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Unique stigmatic hairs and pollen-tube growth within the stigmatic cell wall in the early-divergent angiosperm family Hydatellaceae

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Key Results Trithuria

Methods Scanning and transmission electron microscopy and immunocytochemistry are used to study the structure and composition of both mature and immature stigmatic hairs and pollen-tube growth in Trithuria.

Conclusions The presence of a dry-type stigma in Trithuria supports the hypothesis that this condition is ancestral in angiosperms. Each multicellular stigmatic hair of Hydatellaceae is morphologically homologous with a stigmatic papilla of other angiosperms, but functions as an independent stigma and style. This unusual combination of factors makes Hydatellaceae a useful model for comparative studies of pollen-tube growth in early angiosperms.

Key words: Angiosperm evolution, Hydatellaceae, immunocytochemistry, pollen-tube growth, stigma, Trithuria, ultrastructure.

INTRODUCTION

In the general context of flowering plant reproduction, the rapid and controlled entry of the pollen tube into the stigma is an event of special importance (e.g. Williams, 2008). Pollen tubes, which act as the conduits for non-motile gametes, represent a key trait of some seed plants, which are termed siphonogamous; the three extinct siphonogamous groups are angiosperms, conifers and Gnetales (Rudall and Bateman, 2007). In the majority of extant angiosperms, pollen-tube elongation occurs either through an extracellular layer of mucilage that covers the internalized epidermal stylar surface, as in the hollow styles of many syncarpous monocots and some eudicots (e.g. Campanula), or within the pectin-rich cell walls of a specialized pollen-tube transmitting tract, as in the solid styles of most syncarpous eudicots (e.g. Endress, 1994; Lennon et al., 1998; Erbar, 2003).

The semi-aquatic family Hydatellaceae resembles many other early-divergent angiosperms in possessing ascidiate carpels that are sealed by secretion (Endress and Igersheim, 2000; Endress, 2001; Rudall et al., 2007), but differs in its distinct and unusual pattern of pollen-tube growth. Hydatellaceae lack a stylodium with specialized transmitting tissue, and the bulk of the pollen-tube growth phase occurs on the exposed surface of the unusually long multicellular uniseriate stigmatic hairs that characterize this family (Rudall et al., 2007, 2008, 2009; Sokoloff et al., 2010). Thus, the family Hydatellaceae, containing a single genus, Trithuria of 12 extant species (Sokoloff et al., 2008), is a potentially useful model for pollen-tube growth. The family was formerly ascribed to the monocots, but was recently robustly identified as a member of the waterlily clade, placed phylogenetically as sister to Nymphaeaceae plus Cabombaceae (Saarela et al., 2007). This dramatic phylogenetic reassignment placed the family within a lineage that diverged close to the root of the extant angiosperms, and hence close to the angiosperm stem-group. In a comparative context, their novel placement enhances the significance of Hydatellaceae as a potential model for assessing key aspects of early angiosperm evolution. The three earliest extant lineages of flowering plants, which are sometimes collectively termed the ANITA grade or ANA grade, are represented by three orders (APG III, 2009) containing only about 16 species-poor extant genera: Amborellales (Amborella), Nymphaeales (approx. 10 genera, including Brasenia, Cabomba, Nymphaea, Trithuria) and Austrobaileyales (Austrobailey, Schisandra,
Kadsura, Ilicium, Trimenia). Two other extant early-divergent lineages, Chloranthaceae and Ceratophyllum, are ‘wildcards’ that occupy variable isolated positions in molecular phylogenetic analyses of angiosperms (e.g. Qiu et al., 2000, 2006). A recent record of fossil pollen of Monosulcites riparius from Cretaceous sediments in Siberia (Hofmann and Zetter, 2010), which the authors assigned to Hydatellaceae based on close comparison with surface morphology of extant species (Remizowa et al., 2008), potentially increases interest in the family, though further studies are needed to establish this assignment, ideally including comparative seed morphology.

In this paper, we describe the stigmatic hairs of Trithuria and investigate their role in the germination of pollen grains and guidance of pollen tubes, in the context of comparative studies of stigmatic and transmitting tissue in other early-divergent angiosperms (e.g. Sage et al., 2009). Pollen morphology of all extant species of Hydatellaceae was described earlier by Remizowa et al. (2008), including some aspects of pollen-tube growth (see also Rudall et al., 2008, 2009; Taylor et al., 2010). Here, immunocytochemistry is used to investigate the composition of both mature and immature stigmatic hair cells and pollen-tube growth in Hydatellaceae. In eudicot species such as Arabidopsis, the pollen tube tracks positive signals emitted first by the stigmatic hair cell, then by the transmitting tissue and finally by the ovules (Kandasamy et al., 1994; Lennon et al., 1998). In Arabidopsis, pollen adherence and germination, and pollen-tube growth rate, are less efficient in immature stigmatic papillae (Kandasamy et al., 1994). Directionality and targeting are also affected; tubes only follow their normal path in pistils with a defined stigma possessing elongated papillar cells. If stigmatic cells are removed, basipetal growth is rare and tubes grow randomly, suggesting that mature stigmatic cells produce signals that direct normal pollen-tube growth. Thus, cytochemical differences between immature and mature stigmatic cell walls could indicate how pollen tubes are guided, especially in taxa with unusually long stigmatic hairs such as Trithuria.

MATERIALS AND METHODS

Plant material


Microscopy techniques

For light microscopy using differential interference contrast optics, carpels were dissected on a microscope slide in a drop of a modified version of Herr’s clearing fluid (lactic acid : chloral hydrate : phenol : clove oil : Histoclear, in proportions 2 : 2 : 2 : 1, by weight) and examined using a Leica Diaplan photomicroscope fitted with a Leica DC500 digital camera.

For standard transmission electron microscopy (TEM), appropriately fixed samples were taken through graded ethanol and ethanol : LR White resin series prior to embedding. Ultra-thin sections (50–100 nm) were collected on formvar-coated copper slot grids and post-stained with lead citrate plus uranyl acetate. Samples were imaged in a Hitachi H-7650 TEM with integral AMT XR41 digital camera.

For immunoelectron microscopy, ultra-thin sections (100–150 nm) of resin-embedded material were collected on formvar-coated Ni grids, etched with 5 % hydrogen peroxide, washed with phosphate buffer-saline–Twee (PBST; 20 mM solution of phosphate buffer with 0-9 % NaCl, 0-1 % Tween 20 and 0-02 % Na-azide, pH 7-4) before being blocked with 10 % aqueous nofat dry milk. Incubation with monoclonal antibodies (MAbs) (Plant Probes, Leeds, UK) followed for 1 h at room temperature. MAbs were diluted 1:50 in PBST containing 3 % nofat dry milk. The MAbs used were LM2 for arabinogalactan proteins (AGPs) containing gluconuronic acid, LM19 for nonesterified pectins and LM20 for methyl-esterified pectins. As a negative control, primary antibodies were omitted. Sections were washed in PBST, blocked in 10 % nofat dry milk and then incubated in colloidal gold (12 nm) goat anti-rat IgM complex (Stratech Scientific, Suffolk, UK) diluted 1:20 in PBST containing 3 % nofat dry milk for 1 h at room temperature. The sections were washed twice in PBST, once in deionized water prior to fixation in 2 % glutaraldehyde (aq.) and subsequent washing in water. Sections were stained with uranyl acetate followed by lead citrate.

For scanning electron microscopy (SEM), material was dissected in 70 % or 100 % alcohol, dehydrated through absolute ethanol and critical-point dried using an Autosamdri-815B critical-point drier Tousimis Research, Rockville, MD, USA. It was then mounted onto SEM stubs, coated with platinum using an Emitech (Kent, UK) K550 sputter coater, and examined using a Hitachi (Wokingham, UK) cold-field emission SEM S-4700-II at 1 kV.

RESULTS

Stigmatic hairs

Stigmatic hairs grow from a single cell that undergoes cell division and elongation (Fig. 1A–D). At full expansion, the stigmatic hairs of Trithuria are uniseriate, unbranched and multicellular, consisting of at least 30 cells (Fig. 1F, G). Within each cell, there is a thin layer of cytoplasm surrounding a large central vacuole (Fig. 1F). Occasional dense nodules (vesicles, or wall bodies) of unknown composition are present within the cytoplasm adjacent to the cell wall (see Fig. 4B). Walls between adjacent stigmatic hair cells are very thin, lacking obvious plasmodesmata and middle lamellae. In contrast, the outer stigmatic wall is much thicker and consists of two extra-cellular layers and a distinctly bilayered wall. The extracellular
Fig. 1. Stigmatic hairs and pollen tubes in *Trithuria*: (A–D) *T. submersa* (HK), differential interference contrast of successive stages in development of multi-cellular stigmatic hairs; (E) *T. konkanensis*, scanning electron micrographs (SEM) of part of stigmatic hair with pollen tubes arrowed; (F) *T. submersa* (HK), conventional light micrograph of stigmatic hair with full complement of unexpanded cells; (G) *T. cowieana*, SEM of entire bisexual reproductive unit with outer phyllomes removed to reveal carpels surrounding stamens; (H) *T. cowieana*, SEM of part of stigmatic hair with germinated pollen grain – the pollen tube is arrowed; (I) *T. konkanensis*, SEM of tip of stigmatic hair with overlying pollen tube (arrowed); (J) *T. konkanensis*, SEM of stigmatic hair bases with pollen tube arrowed; (K) *T. submersa* (HK), SEM of stigmatic hair with germinated pollen grain attached; the pollen tube (arrowed) has grown around the top of the grain and downwards along the hair axis; (L) *T. cowieana*, SEM of impression of detached pollen grain left on stigmatic hair cuticle; (M) *T. lanterna*, SEM of part of stigmatic hair with three pollen tubes – the left-hand tube is growing upwards (tip of tube arrowed) and the two right-hand pollen tubes have emerged from pollen grains that have shed their exines, have elongated in intimate contact with each other and cross over each other only near their points of origin. Scale bars: (A–E, H–M) = 10 μm; (F) = 50 μm; (G) = 100 μm.
layers are a thin, discontinuous pellicle overlying a thin cuticle; the cuticle becomes thicker towards the hair base, where fine filaments extend through the cuticle from the cell wall (Fig. 3I). The cell wall has an outer, thin, dark-staining layer, which is often fibrous in appearance, and an inner, thicker, less-densely stained, homogeneous wall layer (Fig. 3J, K). A bilayered cell wall was not observed in other cells of the gynoecium. Below the hair base, the outer walls of gynoecium cells are relatively thin, whereas the intercellular walls are thicker than those between adjacent hair cells, with distinct middle lamellae. Following pollen grain germination and pollen-tube growth, the stigmatic hairs collapse, often a few cells at a time (Figs 1J, 2A and 3E). Preliminary observations suggest that the mode of stigmatic hair senescence could differ among Trithuria species. In material examined of *T. submersa*, stigmatic hair cells show strong longitudinal collapse that results in drastic shortening of the hair (Fig. 2A; see also Rudall et al., 2007; Sokoloff et al., 2008; Taylor et al., 2010). In contrast, in material examined of *T. lanterna*, stigmatic hairs remain as long at the time of fruit dehiscence as at the time of pollination (Fig. 2B; see also Rudall et al., 2007). These differences might be of taxonomic significance and/or may reflect different humidity of the environment of stigmatic hairs after pollination.

**Pollen grains**

The pollen grain surface consists of a sculptured and chambered exine (Fig. 3A–F). The exine cavities contain an extracellular tryphine layer that consists of fine granular material. Pollen grains attach readily and firmly to the stigmatic hairs (Fig. 1H, K). If removed from the hair, the grains leave an exine impression on the hair cuticle (Fig. 1L). Coarse granular material occurs at the pollen–hair interface and in some cases can be seen below the tectum layer, though it rarely meets the tryphine layer, from which it is clearly distinct (Fig. 3D, E). This granular material resembles the adhesion zone material found between pollen grains and the stigmatic papilla surface in Arabidopsis (Elleman et al., 1992; Kandasamy et al., 1994). We found a few cases where a short portion of free pollen tube occurred between the germinated pollen grain and site of penetration into the hair wall. We observed pollen tubes on stigmatic hairs in fixed wild-source material in *T. cowieana*, *T. konkanensis*, *T. lanterna* and *T. submersa* (Fig. 1) as well as in herbarium material of *T. polybracteata* and *T. occidentalis* (see also Sokoloff et al., 2008). In many cases, several pollen tubes were present on a single stigmatic hair (Fig. 1M), and some branched pollen tubes were observed. Once in contact with the hair, the pollen grain becomes polarized, prior to tube emergence (Fig. 3A). Growth of the intine is evident at the pollen sulcus (Fig. 3B). At the site of tube emergence, the sparse cytoplasm possesses relatively few organelles other than vesicles (Fig. 3F). Behind this layer occurs a denser cytoplasm (Fig. 3G). Germinated pollen tubes penetrate the hair cuticle and grow into the outer (darker) layer of the underlying cell wall (Fig. 3J, K). They never break through into the cell cytoplasm, but are apparently entirely accommodated within the wall layer. Pollen tubes grow...
Fig. 3. Transmission electron micrographs (TEMs) of *Trithuria submersa* (WA): (A) section of pollen grain just starting to germinate; (B) aperture of recently germinated pollen grain, showing massive intine; (C) sculptured pollen wall, showing tryphine layer within tectum cavities; (D, E) sections showing granular material forming an adhesion zone between the pollen grain and stigmatic hair; (F, G) transverse sections of pollen tubes located within wall of stigmatic hair; cytoplasm of pollen tube highly vacuolated in (F), relatively dense in (G); (H) longitudinal section of pollen tube elongating downwards through stigmatic hair cell wall – note callose plug; (I) transverse section at bases of two stigmatic hairs – at this point the outer walls are densely staining, dark-staining filaments protruding through the thick cuticles; (J) transverse section of pollen tube located within outer dark-staining layer of stigmatic hair cell wall; (K) transverse section of pollen tube located within stigmatic hair cell wall near base of hair – note thick cuticle with dark filaments. Scale bars: (A) ¼ 10 μm; (B, C, E, F) ¼ 1 μm; (D, G, H) ¼ 2 μm; (I–K) ¼ 500 nm. Abbreviations: c = thin cuticle of stigmatic hair; cp = callose plug; cs = collapsed stigmatic hair cell; e = pollen exine; g = granular layer; i = pollen intine; pt = pollen tube; s = interior of stigmatic hair cell; t = tryphine layer.
along the axis of the stigmatic hair, either to its base or apex (Fig. 1E, H–K, M), usually oriented along the axis of the hair (or rarely slightly obliquely). Pollen tubes that reach the constriction at the base of a stigmatic hair (Fig. 1J) continue to elongate throughout the relatively short distance to the ovule. We occasionally observed germinated pollen grains of other (unknown) species attached to the stigmatic hairs, but in these cases the pollen tubes grew only for a very short distance (approx. 15 μm) and never penetrated the cuticle. Close to the actively growing tip of the pollen tube, the cytoplasm is densely packed with membranous plastids and (mostly rough) endoplasmic reticulum. The pollen-tube wall is relatively thick (65–350 nm), occasionally appearing lamellated. Callose plugs are present at intervals along the pollen tube (Fig. 3H)

**Immunocytochemistry**

For immunocytochemistry (Fig. 4), we used the antibody LM2, which recognizes a carbohydrate epitope, containing β-linked glucuronic acid, of an AGP (Smallwood et al., 1996; Yates et al., 1996). AGPs are hydroxyproline-rich glycoproteins that are structurally complex and ubiquitous in plants, particularly abundant in cell walls, plasma membranes and extracellular secretions, providing recognition signals (Seifert
and Roberts, 2007). The glycan epitopes of some AGPs may be markers for gametophytic cell differentiation (Coimbra et al., 2007, 2009). Other AGPs are necessary for stamen function and pollen-tube growth (Levitin et al., 2008). They are thought to be involved in directional guidance for the pollen tube, being the predominant class of extra-cellular matrix proteins present in the transmitting tract of many plant species (Cheung and Wu, 1999). An increase in LM2 epitopes from the immature stigmatic hairs to the mature hairs was observed. In young stigmatic hairs, LM2 localization was found only around the plasma membrane (Fig. 4A), compared with localization throughout the cell wall in mature stigmatic hairs, showing heavier staining in the outer layer (Fig. 4B, C). These results suggest that in immature stigmatic hair cells AGPs are localized to the plasma membrane, but as the cells age, AGPs increase in concentration and diffuse throughout the wall, becoming more concentrated in the outer layer of the cell wall, which is the site of pollen-tube elongation. The cross walls of the stigmatic hairs are much thinner by comparison, and the concentration of antibody is much lower than in the outer stigmatic hair cell walls, so the signal is much weaker. Also LM2 localization was observed in multivesicular bodies within the cytoplasm. AGPs are thought to be internalized into vesicles and then degraded in the vacuole after they have performed their function.

The MAbs LM19 and LM20, which recognize nonesterified homogalacturonans and highly esterified homogalacturonan domains of pectic polysaccharides, respectively, were also used (Verberbruggen et al., 2009). LM19 localization occurred in variable concentrations throughout the cell wall in mature stigmatic hairs (Fig. 4D–F). As with LM2, a greater concentration of LM19 epitopes was present towards the outside walls, adjacent to the cuticle. LM19 epitopes were less abundant in outer gynoecial walls below the level of the hair base. The greatest density of epitopes was that of LM20, wherein localization occurred throughout the cell walls in both young and mature stigmatic hairs (Fig. 4G–I).

DISCUSSION

Pollens tubes in Hydatellaceae grow within the cell walls of stigmatic hairs (see also Remizowa et al., 2008; Rudall et al., 2008). In most angiosperms, pollen-tube growth and guidance are probably a response to both cell-wall architecture and chemotropic agents that either diffuse freely from their source or are bound to a surface (Heslop-Harrison, 1987; Mascarenhas, 1993; Endress, 1994; Lush, 1999). In dry stigmas, a gradient in water concentration in the immediate environment of the pollen grain plays an important role in pollen-tube guidance (e.g. Lush et al., 2000; Sage et al., 2009). Some studies on pollen-tube growth in the solid styles of tobacco have indicated that a family of transmitting tissue-specific (TTS) AGPs attract pollen tubes and promote tube elongation (Wang et al., 1993; Cheung et al., 1995; Wu et al., 1995, 2000). In this case, the pollen tubes deglycosylate TTS proteins, freeing the carbohydrate from the protein backbone, so the AGPs could be acting as a nutrient source (Wu et al., 1995; Sanchez et al., 2004). Studies on Nicotiana (e.g. Cheung et al., 1995: Wu et al., 1995) indicate that TTS proteins promote pollen-tube growth, but little is known of the mechanism by which AGPs contribute to pollen tube guidance (Sommer-Knudsen et al., 1998; Higashiyama and Hamamura, 2008; Hiscock and Allen, 2008). Our immunocytochemical results suggest that AGPs could play a role in the stigmatic hairs of Trithuria that is similar to their role in the style of Nicotiana. However, the precise nature of that role remains open to debate. In many angiosperm species, elongating pollen tubes use exogenous sugars as nutritional support. Interestingly, in immature stigmas in Arabidopsis and Brassica, pollen tubes grow close to the inner face of the stigmatic cell wall, rather than between the wall layers (Elleman and Dickinson, 1990; Elleman et al., 1992). This is precisely the location of AGPs in immature stigmatic hairs of Trithuria, indicating that these proteins could be involved in pollen-tube attraction, though evidence for this is at best circumstantial.

In Hydatellaceae, the presence of AGPs in the outer cell-wall layer, through which the pollen tubes elongate, suggests that a governing chemical gradient could be present. However, some pollen tubes grow towards the hair apex, then elongate over the tip of the hair and toward its base, ultimately reaching the ovary. This observation indicates that a gradient of glycosylation alone is insufficient for preferential basipetal growth. Growth could be at least partly constrained by cellular microtopography, so that the location and angle of entry of the pollen tube determine the direction of tube elongation. In many other angiosperms, pollen tubes originating from intrastylar pollinations are equally as likely to grow towards the stigma as towards the ovary, indicating that architectural features also play an important role (Mulcahy and Mulcahy, 1987). The secretory transmitting epidermal cells of Lilium possess unesterified pectins, which bind to the pollen-tube wall in combination with a stigma-style cysteine-rich adhesin protein (Lord, 2001). Both components provide an adhesive matrix to which the pollen tubes adhere and grow. Pectins have also been found in the transmitting tracts of other taxa, including Arabidopsis (Thoma et al., 1993; Lennon and Lord, 2000). Our observation of a concentration of unesterified pectins in the outer cell wall layer, through which the tubes elongate, could indicate that unesterified pectins play a similar role in Hydatellaceae. In many angiosperms, governing factors in the route and direction of pollen tubes are largely unknown, owing to the limited accessibility of the transmitting tract within the gynoecium (Endress, 1994). The stigmatic hairs of Hydatellaceae are analogous with both stigmas and styles of other angiosperms, and therefore offer a unique opportunity to investigate pollen-tube growth in vivo, because the majority of pollen-tube growth takes place in the stigmatic hair.

The immunocytochemistry results suggest that AGPs are involved in attracting the pollen tubes through the stigmatic cuticle in Hydatellaceae. AGPs are not localized in the cuticle itself but rather in the underlying cell wall. This localization of AGPs could be the reason that pollen tubes grow through this layer and not through the cuticle, thus differing from other early divergent angiosperms such as Amborella and Illicium (Sage et al., 2009). Once the tubes are safely located within the wall, it is likely that they are channelled down to the hair bases by both physical constraints such as microfibril orientation and the presence of binding factors.
such as unesterified pectins and adhesive proteins. The fact that AGP localization is concentrated in the outer walls could be the reason that in Hydatellaceae pollen tubes never grow in the cross walls of the stigmatic hairs, i.e. between adjacent stigmatic hair cells. In most angiosperms, AGPs are attached to the external surface of the plasma membrane by a glycosylphosphatidyl inositol (GPI) anchor (Lalanne et al., 2004). In response to a cellular signal, a specific phospholipase cleaves the GPI anchor, releasing the AGPs to the periplasmic space, from there to the cell wall, and finally to the extracellular space. This could also be the case with LM2 localization in Trithuria. In immature hairs, AGP is bound to the plasma membrane, but as the hair matures it diffuses through the cell wall, becoming more concentrated in the outer wall layer. Both LM19 and LM20 epitopes are present in the walls between adjacent stigmatic hair cells, but these walls are very thin in comparison with the outer walls. Also both unesterified and highly esterified homogalacturonans were found in the hair-cell walls, but greater amounts of the unesterified type were found in the outer wall layer. The presence of higher concentrations of AGPs and unesterified pectins indicates that the stigmatic hair-cell walls act as a transmitting tissue extracellular matrix, providing chemotactic and haptotactic tube guidance. The greatest concentration of epitopes was that of LM20, which was homogeneously distributed throughout the wall. This result differs from that of another early-divergent angiosperm, Kadsura, in which unesterified epitopes were significantly more abundant than highly esterified epitopes and both were homogeneously distributed (Lyew et al., 2007).

The results of the present study suggest that Trithuria possesses a dry-type stigma, as secretions are absent at maturity. In dry stigmas, pollen adhesion is entirely a property of the pollen (Edlund et al., 2004; Sanchez et al., 2004). Hydration requires direct contact between the pollen and the stigmatic surface, and is facilitated by the pollen coat that extrudes from the exine to form an adhesion zone (Elleman et al., 1992). Uncollapsed stigmatic hairs possess a thin layer of cytoplasm with few organelles, as was observed in Trithuria. Also numerous vesicle-like inclusions were observed in stigmatic cells (Fig. 4B), similar to the wall bodies reported in stigmatic papillae of Senecio; these structures have sometimes been associated with dry or semi-dry stigmas (Hiscock et al., 2002). The stigmatic cell wall in Trithuria is composed of several layers: a thin and discontinuous outer pellicle, a cuticle, and two distinct wall layers, one light-staining and one more electron-opaque. A similar bilayered cell wall structure occurs in Arabidopsis; Ellelman et al. (1992) hypothesized that it represents a specialized adaptation to accommodate pollen-tube growth. These authors found that in the early-divergent eudicot Papaver rhoes, pollen-tube growth occurs through a cuticle that has become distorted, as in the early-divergent angiosperm Amborella (Sage et al., 2009). In Papaver, after the passage of the pollen tubes, the stigmatic papillae become necrotic and collapsed (Elleman et al., 1992), as in Trithuria. Many other angiosperm families, including some submerged marine angiosperms (Ducker and Knox, 1976; Pettitt, 1980), possess a pellicle or superficial protein-lipoidal coating external to the cuticle, which is secreted from the papillae during stigma maturation (Mattson et al., 1974). A pellicle (a protein layer with esterase activity) occurs in both dry and wet stigmas; in a dry stigma, the pellicle and the cuticle remain relatively intact in the functional phase, whereas they lift and rupture in a wet stigma, releasing a subcuticular accumulation of secretion (reviewed by Sage et al., 2009). Immediately after deposition, pollen grains release from their walls proteins and glycoproteins that bind to the pellicle. Unless the cuticle is ruptured or altered, pollen will not germinate even if it is compatible (Heslop-Harrison, 2000). Heslop-Harrison and Heslop-Harrison (1975) hypothesized that the pellicle carries an activator for the cutin-splitting enzymes conveyed by the pollen grains. An active cutinase that occurs in pollen of Brassica napus breaks down the cuticle of the stigmatic papillae (Hiscock et al., 1994; Edlund et al., 2004).

CONCLUSIONS

There are diverse and sometimes contradictory reports of stigma types among the earliest extant angiosperm lineages (Hiscock et al., 2002). Dry stigmas characterize many early-divergent angiosperms, including Amborella (the putative sister to all other extant angiosperms), Ilicium and Trimenia (Heslop-Harrison and Shivanna, 1977; Thien et al., 2003; Bernhardt et al., 2003; Sage et al., 2009). Dry stigmas also occur in Kadsura (Lyew et al., 2007), though both Kadsura and Schisandra (Schisandraceae) were earlier reported as possessing wet-type stigmas (Heslop-Harrison and Shivanna, 1977). There are also diverse records of both dry and wet stigmas in Nymphaeaceae; dry stigmas were reported in Nuphar, Nymphaea and Victoria (Heslop-Harrison and Shivanna, 1977) and wet stigmas in Nuphar, Barclaya, Ondinea, Victoria, Euryale and Nymphaea (Heslop-Harrison and Shivanna, 1977; Meeuse and Schneider, 1980; Schneider and Chaney, 1981; Schneider, 1983). Our observation of a dry-type stigma in Trithuria supports the conclusion of Bernhardt et al. (2003) and Sage et al. (2009) that a dry-type stigma could represent the ancestral (plesiomorphic) condition in angiosperms, though no extant angiosperm outgroups are available to establish this. However, as Hiscock et al. (2002) also noted, this optimization is highly sensitive to interpretation, since relatively few studies have used ultrastructural imaging methods. As noted by Sage et al. (2009), the scenario in which a dry-type stigma (with a cuticle-bound extracellular matrix) is ancestral to a wet-type stigma (with a free-flowing extracellular matrix) contradicts alternative hypotheses in which the pollination droplet found in most gymnosperms is analogized with a free-flowing extracellular matrix (e.g. Frame, 2003).

More data are needed for pollen-tube growth into the mouth of the carpel in Hydatellaceae, but observations by Rudall et al. (2009) indicate intercellular growth in the micropylar region. Carpels of most early-divergent angiosperms remain free from each other, rather than becoming fused together; individual carpels are often sealed at their margins by secretion rather than by post-genital fusion (Endress and Igersheim, 2000; Endress, 2001). In cases of carpel closure by secretion, a canal is present at the top of each carpel; pollen tubes may enter the carpel through the canal. This type of pollen-tube growth occurs in Amborella (Williams, 2009), Austrobailey
et al. (2008), Kadsura (Lyew et al., 2007) and Ceratophyllum (Shamrov, 1983, though the gynoecium could be pseudomonoceros in Ceratophyllum: Shamrov, 2009). In contrast, species of Chloranthaceae also possess an open canal in the carpel, but pollen tubes grow intercellularly in the extracellular matrix between adjacent stigmatic epidermal cells and subsequently along the inner tangential walls of the epidermal cells of the canal (Hristova et al., 2005). A third type of pollen-tube growth in carpels with an open canal is reported in the early-divergent angiosperms Brasenia, Cabomba and Trithuria, in which pollen tubes grow directly through substigmatic ground tissue to reach the canal or ovary locule (Bernhardt et al., 2003; Endress, 2005; Taylor and Williams, 2009; Williams, 2009). In summary, although ascidiate carpels with a secretion-filled canal extending up to the stigma are fairly common among early-divergent angiosperms, pollen tubes do not necessarily grow through this canal, but can elongate through an extracellular matrix between cells. In this respect, they could be analogized with the invasive, nutrient-seeking haustoria of the male gametophytes of some gymnosperms (e.g. cycads and Ginkgo), which evolved before recruitment of the pollen tube to a remarkable new role, siphogamy (Rudall and Bateman, 2007).

Focusing on the stigma itself, the data from this paper show that pollen tubes in Trithuria do not grow along the surface of stigmatic papillae, as in some other early-divergent angiosperms including Nymphaea (Williams et al. 2010). On the other hand, pollen-tube growth on the Trithuria stigma is not surficial, or even immediately subcuticular, as in stigmatic papillae of Amborella (Sage et al., 2009) and Kadsura (Lyew et al., 2007), but occurs deeper within the outer wall of the stigmatic cells, as indicated by antibody localization within the primary walls rather than the cuticle. Each multicellular stigmatic hair of Hydatellaceae is morphologically homologous with a stigmatic papilla of other angiosperms, such as each multicellular stigmatic papilla of Cabomba. However, in terms of function, each stigmatic hair in Hydatellaceae operates as an independent stigma and style with its own cell-wall-bound transmitting tissue (see also Gaikwad and Yadav, 2003). Thus, this family represents a useful model for comparative studies of pollen-tube growth in early angiosperms.

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LITERATURE CITED


