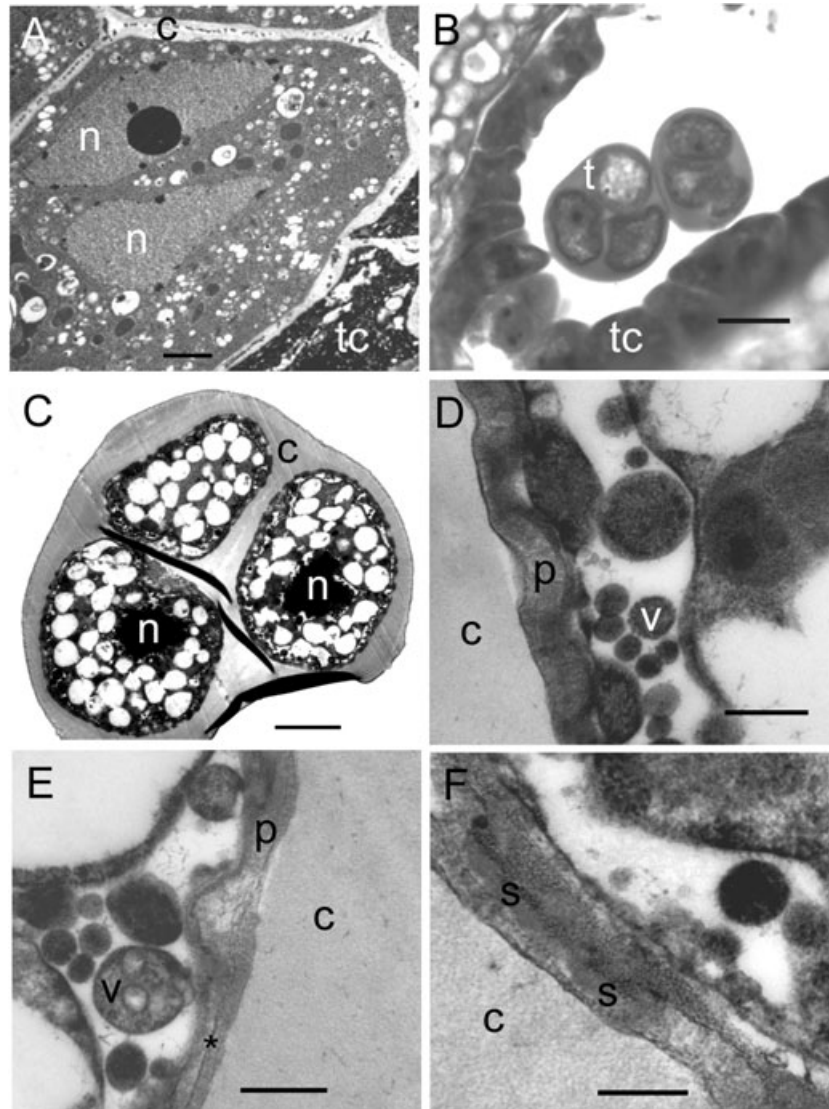




**Figure 1.** Sections of pollen of *Nepenthes ventricosa* × *inermis*. A, tetrahedral tetrads in an anther locule (LM). B, a tetrahedral tetrad (TEM). C, part of a tetrad showing one pollen grain with a vegetative nucleus and generative cell (TEM). D, junction of two pollen grains showing wall layers (TEM). E, junction of three pollen grains showing endexine and an intine bridge (TEM). F, detail of the exine with a spine and the intine (TEM). e = exine, i = intine, gc = generative cell, tm = tapetal material, vn = vegetative nucleus and \* = endexine. Scale bars = 50 µm in A, 5 µm in B, 2 µm in C, 1 µm in D, E and 500 nm in F.

pushed to the outer edge of the microspore adjacent to the wall, and the nucleus is also close to the wall (Fig. 3A, B). The microspores are spheroidal but rather irregular in shape probably due to compression in the locule (Fig. 3A, B). The tapetum has become invasive with tapetal cells invading the anther locule but they have not fused to form a plasmodium (Fig. 3A). This is an unusual, although possibly under-recorded tapetum type. Interestingly, Pacini, Franchi & Hesse (1985) recorded both secretory and plasmodial tapeta in Euphorbiaceae *s.l.* The microspore wall is composed of exine subunits

(Nowicke, 1994) forming a hexagonal or pentagonal reticulum (Fig. 3C, D). Neighbouring subunits are joined at the base but the spheroidal heads are free with gaps in between (Fig. 3C, D). Beneath the subunits is a granular layer embedded in a matrix of amorphous material which appears to be the remains of the primexine (Fig. 3C, D). It is, however, unusual for primexine to be retained this late in pollen development. This matrix also covers the granular layer in the lumina of the reticulum (Fig. 3D). A lamella runs through the centre of the granular layer (as in the primexine; see above) which indicates some of it may



**Figure 2.** Sections of a microsporocyte and tetrads of *Jatropha pohliana* aff. var. *subglaber*. A, a microsporocyte after the first meiotic division with two nuclei (TEM). B, tetrads surrounded by callose in an anther locule (LM). C, a tetrahedral tetrad (TEM). D, vesicles adjacent to the primexine deposited around a microspore (TEM). E, primexine showing lamella and vesicles (TEM). F, two-layered primexine with sporopollenin condensing out of it (TEM). c = callose, n = nucleus, p = primexine, s = sporopollenin, t = tetrad, tc = tapetal cell and v = vesicle. Scale bars = 2  $\mu$ m in A, 20  $\mu$ m in B, 5  $\mu$ m in C, 500 nm in D, E and 250 nm in F.

be endexine (Fig. 3E, F). Lamellae also appear beneath the granular layer (Fig. 3E, F).

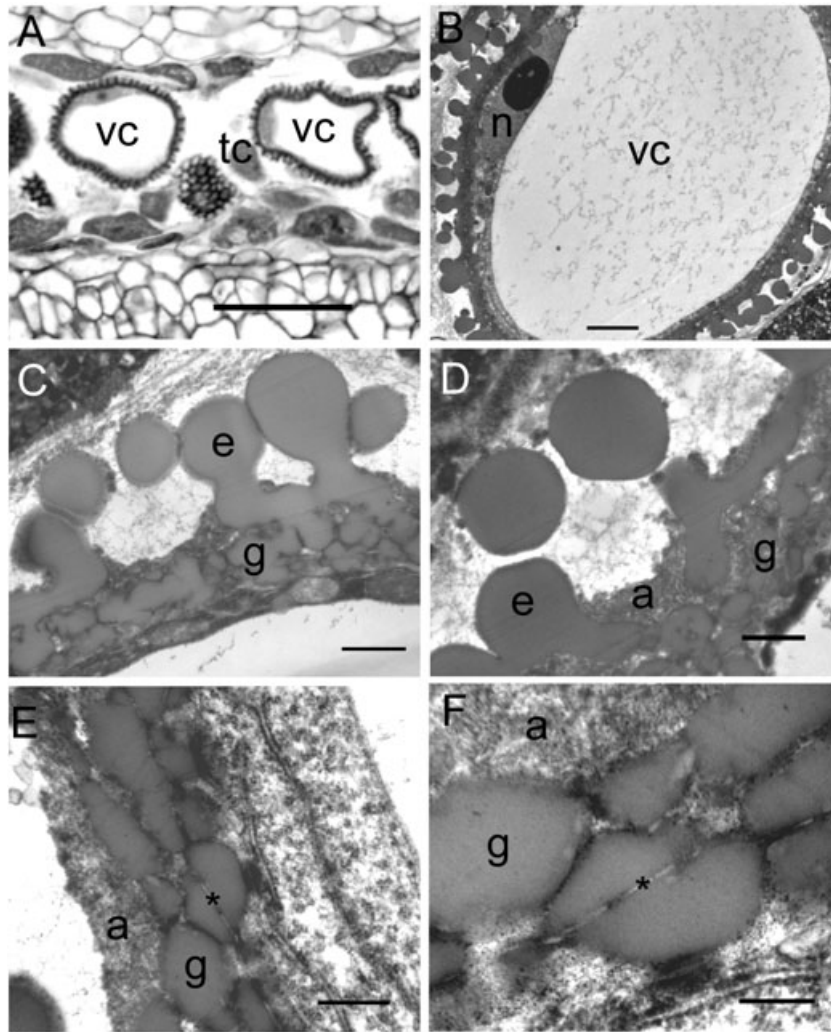
#### POPULUS

Late-stage vacuolate microspores with prominent nuclei were observed in *Populus balsamifera* (Fig. 4A–D). They are spheroidal in shape but are slightly deformed probably due to pressure in the locule given that they lack thick rigid walls (Fig. 4A, B). The exines are thin and composed of a layer of short, irregular columellae supporting irregular pro-

cesses or granules (some pointed) forming a discontinuous tectum (Fig. 4C, D). There is a darker stained endexine layer and beneath this a thin intine layer is deposited (Fig. 4C, D).

#### MATTHIOLA

*Matthiola incana* (Fig. 5A–F) pollen is spheroidal with long columellae and a coarse reticulum (Fig. 5A, C). A section clipping the wall shows the reticulum with large lumina between the muri which are



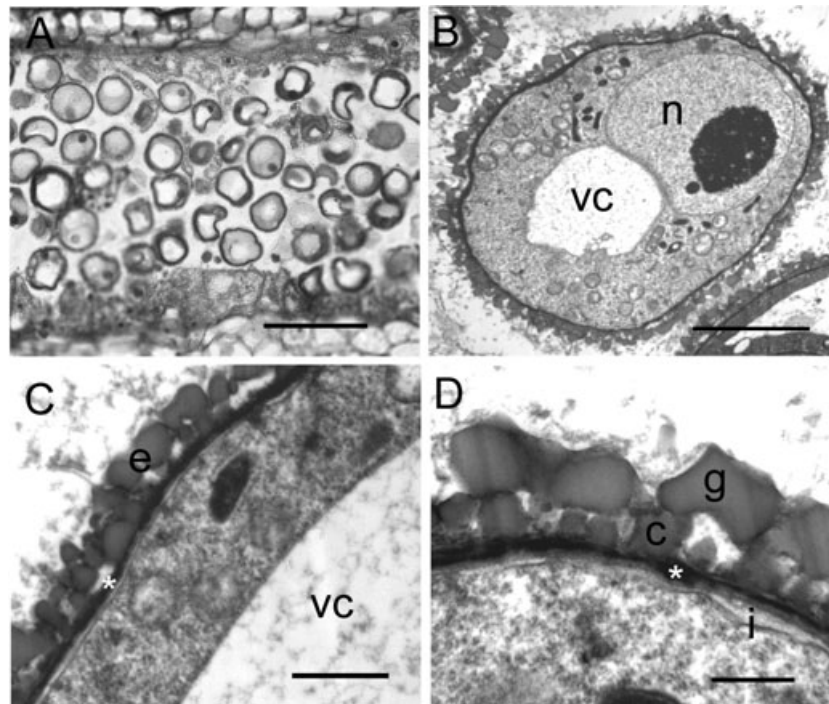
**Figure 3.** Sections of vacuolate microspores of *Jatropha pohliana* aff. var. *subglaber*. A, vacuolate microspores in an anther locule with an invasive tapetum (LM). B, a microspore with a large central vacuole and the nucleus and cytoplasm pushed to the edge (TEM). C, the microspore wall consisting of exine subunits forming a reticulum with a granular layer beneath (TEM). D, detail of the subunits, granular layer and amorphous material in the lumen (TEM). E, a lumen with granules, lamellae and amorphous material (TEM). F, detail of E (TEM). a = amorphous material, e = exine subunit, g = granule, n = nucleus, tc = tapetal cell, vc = vacuole and \* = lamella. Scale bars = 50 µm in A, 5 µm in B, 1 µm in C, D, 500 nm in E and 250 nm in F.

supported by columellae (Fig. 5B). Tapetal material (lipid) is deposited into the spaces between the columellae from the breakdown of the secretory tapetum (Fig. 5A). In vacuolate microspores, the columellae rest on an electron-dense endexine layer which is deposited on lamellae (Fig. 5D, E). The intine is deposited beneath this. By the time the pollen is mature, a two-layered intine has been deposited (Fig. 5F). The endexine appears to stretch as the pollen expands so gaps appear where there is only intine (Fig. 5F).

#### SYSTEMATIC DISTRIBUTION OF INAPERTURATE POLLEN IN EUDICOTS

Inaperturate (and cryptoaperturate) pollen in eudicots falls into two distinct groups. First, complex breeding systems have evolved in core eudicots and several records of inaperturate pollen in these lineages are associated with functional dioecy. In these species, the inaperturate pollen is sterile feeding pollen, or serves as an attractant to pollinators, and is produced by functionally female flowers. This system





**Figure 4.** Sections of vacuolate microspores of *Populus balsamifera*. A, vacuolate microspores in an anther locule with a degenerating tapetum (LM). B, a microspore with a small vacuole and large nucleus (TEM); C, the microspore wall with a granular exine and a dark endexine layer (TEM). D, detail of the wall showing exine granules, short columellae, endexine and thin intine (TEM). c = columella, e = exine, g = granule, i = intine, n = nucleus, vc = vacuole and \* = endexine. Scale bars = 50 µm in A, 5 µm in B, 1 µm in C and 500 nm in D.

has evolved at least six times independently within the core eudicots (Table 1). It is absent from basal angiosperms, including monocots, and from early divergent eudicots. Secondly, fertile inaperturate pollen has also evolved numerous times independently within eudicots (Table 1). The detailed distribution of both of these types of pollen is discussed below.

#### EARLY-DIVERGENT EUDICOTS

In early-divergent eudicots, inaperturate pollen occurs in three families in Ranunculales: Berberidaceae, Menispermaceae and Ranunculaceae (Table 1). In Berberidaceae, there are records of both inaperturate and six-pantocolpate pollen in *Ranzania japonica*, a monotypic taxon restricted to northern Honshu, Japan (Table 1). Other Berberidaceae have tricolpate pollen apart from *Berberis* and *Mahonia*, which are spiraperturate (Kosenko, 1980; Nowicke & Skvarla, 1981; Furness, 1985). In Menispermaceae inaperturate pollen is restricted to two genera, *Disciphania* (Tinosporeae) and *Tiliacora* (Triclisieae). Both have reticulate exines which are common in Menispermaceae pollen. Apertures in this family are

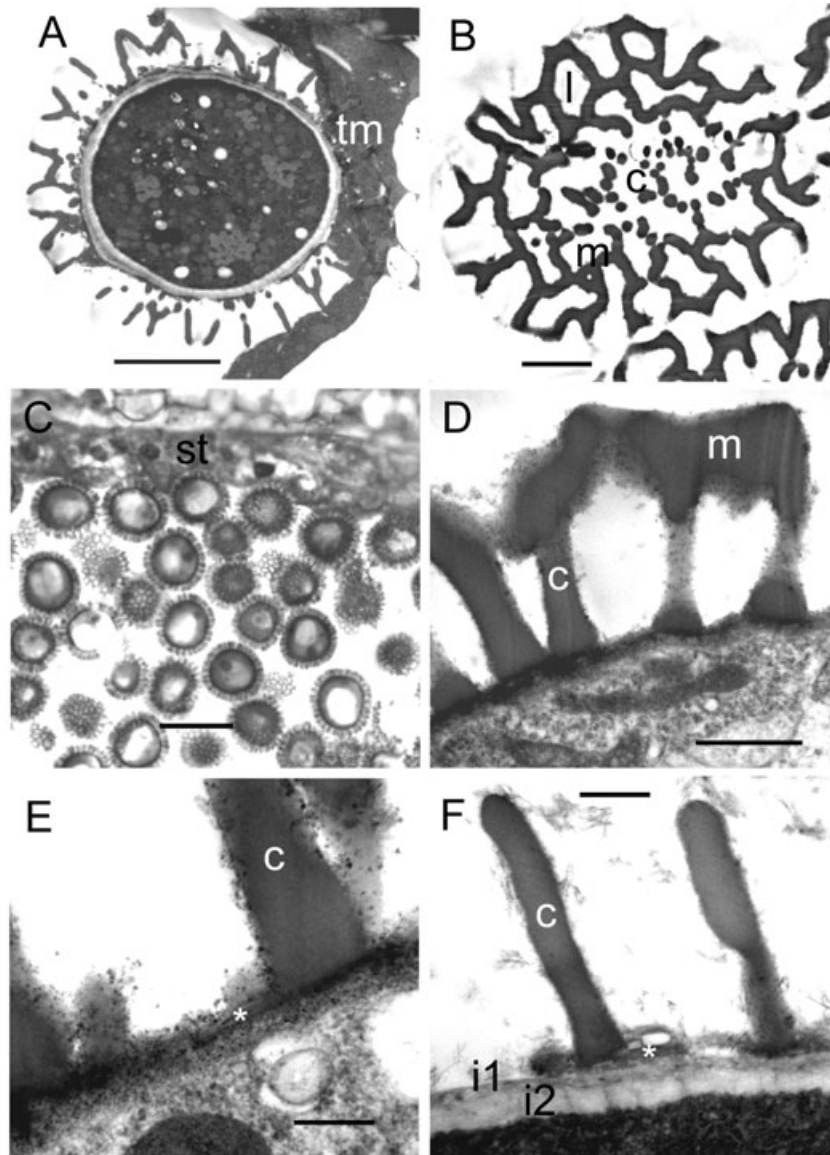
usually tricolpate, sometimes tricolpate (Blackmore, Stafford & Persson, 1995). The monotypic *Souliea vaginata* (Ranuculoideae), a cryptophyte endemic to south-west China, is the only member of Ranunculaceae with inaperturate pollen. The exine is striate-reticulate with spines (Wang, Hong & Li, 1993). Apertures in possibly related genera, e.g. *Cimicifuga*, are tricolpate and the exines are spiny, usually with perforations between the spines (Wang *et al.*, 1993). Pollen of Ranunculaceae is diverse with apertures ranging from tricolpate to pantocolpate and pantoporate, or spiraperturate in *Anemone*, and the colpi may be fused (syncolpate) or irregular (Blackmore *et al.*, 1995). Exine ornamentation is also variable but is most commonly a perforate tectum with granules (scabrae) or small spines (microechinae) (Blackmore *et al.*, 1995).

#### CORE EUDICOTS

##### *Non-rosids and non-asterids*

In core eudicots, dimorphic pollen occurs in some neotropical species of *Tetracera* (Dilleniaceae: unplaced to order; Table 1) that are functionally dioecious. In these species, male flowers have tricolpo-





**Figure 5.** Sections of pollen and vacuolate microspores of *Matthiola incana*. A, a pollen grain with a coarse reticulum with tapetal material (TEM). B, a clip through the reticulate exine showing the muri, lumina and columellae (TEM). C, vacuolate microspores in an anther locule with a secretory tapetum (LM). D, the reticulate exine of a vacuolate microspore showing the muri and columellae (TEM). E, detail of a columella resting on the dark, lamellated endexine layer (TEM). F, the pollen wall with columellae, a discontinuous endexine and two intine layers (TEM). c = columella, i1 = intine 1, i2 = intine 2, l = lumen, m = murus, st = secretory tapetum, tm = tapetal material and \* = endexine lamellae. Scale bars = 5  $\mu$ m in A, 2  $\mu$ m in B, 20  $\mu$ m in C, 1  $\mu$ m in D, 250 nm in E and 500 nm in F.

rate pollen, bisexual flowers (functionally female) have inaperturate, or weakly aperturate pollen with endopores or thinnings where the foot layer is absent, which could be interpreted as cryptoaperturate. There are no other obvious morphological differences (in pollen size, shape or wall sculpturing) between the tricolporate and inaperturate grains (Dickison, Nowicke & Skvarla, 1982). The inaperturate pollen

probably represents feeding pollen offered as a pollinator reward (Anderson & Symon, 1989). Other Dilleniaceae have tricolporate, tricolporate or four-colporate pollen (Dickison *et al.*, 1982).

In Caryophyllales inaperturate pollen is restricted to the insectivorous genus *Nepenthes* (Nepenthaceae: Table 1; Fig. 1A–F), in which pollen is shed as permanent tetrahedral tetrads and has a spiny exine

(Ueno, 1981; Takahashi & Sohma, 1982; this paper, Fig. 1A–F). Pollen in the closely related family Dros-  
eraceae is similar except that it has porate apertures;  
the pores have opercula in some species (Takahashi &  
Sohma, 1982).

The only member of Santalales with inaperturate  
pollen is *Atkinsonia ligustrina* (Loranthaceae:  
Table 1), a monotypic mistletoe endemic to south-east  
Australia. Mistletoes are hemiparasites which  
contain chlorophyll. Pollen is asymmetric, spherical to  
teardrop shaped with a granular surface, and an  
exine composed of a tectum, a thin granular layer, a  
basal layer (foot layer), and a loosely woven endexine  
which is absent in some areas (Feuer & Kuijt, 1980).  
This pollen is unique in Loranthaceae and is autapo-  
morphic for *Atkinsonia*. Most Loranthaceae have  
three apertures which are syncolpate (colpi fused at  
the pole) or diploporate (each ectocolpus with two  
endoapertures), pollen shape is usually triangular  
with concave or convex sides, and exines are granular  
or columellate often with a thick foot layer and a  
thick endexine in polar areas which is thinner at the  
apertures (Feuer & Kuijt, 1980, 1985).

#### *Early-divergent rosids*

*Vitis riparia* and *V. vinifera* (Vitaceae: unplaced to  
order; Table 1) are functionally dioecious with dimor-  
phic pollen. Male plants produce functional spherical  
tricolporate pollen, and females inaperturate pollen  
with the same diameter which cannot fertilize. The  
usual aperture type in Vitaceae is tricolporate  
(Erdtman, 1952).

In Geraniales, inaperturate pollen occurs in only  
one family, Geraniaceae, in which it is restricted to  
the South American genera *Balbisia* and *Wendtia*  
(Table 1).

Pollen of both genera is coarsely reticulate with  
a columellate exine (Bortenschlager, 1967). Other  
pollen types in Geraniaceae are tricolporate, panto-  
porate or tricolpate with reticulate, striate, verrucate  
or spinulate surfaces and columellate exines (Bortens-  
schlager, 1967). Erdtman (1952: 189) commented, 'If  
the short colpi in *Monsonia* and *Sarcocaulon* should  
disappear, a nonaperturate, coarsely reticulate pollen  
type, similar to that in *Balbisia* and *Wendtia*, would  
ensue [cf. parallel features in Cruciferae (*Matthiola*)]'.

#### *Eurosids I*

In contrast, inaperturate pollen occurs in six families  
in Malpighiales, with the largest occurrence in  
Euphorbiaceae s.s. (Table 1; Figs 2A–F, 3A–F). Inap-  
erturate pollen characterizes a well-supported croton-  
oid clade mostly with petals at least in staminate  
flowers (Wurdack, Hoffmann & Chase, 2005). This  
pollen occurs in at least 1500 species, a large group in  
eudicots with inaperturate pollen (Nowicke, 1994).

Pantoporate, tricolpate and tricolporate apertures  
occur in apetalous crotonoids (Nowicke, 1994;  
Lobreau-Callen, Malecot & Suarez-Cervera, 2000;  
Wurdack *et al.*, 2005). 'Crotonoid' pollen is character-  
ized by a typical pattern of exine subunits. According  
to Nowicke (1994) these subunits are raised ectexine  
elements, densely spaced or arranged in more open  
patterns such as rings of five or six, sometimes more.  
Their surfaces are smooth, pitted or striate, and their  
apices are pointed, rounded, flat or attenuate. In  
tangential section the subunits are triangular to  
rounded-triangular and are attached to muri having  
short irregular or granular columellae. The foot layer  
is thin and discontinuous, as is the granular or lamel-  
lated endexine (Nowicke, 1994). Pollen of the remain-  
der of the Euphorbiaceae s.s. is very diverse although  
tricolporate apertures are common (Punt, 1962, 1987;  
El-Ghazaly & Chaudhary, 1993; Lobreau-Callen *et al.*,  
2000; Nowicke & Takahashi, 2002).

Inaperturate pollen is found in three small Asian  
genera of Linaceae (Table 1). Heterostyly and associ-  
ated pollen dimorphism occur in Linaceae (Bahadur,  
Laxmi & Swamy, 1984; Sugawara *et al.*, 2002). In  
*Reinwardtia indica* inaperturate pollen of long-styled  
morphs is significantly smaller than that of short-  
styled morphs and the verrucate and microechinate  
processes found on pollen of both morphs are shorter  
and less swollen but more densely packed in the  
long-styled morphs (Sugawara *et al.*, 2002). Pollen of  
Linaceae is diverse; three-colpate, three-colporate and  
pantoporate apertures occur and the surfaces are  
gemmate, verrucate or reticulate (Saad, 1962; Punt &  
den Breejen, 1981).

Inaperturate pollen is produced by functionally  
female flowers in three genera of Malpighiaceae  
from Madagascar (Table 1). *Microsteira* and *Rhyn-  
chophora* have male flowers with pollen with six  
pores, and morphologically bisexual flowers with  
inaperturate pollen. Both types contain cytoplasm  
although the inaperturate pollen is larger in size  
(Anderson, 2001). Anderson (2001) concluded that  
the 'bisexual' flowers were functionally female and  
the inaperturate pollen was feeding pollen offered as  
a pollinator reward as Malpighiaceae have no nec-  
taries. Inaperturate pollen was also observed in a  
flower of *Digoniopterys* described as female or prot-  
ogynous (Lobreau-Callen, 1983). She described an  
area of thinner exine where the intine was thicker,  
which could represent a vestigial aperture in inap-  
erturate pollen in Malpighiaceae (Lobreau-Callen,  
1983). Other aperture types in Malpighiaceae are  
tricolporate, hexacolporate, syncolporate and panto-  
porate, and some genera produce out-crossing (chas-  
mogamous) flowers with normal aperture pollen  
and self-fertile (cleistogamous) flowers with small  
pollen grains with less well-developed exines and

vestigial apertures (e.g. *Aspicarpa* and *Gaudichaudia*: Lobreau-Callen, 1984). Lowrie (1982) described several genera as polyporate but lacking ectoapertures, e.g. *Bunchosia* and *Heladena*, although his scanning electron microscopy (SEM) images show porate apertures visible in surface view, so these are not cryptoaperturate in the sense of Punt *et al.* (1994) (see Introduction). Halbritter & Hesse (1995) described unusual pollen in *Malpighia* and *Heteropteris* with the exine divided into plates (clypeate) with operculate pores in the grooves between the plates. The intine is thickened beneath the pores.

Some African and South American species of *Phyllanthus* (Phyllanthaceae, formerly included in Euphorbiaceae *s.l.*, e.g. Webster, 1975) have cryptoaperturate pollen (with endoapertures but no ectoapertures) and pilate surfaces (Table 1). These appear inaperturate using SEM as the endoapertures are covered by continuous exine. Punt (1986) accounted for the occurrence of these remarkably similar pollen grains in two continents as due to convergence possibly related to pollination. In the African species a trend towards reduction in the ectoapertures can be seen from distinctly tricolporate (e.g. *Phyllanthus pynaertii*) to indistinctly tricolporate (e.g. *P. polyanthus*) to three-endoporate (with endoapertures but no ectoapertures, e.g. *P. dinklagei*) (Meewis & Punt, 1983; Punt, 1986). This trend could, however, operate in the opposite direction and requires further testing using well-sampled phylogenies. *Phyllanthus*, a genus of about 600 species (Stevens, 2001), has diverse pollen; 3–11-colporate, pantoporate and clypeate (syncolpate with an areolate pattern) occur, and the colpi may be diploporate (with two endoapertures) (Punt, 1980, 1986, 1987; Meewis & Punt, 1983; Lobreau-Callen, Punt & Schmid, 1988; Sagun & van der Ham, 2003). *Phyllanthus* × *elongatus* has unusual pantoporate pollen with polygonal exine shields (clypeate) separated by grooves with pores with thickened intine beneath them located in the intersections of the grooves (Halbritter & Hesse, 1995). This is similar to the clypeate pollen in *Malpighia* and *Heteropsis* (Malpighiaceae: Halbritter & Hesse, 1995; see above).

Erdtman (1952) reported inaperturate pollen in the river-weed family, Podostemaceae, using LM, but this has not been confirmed by more recent studies using SEM and TEM. These describe diverse pollen apertures in the family including tricolpate, tricolporate and pantoporate with tetracolpate, pentacolpate, hexacolpate, zono-aperturate and spiraperturate also occurring (Bezuidenhout, 1964; O'Neill *et al.*, 1997; Rutishauser, 1997; Osborn, O'Neill & El-Ghazaly, 2000; Passarelli, Girade & Tur, 2002; Jäger-Zürn, Novelo & Philbrick, 2006). Unusually in a eudicot, pollen in many species is dispersed as

dyads and these were shown to develop by successive microsporogenesis in *Griffithella* (Razi, 1949). Recently, it has been shown that simultaneous division occurs in Tristichoideae while both simultaneous and (unusually in a eudicot) successive division occur in Podostemoideae (Jäger-Zürn *et al.*, 2006).

Rafflesiaceae, holoparasites lacking chlorophyll which produce exceptionally large flowers smelling of carrion, have recently been placed in Malpighiales based on analyses of mitochondrial DNA (Barkman *et al.*, 2004; Davis & Wurdack, 2004). Pollen of the three genera in the family is inaperturate (Table 1). Previous records of ulcerate pollen and successive microsporogenesis in this family are anomalous in a eudicot (Ernst & Schmidt, 1913; Schnarf, 1931; Takhtajan, Meyer & Kosenko, 1985; Furness & Rudall, 2004). Records of ulcerate pollen are incorrect according to recent data from Blarer, Nickrent & Endress (2004), and microsporogenesis requires re-investigation.

All species of the anemophilous genus *Populus* (Salicaceae) have spheroidal inaperturate pollen (Table 1; Fig. 4A–D). The exine is thin with short columellae and a granular surface (Fig. 4A–D). The pollenkit remains mostly in the anther locule, there is only a little in the exine spaces and the tectum is largely free of pollenkit so the pollen is powdery, an adaptation to pollination by wind (Hesse, 1979; this paper, Fig. 4C, D). In contrast, the insect-pollinated *Salix* has more elongated (prolate) tricolpate or tricolporate pollen with a prominent reticulum and the exine is filled with pollenkit which coats the outer layer of the exine such that the pollen is sticky (Risch, 1960; Hesse, 1979). *Populus* is the only genus in Salicaceae *s.l.* (Chase *et al.*, 2002) with inaperturate pollen; others have mainly tricolporate apertures, although tricolpate, three- to five-colpate and triporate also occur (Keating, 1973, 1975; Lu & Wang, 2004).

#### *Eurosids II*

Brassicaceae (Brassicales: Table 1) have fairly uniform tricolpate, reticulate pollen (Rollins & Banerjee, 1979). The family is stenopalynous, meaning there is little variation in the pollen (Punt *et al.*, 1994). Pollen of *Matthiola incana* examined here lacks apertures and is spheroidal with a reticulate exine (Fig. 5A–F). Erdtman (1952) described pollen of *Matthiola incana* and *M. tricuspidata* as inaperturate and *M. sinuata* and *M. tristis* with weak apertures. *Schizopetalon gayanum* has three faint concavities instead of apertures (Erdtman, 1952). Rollins & Banerjee (1979) described weakly formed or irregular apertures in *Cremolobus suffruticosus*, *Mathewsia peruviana*, *Matthiola incana*, *Pugonium cristatum* and *Schizopetalon biseriatum*. The inaperturate or



weakly aperturate pollen is more spheroidal in shape than the usual tricolpate pollen, which is elongated (prolate).

#### *Early-divergent asterids*

In Cornales, *Hydrostachys* (Hydrostachyaceae), a genus of aquatics from southern Africa and Madagascar, has inaperturate microechinate pollen shed in permanent tetrahedral tetrads similar to those of *Nepenthes* (Table 1).

*Saurauia veraguensis* (Ericales: Actinidiaceae; Table 1) has male flowers producing small functional tricolpate pollen grains and morphologically bisexual flowers producing larger inaperturate grains; these grains stain with lactophenol, indicating the presence of cytoplasm, although they tend to collapse. The inaperturate pollen does not germinate and is probably offered as a food reward to pollinating bees (Haber & Bawa, 1984). Other *Saurauia* species are also functionally dioecious and in these the functionally female flowers produce sterile, collapsed pollen lacking contents, which has weakly developed tricolpate apertures (Soejarto, 1969). A similar situation occurs in *Actinidia chinensis* (Actinidiaceae: Schmid, 1978) where functionally female flowers also produce collapsed pollen with weakly developed tricolpate apertures. Tricolpate is the usual aperture type in Actinidiaceae (Dickison *et al.*, 1982).

#### *Euasterids I*

Erdtman (1952) described inaperturate pollen in three species of Icacinaceae (unplaced to order: Table 1): *Chlamydocarya staudtii*, *Pyrenacantha scandens* and *Stachyanthus zenkeri*. Lobreau-Callen (1972, 1973) described *Chlamydocarya* pollen with six pores in the endexine with the aperture membranes indistinguishable from the rest of the exine, *Pyrenacantha* as cryptoporate with three to five pores, and *Stachyanthus* with six indistinct pores. All have echinate surfaces. These observations indicate the pollen may be cryptoaperturate, as in *Phyllanthus* (Phyllanthaceae: see Table 1), although further work using TEM sections is required to confirm this. Pollen of Icacinaceae is diverse with three- to five-colpate, tricolpate, or three or more porate apertures, and pollen shape and surface sculpturing also vary (Lobreau-Callen, 1972, 1973).

In Gentianales, inaperturate pollen occurs throughout two subfamilies of Apocynaceae *s.l.*, Secamonoideae and Asclepiadoideae (Table 1). Secamonoideae are composed of nine genera with *c.* 170 species, and Asclepiadoideae about 200 genera and *c.* 2300 species (Stevens, 2001). Unlike that of other eudicots, pollen in both these subfamilies is shed in pollinia. In Secamonoideae, there are two pollinia (massulae) per anther locule (a total of four

pollinia per anther) composed of inaperturate tetrads without an outer wall enclosing the pollinium, and in Asclepiadoideae (excluding *Fockea*) there is one pollinium per locule (two per anther) composed of inaperturate single pollen grains with an outer wall enclosing the pollinium (Verhoeven & Venter, 2001; Vinkier & Smets, 2002). In *Fockea* the pollinia are composed of inaperturate tetrads not covered by a pollinium wall and are similar to those of Secamonoideae except there are only two pollinia per anther (Verhoeven, Leide & Endress, 2003). Apertures occur in pollen of the other subfamilies of Apocynaceae *s.l.* Rauvolfioideae, considered to be first-branching (Endress & Bruyns, 2000), have three to four colpate single pollen grains, Apocynoideae have three to four porate, occasionally aperturate or polypantoporate single pollen grains or porate tetrads, and Periplocoideae have four to six porate tetrads, multiporate tetrads, or pollinia composed of porate tetrads (Vinkier & Smets, 2002). The type of pollinium and translator (which together form the pollinarium) in Asclepiadoideae was considered to be the most advanced by Verhoeven & Venter (2001). This implies a trend throughout the family from aperturate to inaperturate pollen. However, the position of Periplocoideae as sister to Secamonoideae plus Asclepiadoideae is uncertain and details of the evolution of the pollinarium of the latter two subfamilies are unclear according to Stevens (2001).

Rubiaceae (Gentianales) are a large family with diverse pollen morphology. Inaperturate pollen occurs in five heterostylous genera in Rubioideae (Table 1). Heterostyly is common in Rubioideae (Stevens, 2001). Inaperturate pollen is found only in heterostylous Rubioideae but not all heterostylous Rubioideae have inaperturate pollen. *Hymenocoleus* is characterized by inaperturate pollen with a granular surface and, as is usual in heterostylous plants (e.g. *Reinwardtia indica*, see Table 1), pollen of the short-styled form is larger than that of the long-styled form, which also applies to other heterostylous Rubioideae (Robbrecht, 1975). Inaperturate pollen in *Palicourea*, *Psychotria* and *Rudgea* has psilate, rugulate, reticulate, retipilate or echinate surfaces (Jung-Mendaçolli & Melham, 1995). Tricolpate pollen also occurs in *Psychotria* and tricolpate in *Rudgea* (Jung-Mendaçolli & Melham, 1995).

In Martyniaceae (Lamiales: Table 1), the apertures in *Craniolaria* are difficult to interpret and pollen may possibly be inaperturate. There are unusual circular 'aperturoid' areas scattered over the pollen surface surrounded by raised rings of ectexine (Erdtman, 1952; Bretting & Nilsson, 1988). However, the intine does not appear to thicken in these aperturoid areas, although studies using fresh pollen are required (Bretting & Nilsson, 1988). The other



genera, *Ibicella*, *Martynia* and *Proboscidea*, have clypeate pollen with shield-shaped areas of exine (areolae) surrounded by the apertural areas (Bretting & Nilsson, 1988; Halbritter & Hesse, 1995).

Inaperturate pollen is found in *Callitriche* (Lamiaceae: Plantaginaceae; Table 1). Both aerial and underwater pollination occur in this genus of around 50 species and are reflected in the different pollen types as described by Osborn and co-workers (Osborn & Philbrick, 1994; Cooper, Osborn & Philbrick, 2000; Osborn *et al.*, 2001). The obligately submerged species, e.g. *Callitriche hermaphroditica* and *C. truncata*, have underwater pollination and inaperturate pollen with a reduced exine or exine is absent (exineless pollen). The intine is thick but it does not appear to contain channels (Cooper *et al.*, 2000; Osborn *et al.*, 2001). Channels commonly occur in the thickened intine of omniaperturate monocot pollen (see Introduction). Amphibious *Callitriche* species have pollen with weakly defined apertures and well-developed, intectate exines. Pollination is by anemophily, typical geitonogamy or internal geitonogamy in which the pollen grains germinate within indehiscent anthers and the pollen tubes grow directly to the ovules, e.g. in *C. heterophylla* var. *heterophylla*. Terrestrial species are geitonogamous with tricolpate pollen with a well-developed exine, e.g. *C. peploides*.

Unusual cryptoaperturate pollen occurs in *Mandragora* (Solanaceae: Table 1). There are no ectoapertures but a pattern of endexine thinnings or endoapertures divides pentagonal, hexagonal or sub-circular plates or shields of thicker endexine. There is a slight increase in the thickness of the intine below these endexine thinnings. The ectexine layer overlies the whole endexine and is composed of fine gemmate-clavate elements interspersed with large, widely spaced spines (Diez & Ferguson, 1984). This type of pollen is not found in any other Solanaceae, which typically have tricolporate pollen. The most frequent occurrence of inaperturate pollen in Solanaceae, however, is in functionally dioecious species of *Solanum* (Table 1; reviewed by Anderson & Symon, 1989; Knapp, Persson & Blackmore, 1998). Both male and female flowers are morphologically hermaphrodite although functionally unisexual. The males have ovules which never produce fruits or seeds and the females have pollen but while that of the males is tricolporate that of the females is usually inaperturate. The inaperturate pollen functions as a pollinator reward as the flowers are nectarless. Not all functionally dioecious *Solanum* species produce inaperturate pollen. The female flowers of *Solanum crotonoides* have tricolporate pollen similar to that of the males but the apertures have a narrow, pinched appearance, while *S. polygamum* does not produce any pollen in the anthers of female flowers. The inaperturate pollen

usually has a thicker exine, with less differentiation into sexine and nexine, than the pollen of male flowers. Studies on pollen development have demonstrated that meiosis can be abnormal, apertural shields can be lacking and no mechanism of aperture formation remains in the inaperturate pollen (Knapp *et al.*, 1998). The inaperturate pollen does not produce normal pollen tubes due to the exine acting as a barrier to the stimuli that initiate pollen tube growth (Zavada & Anderson, 1997; Zavada, Anderson & Taylor, 2000).

#### *Euasterids II*

Pollen dimorphism associated with functional dioecy with inaperturate pollen (or pollen with poorly developed apertures) produced by female flowers has been reported in the New Caledonian endemic *Polyscias pancheri* (Apiaceae: Araliaceae; Table 1). The male flowers have tricolporate pollen, as do most other Araliaceae (Erdtman, 1952; Schlessman, Lowry & Lloyd, 1990).

#### TAXA OF UNCERTAIN POSITION

The taxonomic position of Apodanthaceae (holoparasites lacking chlorophyll) is uncertain although recent analyses using mitochondrial and nuclear genes suggest they are related to either Cucurbitales or Malvales (Nickrent *et al.*, 2004). Pollen of *Berlinianche* (Apodanthaceae) is inaperturate (Table 1). Other Apodanthaceae (*Apodanthes* and *Pilostyles*) have tricolporate pollen (Blarer *et al.*, 2004).

#### FUNCTIONAL SIGNIFICANCE OF INAPERTURATE POLLEN

Sterile inaperturate pollen produced by female flowers is a core eudicot character related to the evolution of complex breeding systems in these lineages and functions as a pollinator reward or attractant (Table 2). Fertile inaperturate pollen has evolved numerous times independently throughout eudicots and in some it clearly has a functional significance (Table 2).

#### AQUATIC HABIT, PARASITISM, INSECTIVORY, HETEROSTYLY, ANEMOPHILY, POLLINIA

Inaperturate pollen has evolved in aquatic eudicots in two unrelated families, Hydrostachyaceae and Plantaginaceae (*Callitriche*) (Table 2). As in submerged monocots such as seagrasses (Cymodociaceae, some Hydrocharitaceae and Zosteraceae), a thick exine is not necessary as the pollen is not subject to dehydration but remains fully hydrated. In submerged *Callitriche* species, most of the wall is composed of intine

**Table 2.** Distribution of significant or possibly significant characters associated with inaperturate (including cryptoaperturate) pollen in eudicots

	Functional dioecy	Aquatic habit	Parasitism	Insectivory	Heterostyly	Anemophily	Pollinia
Menispermaceae							
Ranunculaceae							
Dilleniaceae	+						
Nepentheaceae				+			
Loranthaceae			+				
Vitaceae	+						
Geraniaceae							
Euphorbiaceae							
Linaceae					+		
Malpighiaceae	+						
Phyllanthaceae							
Rafflesiaceae			+				
Salicaceae						+	
Brassicaceae							
Hydrostachyaceae		+					
Actinidiaceae	+						
Icacinaceae							
Apocynaceae							+
Rubiaceae					+		
Martyniaceae							
Plantaginaceae		+					
Solanaceae	+						
Araliaceae	+						
Apodanthaceae			+				

and pollen tube germination can presumably take place at any point. Although the submerged aquatic Podostemaceae lack inaperturate pollen, functionally similar spiraperturate pollen, in which the spiral aperture increases the area for potential pollen tube germination (Furness & Rudall, 1999a), does occur in this family. The occurrence of inaperturate pollen in two eudicot families of holoparasites (Rafflesiaceae and Apodanthaceae) and one hemiparasite (*Atkinsonia* in Loranthaceae) is reminiscent of the possession of inaperturate pollen by mycoheterotrophic taxa in monocots (Table 2). Reduction of pollen exine and accompanying loss of apertures may be favoured in mycoheterotrophs and parasites due to the cost of exine production and could be part of an iterative trend towards morphological reduction in these taxa. Interestingly, the insectivore *Nepenthes* (Nepentheaceae) also has pollen with a thin exine lacking apertures, which may also be related to resource allocation in a challenging (nutrient-poor) habitat (Table 2; Fig. 1A–F). The areas close to the junction of the grains in the *Nepenthes* tetrad, where the ectexine thins and the endexine becomes apparent, may be weaker zones where the pollen tube could potentially emerge (Fig. 1D).

Inaperturate pollen occurs in several heterostylous taxa although possible reasons for this are unclear (Table 2). Loss of apertures in *Populus* (Salicaceae: Table 2; Fig. 4A–D) is related to wind pollination and there is a striking contrast with the pollen of *Salix* (see page 34). Dry *Populus* pollen does not need a thick exine with large cavities for storage of pollenkit and the reduced volume of exine may make the pollen more aerodynamic. It may be possible for the pollen tube to emerge at any point through the thin granular exine so apertures are unnecessary, although this has not been documented. Studies of pollen germination in *Populus* do not demonstrate from which part of the wall the tube emerges (Gaget *et al.*, 1984; Gaget, Villar & Dumas, 1989; Zhu & Li, 1989). In monocots, thin exines and weakly defined apertures occur in wind-pollinated Cyperaceae pseudomonads, whereas there is a shift to spherical monads with thicker exines and prominent ulcerate apertures in probable animal-pollinated Cyperaceae such as *Mapania* (Simpson *et al.*, 2003). Linder (1998, 2000) described pollen characters associated with anemophily: dry, smooth pollen, 20–50 µm in size, with circular rather than elongated apertures. Pollen of *Populus* has all these characters except the apertures are absent.

Covering the whole grain with a thin exine layer may prevent excessive desiccation. It is not known if aperture loss occurred after wind pollination evolved in *Populus* or if the pollen was preadapted to wind pollination. *Populus* is a model organism whose genome has been sequenced (Brunner, Busov & Strauss, 2004). This could provide insights into the genetic control of these changes in the pollen.

Pollen lacking apertures also appears to be associated with the evolution of pollinia (Table 2); in fact the greatest number of species with inaperturate pollen occurs in the two families with pollinia, Apocynaceae *s.l.* in eudicots and Orchidaceae in monocots. Pollinia, often with inaperturate pollen, are characteristic of many Epidendroideae, Neotioideae and Orchidoideae (Orchidaceae: Furness & Rudall, 1999a). However, pollen in pollinia may possess apertures, as in some Periplocoideae (Apocynaceae *s.l.*) with porate tetrads (see page 40), and some Epidendroideae, Neotioideae and Orchidoideae with ulcerate or porate pollen (Zavada, 1983). Reduction of the pollen exine occurs in pollinia and the loss of apertures is possibly related to this. In Secamonoideae and *Fockea* (Apocynaceae *s.l.*), the exine of the walls that separate tetrads on the inside of the pollinium (the proximal walls) consists of only a granular layer (Verhoeven & Venter, 2001). Pollen in orchid pollinia may be exineless, or exineless in the interior of the pollinium (Zavada, 1983).

#### APERTURE REDUNDANCY

Brassicaceae contains the model organism *Arabidopsis thaliana*, which has pollen very similar in morphology and ultrastructure to that of *Matthiola incana* examined here (Fig. 5A–F) except that it has tricolpate apertures (Owen & Makaroff, 1995). However, these apertures are not always used as exits for the pollen tube. Edlund *et al.* (2004) demonstrated that *Arabidopsis* pollen tubes often break directly through the interapertural exine walls precisely at the site of contact with the stigma surface. This may be because after pollen imbibition the increased turgor pressure of the grain or gel-swelling forces are focused on an area of exine partially degraded by enzymes at the pollen–stigma interface, which then ruptures (Edlund *et al.*, 2004). The thinner exine in the lumen of the reticulum may be preadapted as a site for this. Although the pollen tube does not exit from the apertures in *Arabidopsis* they could still function in water uptake before germination and in harmomegathy. However, the lack of apertures in *Matthiola* suggests that they may not be necessary for these functions. Water could ingress through the largely intinous lumina walls, which may be flexible enough to accommodate volume changes (Fig. 5F).

The situation in Brassicaceae suggests that apertures could become redundant in some eudicots. Interestingly, a coarse reticulum occurs in other inaperturate pollen, e.g. in *Balbisia* and *Wendtia* (Geraniaceae). Similarly, in pollen with verrucate or microechinate protuberances, e.g. in *Reinwardtia indica* (Linaceae), the spaces between the protuberances could take over the function of apertures, and the pollen tube could force its way between the protuberances. These pollen grains are all spheroidal, which is probably the most common shape for inaperturate pollen (see Figs 3A, B, 4A, B, 5A, C), and this may have harmomegathic significance as the stresses will be evenly distributed in a sphere.

Relatives of taxa with inaperturate pollen have a diverse range of apertures (see Systematic Distribution, above). These include polyporate, clypeate and spiraperturate (as in monocots: Furness & Rudall, 1999a), although aperture types not found in monocots, such as tricolpate and tricolporate, also occur. Loss of apertures and the transfer of their function to other parts of the pollen wall has occurred numerous times independently in eudicots and may be an iterative trend. However, generally the number of taxa in which it occurs is relatively low and is mostly restricted to a few genera or species in any particular family, although notable exceptions occur in Euphorbiaceae *s.s.* and Apocynaceae *s.l.* (Table 1).

The petaliferous crotonoid Euphorbiaceae *s.s.* comprise a whole clade of some 1500 species characterized by inaperturate pollen with a distinctive ‘crotonoid’ exine (Table 1). Here the pollen tube can probably emerge through the thinner exine, consisting of a loose aggregation of granules, in the lumina between the exine subunits (Fig. 3C–F). This strategy is similar to that of *Matthiola* and is probably common in inaperturate eudicot pollen. Interestingly, Malpighiales contain more families with inaperturate pollen than any other eudicot order (Table 1). This relatively novel taxonomic grouping, discovered using molecular sequence data, is highly diverse and in addition to Euphorbiaceae includes some parasites, plus the wind-pollinated poplar (Stevens, 2001).

#### CRYPTOAPERTURATE POLLEN

Cryptoaperturate pollen, with only endoapertures, has a restricted distribution (Table 1): in some *Phyllanthus* (Phyllanthaceae), possibly some Icacinaceae and *Mandragora* (Solanaceae). A specialized area of the exine (the endoaperture) functions as an aperture although this is not visible on the surface since it is covered with continuous exine. This is similar to, although structurally different from, functionally monoaperturate pollen in monocots in which the aperture (a localized thickening of the intine) is also

hidden beneath continuous exine (Furness & Rudall, 1999a, 2000). Both these types of aperture are also similar to, and may be related to, operculate apertures, which occur in both monocots and eudicots (Furness & Rudall, 2003). Here the aperture is covered by a separate piece of exine, like a lid, which protects against desiccation and entry of pathogens (Furness & Rudall, 2003). The exine covering the aperture in cryptoaperturate (and functionally monoaperturate) pollen probably has the same function. Some sterile feeding pollen is also cryptoaperturate, e.g. in some female *Tetracera* (Dilleniaceae) flowers, and probably results from incomplete or abnormal aperture development.

## DEVELOPMENT OF INAPERTURATE POLLEN

### MICROSPOROGENESIS AND TAPETUM

Fertile inaperturate pollen develops from tetrahedral tetrads produced by simultaneous microsporogenesis in eudicots (Figs 1A, B, 2A–C). This microsporogenesis type occurs throughout eudicots, although inaperturate pollen can develop from either the simultaneous (with tetrahedral tetrads) or the successive type (with tetragonal tetrads) in monocots and basal angiosperms, so tetrad orientation does not influence the development of inaperturate pollen (Furness & Rudall, 1999a, b; Furness *et al.*, 2002b). A colpal shield of endoplasmic reticulum or other organelles is absent in the tetrad stage of development of fertile inaperturate pollen (Fig. 2D–F). In sterile inaperturate pollen, e.g. *Solanum*, meiosis may be irregular and apertural shields are absent (Knapp *et al.*, 1998). However, in aperturate pollen the position of the colpal shield is related to that of the microtubule organizing centres (MTOCs) of the meiotic spindle and hence to the tetrad cleavage planes (Heslop-Harrison, 1971; Sheldon & Dickinson, 1983, 1986).

There is no evidence to suggest that pollen apertures are correlated with particular tapetal types, and as expected, inaperturate pollen is not correlated with any particular type of tapetum, either in monocots and basal angiosperms (Furness & Rudall, 1999a, 2001) or in eudicots; this is confirmed by the occurrence of both secretory and invasive tapeta in inaperturate eudicots (Figs 2B, 3A, 4A, 5C).

### DEVELOPMENTAL CONTROL

The occurrence of inaperturate pollen in *Populus*, and in *Matthiola*, a relative of *Arabidopsis*, indicates the potential for evolutionary–developmental studies on the loss of pollen apertures. Evo-devo of pollen

lacking apertures could potentially provide valuable insights into the genetic control of aperture development.

In inaperturate pollen, the presence of vesicles and primexine deposition between the plasma membrane and callose wall is uninterrupted around each microspore, due to the absence of a colpal shield in the tetrad stage of development. The development of the patterned exine in *Arabidopsis* is controlled by the plasma membrane and the vesicles found between the primexine and the callose wall (Paxson-Sowders, Owen & Makaroff, 1997). In the *defective exine* (*dex1*) mutant, normal primexine development is disrupted, which affects the conformation of the plasma membrane and sporopollenin deposition so that sporopollenin forms large aggregates (Paxson-Sowders *et al.*, 2001). The *dex1* gene is essential for early pollen wall formation and the DEX1 protein is probably a component either of the primexine matrix or the endoplasmic reticulum and is involved in the assembly of primexine precursors (Paxson-Sowders *et al.*, 2001). Whether DEX1 is lacking or modified in areas destined to be apertures in wild-type *Arabidopsis* pollen does not appear to have been investigated to date. It would also be expected that DEX1 would be functional during the development of inaperturate pollen such as in *Matthiola*.

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