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Pollen–pistil interaction in wheat

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SUMMARY

General organization and ultrastructure of pistil transmitting tract (PTT) for pollen tube pathway in wheat (*Triticum aestivum* L.) and pollen tube growth were studied. The peculiar features of cell ultrastructure in different parts of the PTT were shown and point to heterogeneous pollen tube pathway. Stylodia TT cells show protein and carbohydrate secretion. In stylar TT cells dark globules are secreted. In the ovarian cavity fibrillar material is present.

The organization of pollen tube cytoplasm also changes during its growth towards the ovule. The thickness of the callose layer of the pollen tube wall decreases in the direction of the ovule. In the stylar TT the pollen tube plasma membrane forms finger-like protrusions, or plasmotubules, which can connect the neighbouring pollen tubes. The role of these plasmodesmata-like formations in the pollen tube and their role in the interaction of pollen tubes with PTT in different parts of pollen tube pathway in wheat are discussed.

Key-words: plasmodesmata, pollen tube, transmitting tract for pollen tube route, wheat.

INTRODUCTION

For several cereals such as *Hordeum vulgare, Secale cereale, Zea mays* and *Alopecurus pratensis* (Cass & Peteya 1979; Heslop-Harrison 1979a,b; Kroh *et al.* 1979; Heslop-Harrison & Heslop-Harrison 1980, 1981, 1982; Shivanna *et al.* 1982; Heslop-Harrison *et al.* 1984a,b; Heslop-Harrison *et al.* 1985), structural and functional aspects of pollen–stigma interactions have been studied. Since wheat is involved in a number of intergeneric and interspecific crosses in breeding programmes, there are still important questions about the organization of the transmitting tissue (TT), mode of pollen tube growth, mechanisms of pollen tube guidance and regulation of pollen tube number in compatible as well as in incompatible pollinations.

This paper describes the organization and fine structure of wheat pistil transmitting tract (PTT) as well as the interaction between growing pollen tubes and PTT that leads to the reduction in number of pollen tubes to one, which will finally penetrate the micropyle.

Abbreviations: a, amyloplast; cc, carpel cuticle; ii, inner integument; oc, ovarian cavity; oe, ovarian epidermis; oi, outer integument; p, parenchyma; pd, plasmodesmata; p-p, p-particle; pt, pollen tube; tt, transmitting tissue; v, vacuole.

MATERIALS AND METHODS

Plants of wheat var. Chinese Spring were grown in the glasshouse of the Department of Plant Cytology and Morphology, at the Agricultural University of Wageningen. The minimum night temperature was 15°C and the day temperature was *c.* 22°C. Florets were emasculated 3 days before anthesis and pollinated with freshly collected pollen on the day of anthesis. Dissected pistils were fixed 20 min and 2·5 h after pollination. To stain callose, pistils were fixed in ethanol-acetic acid mixture (3:1), rinsed in 96% ethanol overnight, softened in a saturated solution of NaOH, rinsed in some changes of water and stained with 1% water-soluble aniline blue. Observations were made using a Nikon UV fluorescent microscope.

For electron microscopy, unpollinated pistils and pistils taken 20 min and 2·5 h after pollination were fixed for 4 h in 2·5% glutaraldehyde in phosphate buffer (Ph=7·2) at room temperature, rinsed three times in buffer and post-fixed for 2 h with buffered 1% osmium tetroxide. Specimens were embedded in Epon 812. Ultrathin sections were post-stained with uranylacetate and lead citrate, and observed in JEOL JEM 1200EX II electron microscope. Semithin sections (1–3 μm) were alternated with ultrathin sections and stained with 1% toluidine blue for observation in a bright-field light microscope. For protein detection, sections and total pistils were stained with Coomassie blue R 250.

The number of organelles was counted in longitudinal sections of 3–4 cells. The number of ribosomes was counted over an area of about 150 μm^2.

RESULTS

Organization of the pistil transmitting tract

As in most grasses, the wheat pistil consists of a two-branched feathery stigma, a short style and a unilocular ovary. To avoid confusion, we designate the parts of wheat stigma as given previously for plumose stigma of Gramineae (Heslop-Harrison & Shivanna 1977; Heslop-Harrison & Heslop-Harrison 1981): two primary branches (stylodia) with secondary papillate branches (hairs) (Fig. 1). PTT includes all stigmatic hairs capturing pollen, TT of stylodia and style and the passage between outer integument and ovarian epidermis in the ovarian cavity.

The cells of TT have an elongated form, are tightly packed and are sharply distinguishable from the parenchymae surrounding TT in the lower part of the stylodia and in the style (Fig. 2a,c). Stylar TT continues into the stylodia TT and consists of two bundles coming together from two stylodia. These bundles have about 20 cell rows of TT each, counted on the medial section of the pistil, and are demarcated by carpel cuticle (Fig. 2b).

In longitudinal section the stylar TT bundle consists of 5–7 cell rows at the base of the style (Fig. 2c) where the outline of the TT appears funnel-shaped. The basal part of the style has an asymmetrical TT organization due to the asymmetrical ovule position. In unpollinated pistil the outer integument is partly squeezed to the ovary wall on one side and a space between the ovule and the ovary wall is formed. One bundle of stylar TT turns slightly in the direction of this space forming a kind of elongated branch. The number of TT cell rows in this branch on the medial section of the pistil gradually reduces to one or two (Fig. 2c, arrow). Here, TT reaches the cells of the inner ovary epidermis. The other bundle of the TT has no well-defined reduction in the number of cell rows and ends before the outer integument.

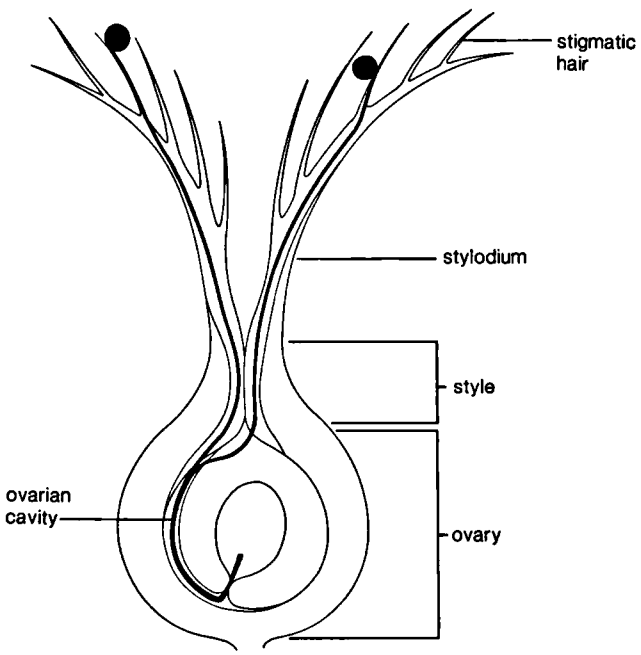


Fig. 1. Schematic drawing of a median longitudinal section of wheat pistil. Possible pathways for pollen tubes to the ovule are shown with thick lines.

After passing through TT, pollen tubes enter the ovarian cavity and grow in a space between the ovary and the ovule walls towards the micropyle. After pollination, this passage becomes much narrower, probably because of water uptake by the ovule and the surrounding tissue.

Fine structure of the pistil transmitting tract

In cross-section, the stigmatic hair consists of 4–5 papillar cells (Fig. 3a). These cells are rectangular, vacuolate and contain a round nucleus. Two to four proplastids are visible per cell section with small globules and occasional starch grains. Mitochondria have small tubular cristae and ribosomes. RER cisternae, SER and electron-transparent vesicles can be observed. The large vacuole contains thin fibrillar material. At the base of the hair rough dark fibrils in the vacuole are seen, together with fine fibrils observed in the upper parts (Fig. 4c). A few dictyosomes are in an inactive condition. Only 1–2 lipid bodies per section can be observed. Ground plasma contains fine fibrils, which make it moderately electron-transparent. The plasma membrane is straight.

The walls of papillar cells show four layers: pellicle, cuticle, the primary cell wall with a slight fibrillar pattern, and a homogenous, highly electron-transparent secondary wall layer (Fig. 3a). Plasmodesmata are sometimes seen between papillae (Fig. 4a).

Cells of stylodia TT are elongated and folded in both longitudinal and cross-sections. They contain a lobed nucleus and chloroplasts with starch grains and very well-defined thylacoids. The number of mitochondria is about the same as in papillar cells while the density of ribosomes is higher. A large vacuole is spread throughout the cell length and contains a strand of dark fibrillar material, connected with the network of finer fibrils

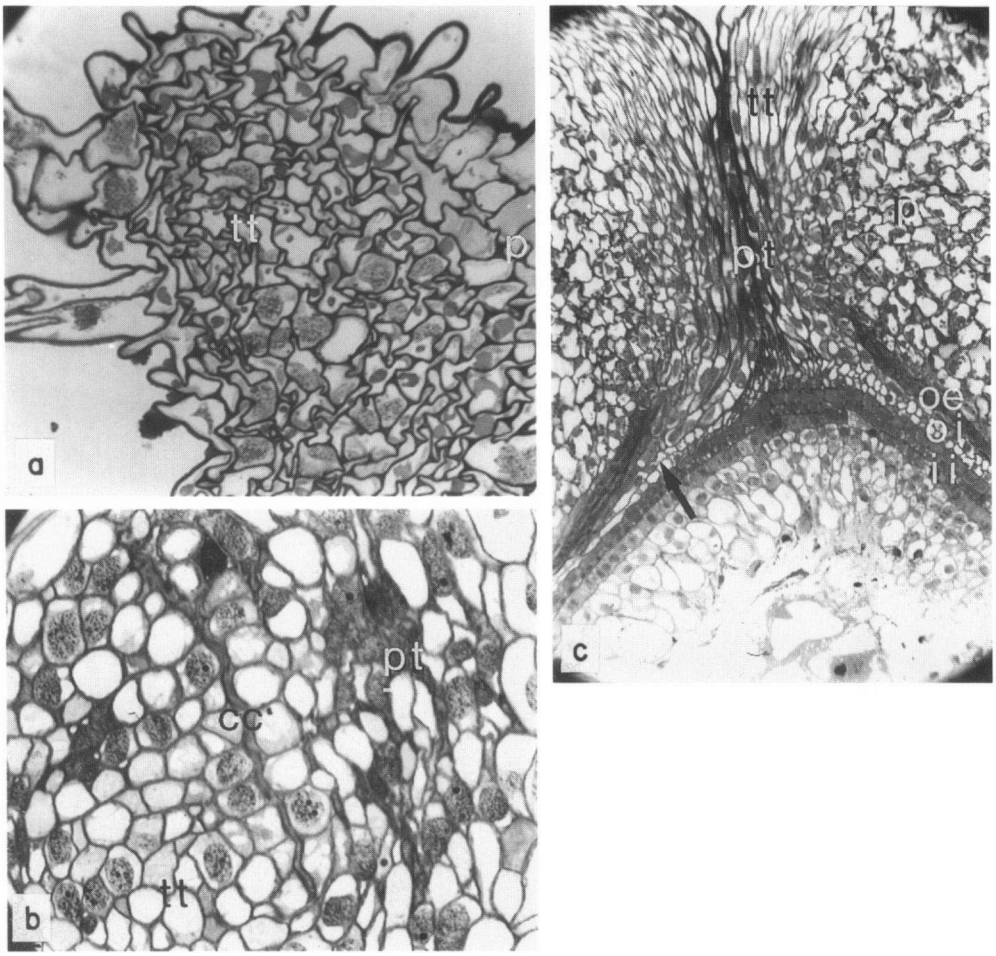
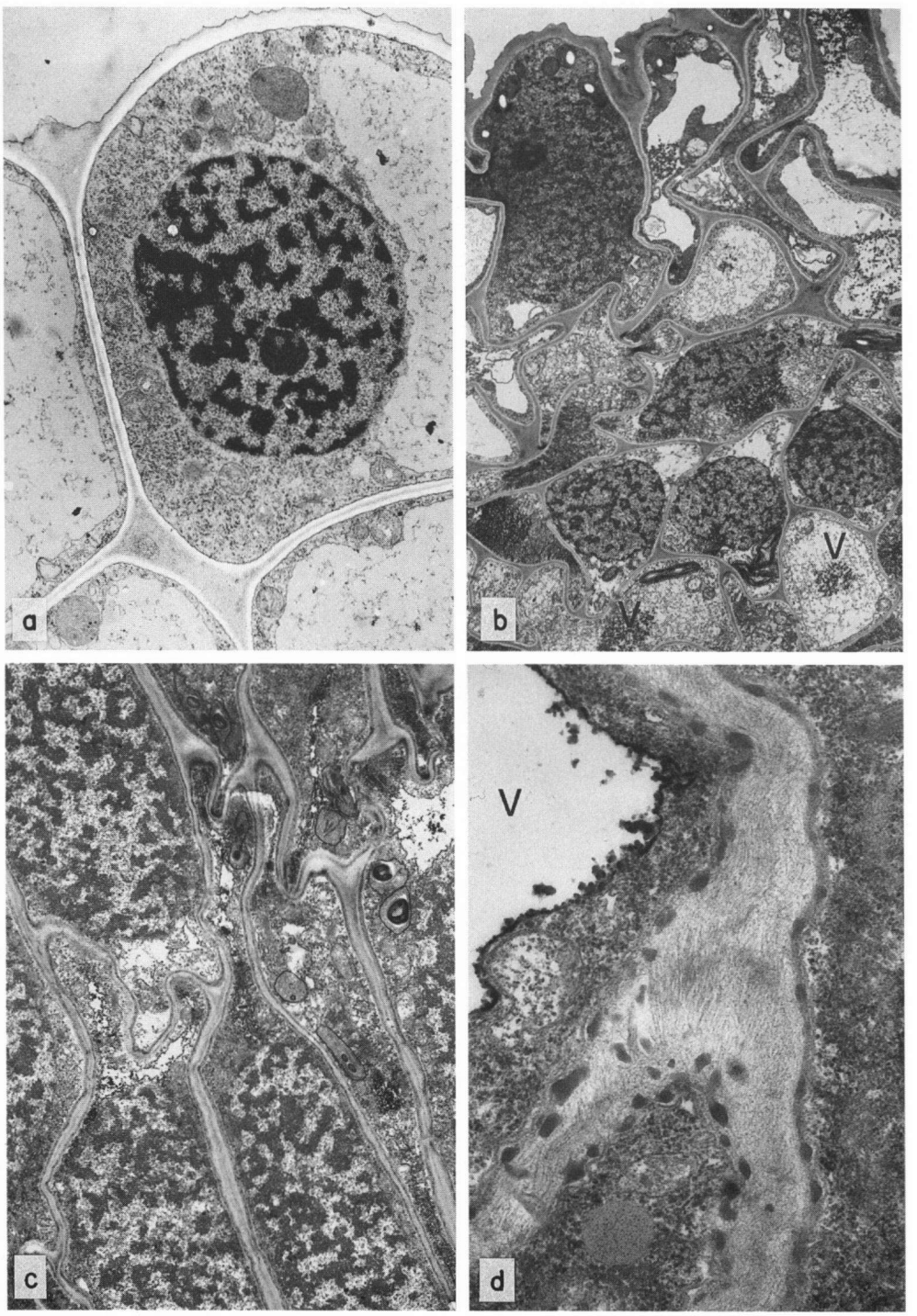


Fig. 2. (a) Semithin sections of wheat pistil, toluidine blue staining. Cross-section of the base of the stylodia. Stigmatic hairs enter stylodia from inner side, where cells of TT are situated. Outer stylodia part consists of parenchymatous tissue. × *c.* 250. (b) Cross-section of the basal part of the stylar TT. The cells of one part of TT have roundish outlines, while the other part has irregular shaped elongated cells. Carpel cuticle of stylodia epidermis dividing stylar TT is seen in the centre. × *c.* 625. (c) Median longitudinal section of stylar TT. Note the diminution of TT cells rows below and the asymmetrical pattern of stylar TT termination. Arrow shows the site of stylar TT and ovarian epidermis connection. × *c.* 65.

(fig. 3b). Large lomasome-like structures with fibrillar material are often visible in cross-sections. Cells of stylodia TT showed staining for common protein (Coomassie blue) as compared to other parts of the pistil.

Cells of stylar TT have some differences compared with stylodia TT cells. Their folds are visible only on longitudinal sections, on polar ends of the cells (Fig. 3c). Amyloplasts contain 2–3 starch grains. The number of mitochondria per cell section and density of

Fig. 3. Electron micrographs of PTT cells in unpollinated pistil. (a) Cross-section of the stigmatic hair. × *c.* 6300. (b) Cross-section of stylodia TT. Note fine fibrillar content of vacuoles. × *c.* 3150. (c) Longitudinal section of stylar TT. Note folded form of the cells. × *c.* 5000. (d) Globules between plasma membrane and cell wall in stylar TT cells. Note the fibrillar material against the tonoplast in vacuole. × *c.* 25 200.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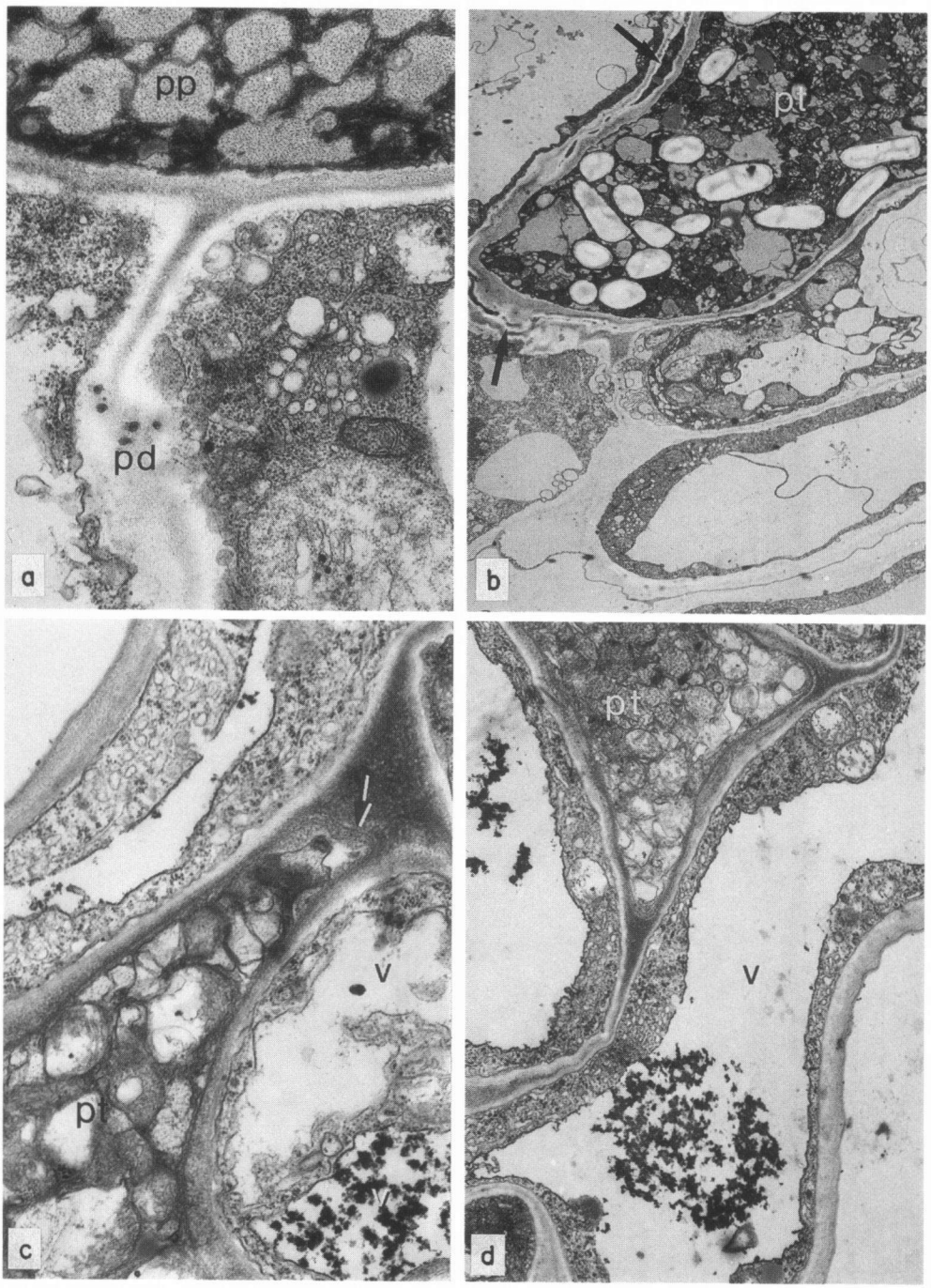
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ribosomes are higher than in stylodia cells. The content of the vacuole consists of a dark granular material which is visible only on the periphery of the vacuole against the inner side of the tonoplast (Fig. 3d). Ground plasma is dense due to the microfibrillar content. Numerous dark globules are visible between the plasma membrane and cell wall. These globules are most abundant in the regions of cell junctions (Fig. 3d).

Cell walls of stylodia and style TT have two layers: an outer primary wall with relatively tightly packed fibrils and an inner, secondary wall which is electron-transparent with a loose fibrillar pattern. Plasmodesmata are present both in transverse and lateral walls and are most numerous in stylar part of TT (Fig. 5a).

Cells of the inner ovary epidermis bordering the ovarian cavity are elongated with a long prominent nucleus, small vacuoles and large vesicles (Fig. 5d). Distinguishing them from the TT cells are dividing mitochondria and plastids, 6–7 large lipid bodies and 6–8 active dictyosomes per cell section. The contents of the vacuoles are electron-transparent. Plasmodesmata connect these cells to the cells of adjacent ovary wall layer containing numerous chloroplasts. The stylar TT and the ovarian epidermis are connected by plasmodasmata (Fig. 5c). A carpel cuticle covers the cells of the ovary epidermis.

Cells of the outer integument have rectangular or roughly isodiametric form (Fig. 5c,d). The round nucleus is surrounded by the vacuole. Dark globules are visible between the plasma membrane and cell wall, as well as against the tonoplast in the vacuole (Fig. 5b). Similar globules can be observed in the intercellular space. Dividing mitochondria and proplastids are observed. Six to seven large lipid bodies are present in each cell section, as well as numerous dictyosomes with small vesicles. The plasma membrane shows undulations and excretion of dictyosome vesicles takes place. Cell walls have a distinct loose fibrillar pattern and the cuticle is locally disrupted. Plasmodesmata connect all cells of the two layers of the outer integument.

Fine fibrillar material is visible in the ovarian cavity between the ovary wall and outer integument (Fig. 5c).

Pollen tube organization

After pollen grain germination on stigmatic hair, pollen tube grows in the intercellular space. Some pollen tubes could enter a hair, but only one will grow through. Others will flatten and die (Fig. 4b).

After pollen arrival, some changes in papillar cells are observed. The nuclear chromatin becomes less dense, dictyosomes are active and many electron-transparent vesicle of various shapes appear in the cytoplasm (Fig. 4a,b). Tubular and vesicular elements of SER occur, frequently close to or in contact with the plasma membrane (Fig. 4c). Lipid globules are not observed and the number of ribosomes decreases. The ground plasma shows a decrease in fibrillar components. The plasma membrane

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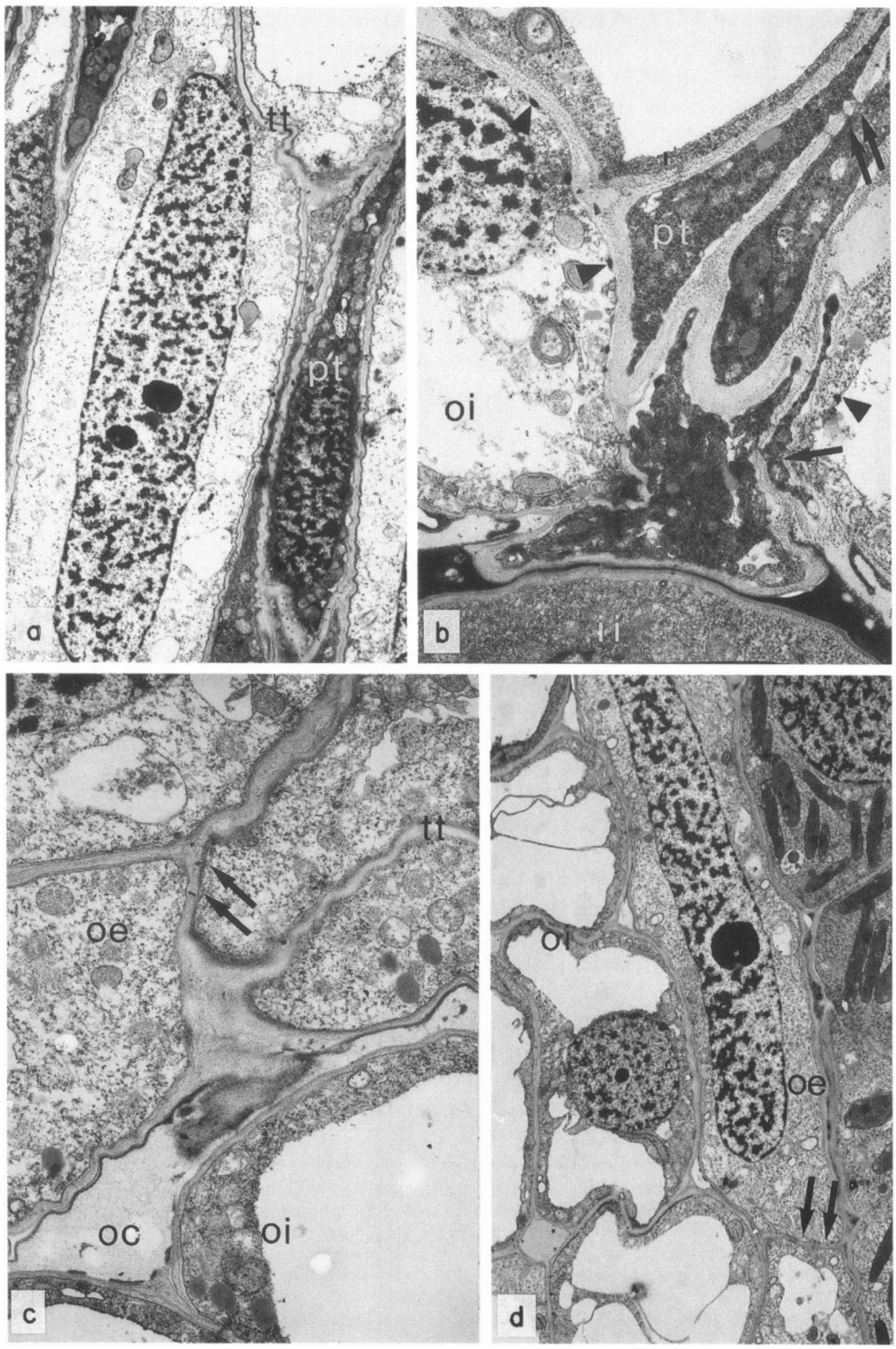
Fig. 4. Electron micrographs of pollen tubes growing in the stigmatic hairs (a–c) and stylodia TT (d). (a) Fragment of pollen tube near the tip. The main content of the tube cytoplasm p-particles represent. Note vesicles and paramural bodies in stigmatic papillar cells. × *c.* 18 200. (b) The apical part of growing pollen tube, containing amyloplasts. Some flattened tubes are seen (arrows). × *c.* 5050. (c) Proximal to the pollen grain part of the tube growing in the base of the stigmatic hair. Pollen tube wall is partly discernible (arrow). Note vacuole content in papillar cell and electron density of intercellular material. × *c.* 18 900. (d) Fragment of pollen tube growing in stylodia TT. Vacuoles of TT cells contain only rough fibrils, fine fibrillar material is not seen longer. × *c.* 11 200.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develops numerous undulations in which vesicles and lomasomes are observed (Fig. 4a,b). Electron density of the intercellular matrix increases after the passage of pollen tubes (Fig. 4c).

In cross-section, the content of pollen tubes growing in stigmatic hairs differs, depending on the level of the section. The apical part of the tube contains a large amount of p-particles, amyloplasts, mitochondria, cisternae ER and ribosomes (Fig. 4a,b). Ground plasma is generally opaque because of thin fibrils. Two sperm cells in close connection with each other and to the vegetative nucleus can be observed in this part of the pollen tube. The plasma membrane forms only slight undulations. Cortical microtubules sometimes are visible in the vicinity of plasma membrane. The thin electron-lucent wall layer bordering the plasma membrane is callose. No callose plugs are observed. The outer fibrillar layer of pollen tube wall is not sharply demarcated from primary wall of papillar cell (Fig. 4a).

The basal part of the tube contains p-particles, mitochondria, ribosomes, large electron-transparent vesicles and small vacuoles with thin fibrillar content (Fig. 4c). Sometimes multi-vesicular and multi-membrane structures are visible. As the pollen tube elongates, vacuoles become larger. They fuse and form a large vacuole, which occupies the tube volume. Thin cytoplasmic layer containing ribosomes, scarce mitochondria, proplastids and p-particles make up the pollen tube content in its basal part. The callose wall layer of the tube is more clearly distinguishable.

Interaction of the tube with the pistil transmitting tract

Passage of pollen tubes through stylodia induces changes in TT cells. The fine fibrillar material disappears from vacuoles and electron density of rough fibrils decreases (Fig. 4d). Lomasomes with fine fibrils are no longer visible. Other changes after pollination are comparable with those in papillar cells. Some pollen tubes cease growth in stigmatic hairs and show a thick callose layer.

While the pollen tube grows into the intercellular substance of stylodia TT, the number of starch grains and p-particles in its cytoplasm decreases. Numerous mitochondria, ribosomes, active dictyosomes, scarce microtubules, small vacuoles and cisternae RER are present. The plasma membrane is straight (Fig. 4d). Some pollen tubes cease growth in stylodia TT, accompanied by thickening of the callose layer of the tube wall. Occasionally callose is not seen, but the wall becomes folded and thickened suggesting termination of the pollen tube growth.

In stylar TT, the pollen tubes pass through the intercellular material and, 20 min after pollination, they can be observed in the base of the style. Most changes are comparable with the events in stylodia TT. The folded form of stylar TT cells changes to rectangular form. Dark globules between plasma membrane and cell wall

Fig. 5. Electron micrographs of the longitudinal sections of stylar and ovarian parts of PTT. (a) Pollen tubes growing in the style and cell of TT 20 min after pollination. Note numerous plasmodesmata in the longitudinal wall of the TT cell. × *c.* 2750. (b) Pollen tube inhibition and rupturing in intercellular spaces of outer integument. Plasmodesmata-like formations connect the stopping pollen tubes (arrows). The dark globules are seen on cytoplasm periphery in the cell of outer integument (arrowheads). × *c.* 6300. (c) The site of the connection of the stylar TT cells and the cell of ovarian epidermis. Plasmodesmata (arrows) link the cells. Fine fibrillar material in the space of ovarian cavity is visible. × *c.* 7600. (d) Cells of ovarian epidermis and outer integument bordering the space for pollen tube passage in unpollinated pistil. Note the plasmodesmata between the cells of ovarian epidermis (arrows). × *c.* 3000.

observed before pollination are no longer visible. Dark granular material in vacuoles becomes much less conspicuous (Fig. 5a).

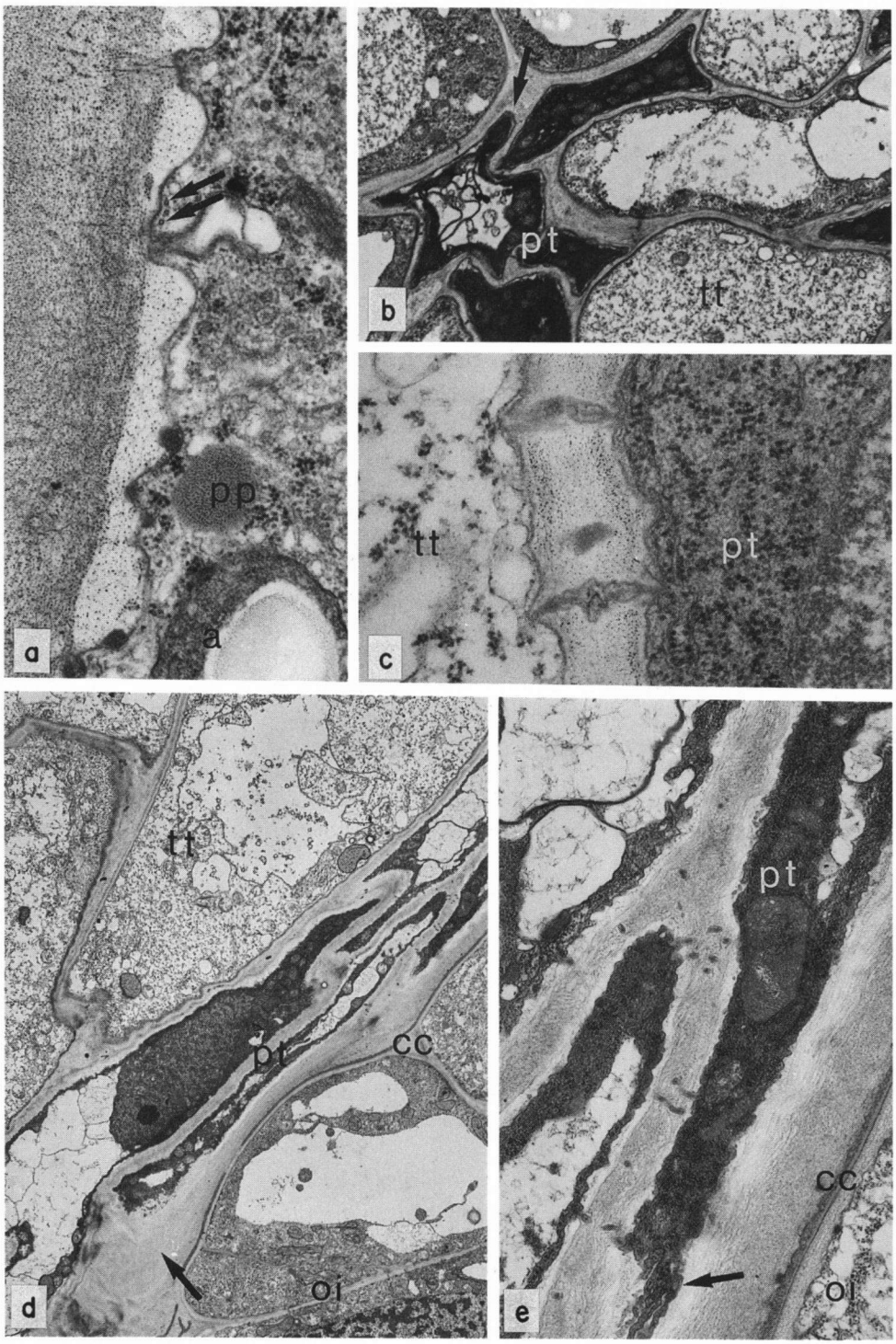
The most common changes in the cells of stigmatic hairs and TT 20 min after pollination indicate the activation of their cytoplasm. However, 2·5 h after pollination, after many pollen tubes have passed this pathway, other changes are noted. The number of ribosomes decreases to about one-tenth that observed in unpollinated pistil. Starch grains in plastids and lipid bodies disappear, profiles of ER and Golgi are scarce or absent and mitochondrial cristae become almost indiscernible. Plasmodesmata are not seen and plasma membrane and tonoplast often lost their integrity.

After reaching the base of the style, pollen tubes grow in the elongated stylar part; they bend to penetrate the carpel cuticle and to enter the space between the ovary epidermis and outer integument. Pollen tubes growing in the non-elongated part of stylar TT and reaching the base of the style show different behaviour. After penetration of carpel cuticle, some tubes will turn 90° and grow in the direction of the main pathway. Others do not turn but grow between cells of the outer integument and then stop (Fig. 5b). Residues of burst pollen tube cytoplasm are often seen between the outer and inner integuments (Fig. 5b). Carpel cuticle appears to be disrupted after pollen tube penetration.

The composition of cytoplasm of pollen tubes during their growth in the stylar TT, differs from that described for stigma pathway. The amount of starch grains and p-particles is diminished and many active dictyosomes, producing vesicles of intermediate electron opacity, are observed in the apical part of the pollen tube (Fig. 6a), as well as vacuoles with fine fibrils (Fig. 6d). Plasma membrane of the apical part of the tube forms prominent protrusions (Fig. 6a,e). These protrusions occasionally cross the pollen tube wall and are observed in proximity to similar formations of neighbouring pollen tubes, probably coming in to contact with them (Fig. 6e). Interconnections between arresting pollen tubes via these protrusions were definitely seen (Fig. 5b). Contact of pollen tube plasma membrane and plasmadesmata of TT cells is occasionally noticeable (Fig. 6c). No signs were observed of callose in pollen tube walls in the style base or further in the pathway.

During pollen tube growth, only a few changes are observed in the cells of the ovary epidermis, mainly an increase of the number of small dictyosome vesicles and some activation of plasma membrane. More obvious changes are observed in the cells of the outer integument bordering the passage of the pollen tube route. Active processes of ectocytosis of different kinds of vesicles and dark globules are observed (Fig. 5b). Some excreted globules are visible in intercellular space and dark granules are seen on the inner surface of the tonoplast (Fig. 5c). When examining the cross-sections, it should be noted that the volume of each pollen tube appears to decrease while it is growing in PTT. In cross-sections of the style the pollen tube area is 6–12 times less than in the stigmatic hair. The density of cytoplasmic organelles in sections of pollen tube growing in style is rather high compared to the neighbouring TT cells. 20 min after pollination

Fig. 6. Electron micrographs of cross- (a) and longitudinal- (c–e) sections of pollen tubes growing in stylar TT. (a) Fragment of the pollen tube cytoplasm. Plasma membrane forms protrusions. Some cortical microtubules are seen (arrows). × *c.* 31 500). (b) Pollen tubes growing in intercellular substance. Three pollen tubes cut in apical parts, while one (arrow) is cut in the basal part. × *c.* 4750. (c) Pollen tube passing plasmodesmata of TT cells. × *c.* 37 800. (d) Pollen tubes growing in the base of the style follow the bending of TT. One inhibited pollen tube with the thickening of the wall is seen (arrow). × *c.* 2500. (e) Enlarged portion of the tubes. Note numerous cytoplasmic finger-like protrusions of the plasma membrane (arrows). × *c.* 12 500.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pollen tubes have a mitochondrial density 3–3·5 times and ribosomal density 1·5 times greater than that observed in TT cells (Fig. 6b,c,d).

Very few pollen tubes enter the narrow space of the ovarian cavity. Signs of pollen tube arrest can be observed, e.g. wall folds and thickening. The entire space is occupied either by growing pollen tubes or tubes whose growth have been inhibited.

DISCUSSION

In previous studies of pollen–stigma interactions in grasses (Cass & Peteya 1978; Heslop-Harrison 1979b; Heslop-Harrison & Heslop-Harrison 1980, 1981) it was shown that there was no defined TT in stigmatic hairs. Organization of further pathway for pollen tubes was not shown.

Organization of the pistil transmitting tract before and after pollination

Differences in cell organization in stigmatic hairs, stylodia and stylar parts of wheat PTT point to a heterogeneous pollen tube pathway.

Before pollination the characteristic features of stylodia TT cells are chloroplasts and large vacuoles with fine and rough fibrils, which are proteinaceous as in rye (Heslop-Harrison & Heslop-Harrison 1980). The presence of lomasomes with fine fibrillar material in stylodia cells, the undulations of the plasma membrane and the disappearance of the material from vacuoles after pollination can be interpreted as signs of protein secretion, as is suggested in rye and barley (Heslop-Harrison & Heslop-Harrison 1980) and in *Nicotiana alata* (Sedgley & Clarke 1986).

In TT cells the disappearance of starch, activation of dictyosomes, close juxtaposition of SER elements to plasma membrane and exocytosis suggest a mechanism for carbohydrate delivery to the cell exterior via derivates of ER, as observed in some plant and animal cells (Gunning & Steer 1975).

A characteristic of stylar TT cells is the presence of dark globules of an unknown nature outside the plasma membrane before pollination. The disappearance of these globules after pollination probably indicates that the material is transferred to the cell wall or intercellular space.

Another product is the fine fibrillar substance of the ovarian cavity which has an unknown composition. Numerous active dictyosomes in cells bordering this space, dark excreted globules and vacuole content in cells of outer integument are probably involved in the formation of this substance.

PTT cells have plasmodesmata in both lateral and transverse walls; in several species with solid styles, plasmodesmata exist only in transverse walls in mature pistils (Knox 1984).

Such differences in the ultrastructure of cells composing PTT suggest a possible difference in secretion products along the PTT.

Pollen tube interaction with the pistil transmitting tract

Variations in the mode of pollen tube growth also reflect the differences in properties of stigmatic, stylar and ovarian parts of PTT in wheat pistil.

During growth of the stigmatic hairs and stylodia TT, pollen tubes have a relatively conspicuous callose wall layer. In stylar TT, the callose layer of the pollen tube wall is nearly absent. An incomplete callosic layer was also noted in *Petunia hybrida* (Herrero

& Dickinson 1981) and *Gasteria verrucosa* (Willemse & Franssen-Verheijen 1986, 1988). In *Gasteria* it is assumed to be connected with the velocity of pollen tube growth and a quick uptake of nutrients.

In wheat pistil the plasma membrane of pollen tube forms finger-like protrusions, also observed in rapidly growing pollen tubes of *Nicotiana sylvestris*, which are called plasmotubules (Kandasamy *et al.* 1988). However, in wheat there is plasmotubule contact with TT intercellular material and apparently with plasmodesmata. The formation of these plasmodesmata-like structures begins in the stylar part of the route and coincides with the restriction of PTT volume and probably with a deficiency of nutrients. These facts and the high speed of pollen tube growth allow the surmise that the plasmotubules of the pollen tube plasma membrane provide opportunities for an intensive and direct interaction with TT cells. It is not clear whether there are symplastic communications between growing pollen tubes and PTT cells via these plasmodesmata-like formations. Interconnections observed between arrested pollen tubes show features of coenobium. Formation of secondary plasmodesmata is known for other types of cells (Jones 1976), as in the interaction between the dwarf mistletoe *Arceuthobium pusillum* and its host *Picea mariana* (Tainter 1971). Cell walls of this parasite and host parenchyma cells come so close together that no separation between walls is observed, and plasmodesmata are formed between these cells followed by symplastic transport. The mechanism of pollen tube interaction with TT plasmodesmata remains controversial. Carr (1976) points to the participation of plasmodesmata in the exchange of molecular informations. However, there are reports that such transfer of macromolecules via plasmodesmata may be quite limited (Heslop-Harrison & Linskens 1984).

Ultrastructural data about the organization of PTT and pollen tube in the wheat pistil make it clear that special types of pollen tube—PTT interactions occur in the style. These involve specificity of secretion in the stylar TT cells, a change in pollen wall organization, and formation of pollen tube plasma membrane protrusions which provide plasmodesmatal-like contact with the intercellular material of TT.

In most descriptions of grass pistil the presence of the style is not mentioned. Our data revealed that in wheat it is funnel-shaped, formed by slightly asymmetrical partial coalescence of the carpels. These anatomical features reducing the volume of the TT play a significant role in the regulation of pollen tube number. The data for wheat are generally in agreement with those of Heslop-Harrison's *et al.* (1985) for *Zea mays*. Next to mechanical guidance, the functional control of pollen tube growth would be expected at the base of the style or just after the exit from the stylar TT, the site of pollen tube inhibition and bursting. Such rupture of compatible pollen tubes was shown earlier for *Petunia hybrida* (Herrero & Dickinson 1981) where pollen tubes burst because of the absence of a rigid callosic layer. In incompatible crossings, another mechanism would be involved for the elimination of alien pollen tubes.

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