

Wall Projections in the Vegetative Cell of *Parietaria officinalis* L. Pollen

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Summary

Ultrastructural aspects of pore development in *Parietaria officinalis* L. pollen are described. The main feature is the presence of intine wall projections formed in correspondence to the pores during the late microspore stage and persisting in the shedding pollen. This structure coexists with the tubules occurring in the pore and first appearing as evaginations of the plasma membrane.

The morphological evidence and other features such as the occurrence of mitochondria next or in contact with wall projections lead us to compare the pollen vegetative cell to a transfer-cell. The possible functions of these transfer-cell type structures are discussed.

Keywords: *Parietaria*; Pollen pore; Transfer-cells.

1. Introduction

The purpose of this paper is to describe the wall projections formed in the poral zones of *Parietaria officinalis* L. pollen. These wall projections are similar to those found in transfer-cells, and are probably not to be confused with the tubular plasmalemma evaginations frequently observed in the poral intine of many species until now described (KNOX and HESLOP-HARRISON 1970, HESLOP-HARRISON *et al.* 1973, HESLOP-HARRISON 1975, PACINI and CRESTI 1977, PACINI and JUNIPER 1979).

2. Materials and Methods

Anthers at various stages of development (from tetrad formation to pollen shedding) were gathered at bimonthly intervals between September 1978 and September 1979 from *Parietaria officinalis* L. plants, growing spontaneously in the Siena district, where they are continuously blooming except in the frost periods.

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The anthers were pre-fixed for 1 hour in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) and post-fixed in 1% OsO₄ in the same buffer, then dehydrated in ethanol and embedded in an Epon/Araldite mixture.

Sections were cut on an LKB Ultratome III, stained with uranyl acetate and lead citrate, and examined in either a Zeiss EM9A or a Jeol JEM 100B transmission electron microscope.

Sections 1 to 3 µm thick were cut from the same blocks from which ultrathin sections had been made, and tested for polysaccharides by the PAS reaction (JENSEN 1962).

3. Observations

The ultrastructural features of normal, ripe, viable pollen grains do not vary through the year, except as far as the number of starch grains is concerned. There may even be pollen grains completely devoid of starch, the percentage of starchless pollen grains reaching a maximum in September (our unpublished data). The increase in the percentage of starchless pollen grains occurs together with an increase in the percentage of unviable grains. Not considering this type of variation the following descriptions apply to all pollen grains independently of time of collection.

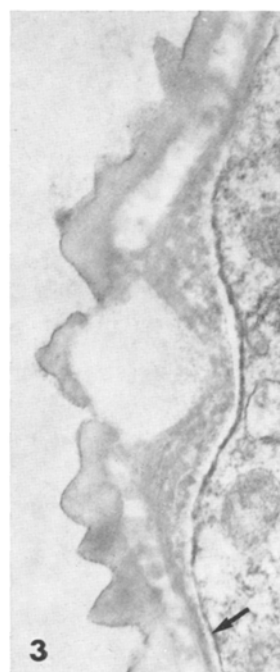
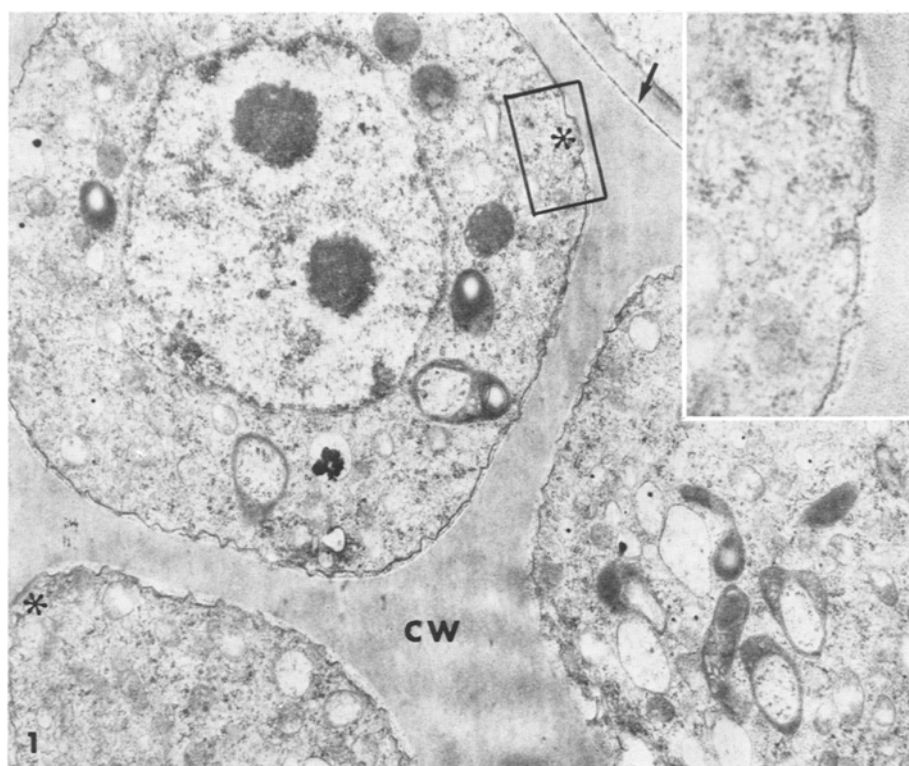
Tetrad stage. Both tetrads and microspores are tetrahedral, the microspores being arranged along the axes of a tetrahedron. At this stage the microspores are surrounded by a callosic wall (Fig. 1), the four microspores being still contained within the primary pectocellulosic wall of the microspore mother cells. Plastids show the starch grains derived from the first amylogenesis. The position of the pores is now established: in fact the plasmalemma of the microspores detaches from the callosic wall to allow primexine deposition, this phenomenon occurring on the whole surface of the microspore, with the exception of the three points where pores will be formed, *i.e.*, each vertex of the outer face of each microspore (Fig. 1).

Early microspore stage. (Intine uncompletely formed—no “tubules” are present.) Exine is rapidly formed after the microspores are released from the tetrads; each pore is surmounted by an operculum, bearing in section one or two spinules similar to those on the exine surface (Fig. 2). The microspore

Fig. 1. Tetrad with incipient primexine formation. Three microspores are present, separated by a callosic wall (CW). The primary pectocellulosic wall of the mother cells is still present (arrow). Asterisks mark the positions where the pores will be formed; the plasmalemma in the interpore zone detaches itself from the callosic wall allowing the primexine to be deposited, whilst in the pore zone the plasmalemma is still in contact with the wall (inset). In the space between plasmalemma and callosic wall a granular material is evident (inset). $\times 13,000$, inset $\times 39,700$

Fig. 2. Early microspore stage. The nucleus has a parietal position, owing to the formation of a big vacuole. Exine is already formed; each pore is surmounted by an operculum (arrow), bearing in section two spinules similar to those on the exine surface. $\times 9,500$

Fig. 3. Microspore at the same stage of the preceding plate. Intine deposition has just started (arrow); endexine in the pore zone is thick and “fringy” on the cytoplasm side. $\times 27,500$



Figs. 1-3

has now a big vacuole; no starch grains are present. The formation of the pectocellulosic part of the pore occurs simultaneously with intine deposition along the rest of the grain wall. The whole plasmalemma retracts from the endexine to let the intine be deposited (Fig. 3), this retraction being greater in the poral zones, where the endexine is thicker and less homogeneous than in the interpore area (Fig. 3).

Middle microspore stage. (Intine completely formed—"tubules" present.) At this stage, the general organization of the microspore cytoplasm is the same as in the preceding stage. Intine formation is nearly completed (Fig. 4); in front of the pores the intine is much thicker than in the rest of the pollen grain, and the plasmalemma has moved further away, but leaving inside the poral wall some very thin, unbranched evaginations (Fig. 4), usually called "tubules" (HESLOP-HARRISON *et al.* 1973), which have a faintly electron-dense content. Besides the tubules also a microfibrillar substance can be seen in the poral intine (Fig. 4).

Late microspore stage. (Formation of poral wall ingrowths.) At this stage the vacuole is reduced in volume, and the second amylogenesis has started. The intine is now entirely formed; under the germinative pores a deeper irregular detachment of the plasmalemma has occurred, so that the intine, while being deposited, has formed a set of projections. The spaces between the projections are wider than the preexisting tubules, with which they are however continuous, and have a labyrinthic appearance (Figs. 5–7). The wall projection apices are often in contact or close to mitochondria (Figs. 5 and 6),

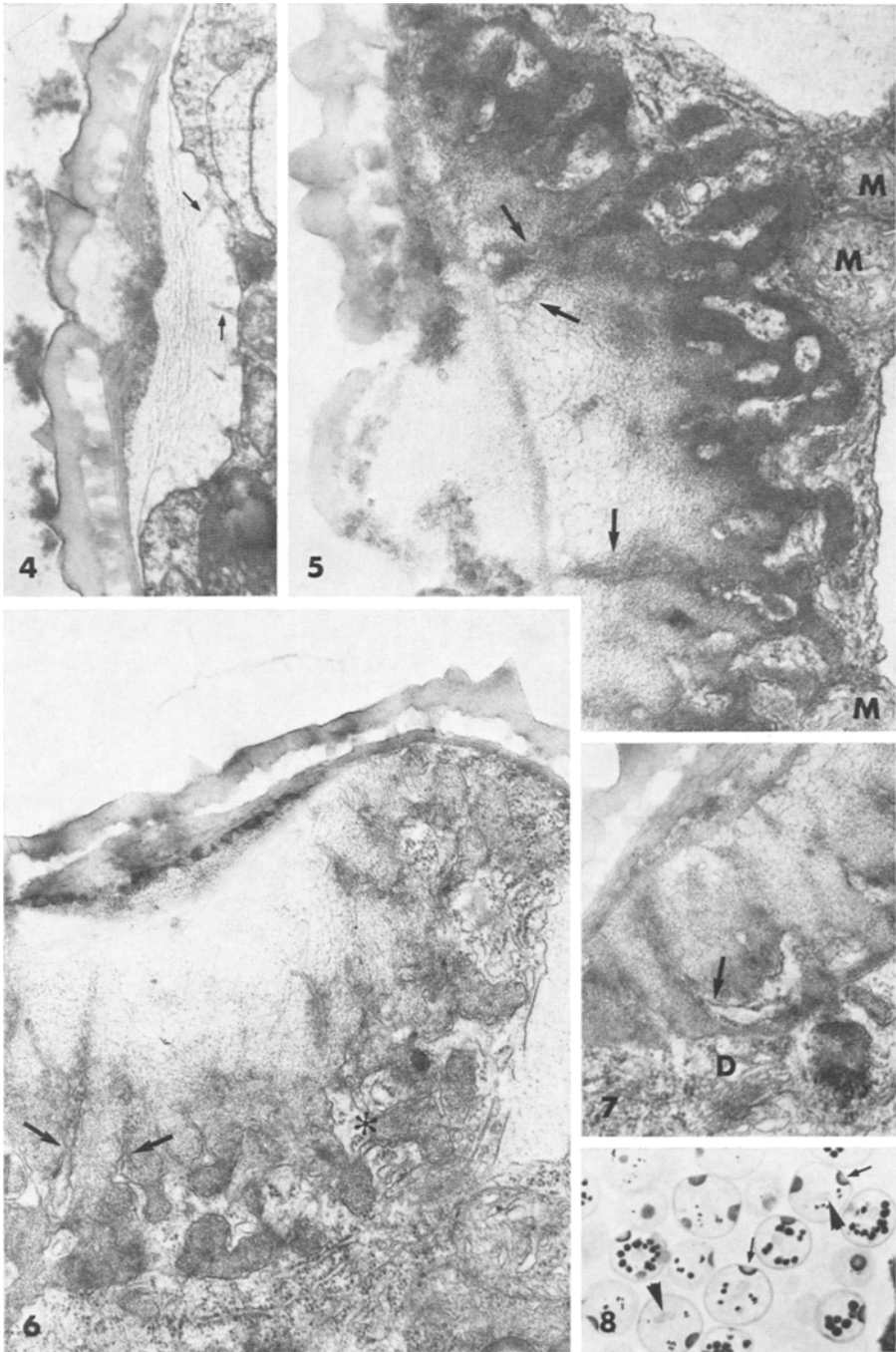
Fig. 4. Middle microspore stage. In correspondence to the pore the intine is quite thick and filled with a microfibrillar substance; some plasmalemma evaginations (arrows), commonly named "tubules", penetrate into the intine. The debris in the locular fluid derive from tapetal cells damaged during handling. $\times 24,100$

Fig. 5. Late microspore stage. The pore is completely formed. Both the tubules (arrows) formed during the preceding stage and wall projections are evident. Three mitochondria (*M*) are close to the wall projections tips. $\times 30,700$

Fig. 6. Almost ripe grain: glancing section not intercepting the operculum. The tubules have a faint electron-dense content and are continuous with the cytoplasm (arrows). The wall projections are granular and electron-dense on the cytoplasm side, their electron-density decreasing towards the microfibrillar part of the intine. Ribosomes and polysomes (asterisk) are present in the cytoplasm between wall projections. Flat cisterns and two mitochondria are evident in the cytoplasm near the wall projections tips. $\times 34,300$

Fig. 7. Almost ripe grain. A dilated endoplasmic reticulum cistern, still bearing the ribosomes, apparently creeps through the cytoplasm between the wall projections, towards a tubule (arrow). A dictyosome (*D*) is evident near some wall projection tips. $\times 29,400$

Fig. 8. PAS test on a section of anther at the same stage as the preceding plate. The inner part of the pore, on the cytoplasm side, is much more PAS positive than the outer part (arrows), the interpore intine, and the generative cell wall (arrow heads). Starch grains are present in different amounts in the various pollen grains, as the second amylogenesis, from which they derive, is asynchronous. $\times 600$



Figs. 4-8

while the cytoplasm inside the spaces between the projections contains only ribosomes (Figs. 6 and 7) and dilated endoplasmic reticulum cisterns (Fig. 7). The material constituting the wall projections is granular and electron-dense near the cytoplasm, but its electron-density decreases towards the outer microfibrillar layer of the intine, where the first tubular evaginations had formed (Figs. 5 and 6). The outer part of the projections is also less PAS positive than the apical portion in contact with the cytoplasm (Fig. 8).

The germinative pores are completely developed shortly before the first haploid mitosis, remaining then unchanged until germination (Figs. 6 and 7).

4. Discussion

The wall projections occurring in the pore regions of *Parietaria officinalis* pollen, on account of their morphology and their frequent association with mitochondria, are similar to the wall projections occurring in transfer-cells, as originally described by GUNNING and PATE (1969); indeed the vegetative cell of *Parietaria* pollen could somehow be considered as a transfer-cell engaged in the flow of substances in and/or out of the pollen grain. This is the first report of transfer-cell structures in pollen: in fact the structures described as wall projections in *Nuphar* and *Epilobium* unripe pollen (ROWLEY and FLYNN 1971) are not considered of the transfer-cell type by GUNNING and PATE (1974), even if they "enhance the area of contact between microsporangium and microspores". Probably in the latter species the poral region of the wall is characterized not by wall projections, but by the tubular plasmalemma evaginations often formed in pollen grains. In most species these tubules, which are often wide and branched, become separated—at least in the ripe pollen—from the cytoplasm by an additional, newly formed, wall layer (KNOX and HESLOP-HARRISON 1970, HESLOP-HARRISON *et al.* 1973, HESLOP-HARRISON 1975, PACINI and CRESTI 1977, PACINI and JUNIPER 1979). Such tubules have commonly been considered as the storage sites of gametophytic proteins involved in the pollen-stigma recognition process, even if at first, during pollen development, by amplifying the protoplast surface, they could be involved in the exchange of materials between anther loculum and pollen grain. In *Parietaria officinalis* instead, the tubules derived from plasmalemma evaginations are never sealed off, but are integrated with the wall projections. It seems therefore that the final structure is mainly a transfer structure.

Transfer-cells either absorbing from or secreting to the external medium have already been described and recently reviewed by GUNNING and PATE (1969, 1974). For example epidermal transfer-cells occur in submerged leaves of many water plants and it is reasonable to suppose that they are involved in ion absorption. At the same time wall ingrowths are possessed by many nectaries, salt glands, and other secretory structures (again see the reviews by GUNNING and PATE, 1969 and 1974).

As it is not possible, on purely morphological grounds, to decide whether a transfer-cell is absorbing or secreting (GUNNING and PATE 1974), in the case of the wall ingrowths of *Parietaria* pollen the following hypotheses can be put forward as regards their function:

1. while the pollen grain is still inside the anther, the wall ingrowths may act to facilitate a more speedy absorption of substances present in the locular fluid, and derived from the degenerating tapetum;
2. still while the pollen grain is inside the anther, they could facilitate a more speedy dehydration before shedding;
3. when the pollen is on the stigma, they could facilitate a more speedy rehydration;
4. after rehydration, they might speed up the release of gametophytic proteins.

These four hypotheses, still to be verified, regard four events staggered in time, which theoretically are all compatible with each other; similarly, cases are reported of gland cells which besides secreting materials out of the plant can also "absorb solutes presented to their secretory surfaces" (GUNNING and PATE 1969).

Nevertheless, the fourth hypothesis seems the more attractive one. In fact, in pollen grains two kinds of diffusable proteinaceous complexes may be present: the gametophytic and the sporophytic one (HESLOP-HARRISON *et al.* 1973, HOWLETT *et al.* 1979); in *Parietaria officinalis*, from our ultrastructural observations, we can say that only the former is present. As in *Parietaria officinalis* pollen the poral tubules are extremely reduced, as compared to those of other species, and as the poral tubules are thought to be the storing site of diffusable gametophytic proteins, it derives that a very small amount of these substances is stored in the poral wall of *Parietaria*. As the allergenic fraction of the proteins released by *Parietaria* pollen is only 0.5–2% of the total (FALAGIANI 1976), and as *Parietaria* pollen is strongly allergenic (STANLEY and LINSKENS 1974, SERAFINI 1974), one must suppose that the total amount of released proteins must be quite considerable and greater than the quantity that can be stored in the poral tubules. Therefore the major part of diffusable proteins must come from inside the cytoplasm. Besides, FALAGIANI (1976) reports that placing pollen in water, the greatest amount of allergens is released within the first 10", and that practically all allergenic proteins are eluted in the first 32": later on only a very small increase of eluted allergens can be noticed. Such elution time is very short if compared with the times reported by HESLOP-HARRISON *et al.* (1975) for the gametophytic proteins of some species, time intervals which range between 30" and 20'.

For these reasons, that is the presumably great quantity of proteins which must be released on the stigma, and the comparatively great speed with which

this process occurs, the hypothesis that the pollen wall ingrowths may be involved in active transport mechanism of gametophytic proteins to the external medium seems the more probable one.

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