

Ultrastructure of *Linaria vulgaris* pollen grains

M. CRESTI, C. MILANESI, A. TIEZZI, F. CIAMPOLINI
and A. MOSCATELLI

Dipartimento di Biologia Ambientale, Università di Siena, Via P. A. Mattioli n. 4, 53100 Siena, Italy

SUMMARY

Both the internal anatomy and the external morphology of the mature pollen grain of *Linaria vulgaris* were studied.

A peculiar feature of mature pollen is the occurrence in the vegetative cytoplasm of a large quantity of stacked endoplasmic reticulum. The generative cell contains a complex system of longitudinally arranged microtubules; their roles in the shaping and movement of the generative cell are discussed.

Key-words: cytoskeleton, *Linaria vulgaris*, pollen.

INTRODUCTION

The essential function of the pollen grain is to deliver the gametes into the embryo sac through the pollen tube. Mature pollen may also be considered as a storage organ that supplies nutrient materials to the growing pollen tube (Jensen *et al.* 1974; Cresti *et al.* 1975; for a complete review see Heslop-Harrison 1987). It is also important to study the cytoskeleton of the pollen grain and the pollen tube in order to understand better the generative cell and sperm cells quality and motility. In the styles and leaves of *Linaria vulgaris* a paracrystalline nuclear inclusion, probably existing of tubulin, was observed (Ciampolini *et al.* 1980). In order to clarify the nature and function of these structures we have studied the ultrastructure of the pollen grain to verify whether these structures are present in the vegetative or in the generative nucleus.

MATERIAL AND METHODS

Transmission and scanning electron microscopy. Mature pollen was gathered from the anthers of *Linaria vulgaris* grown in the greenhouse of the Department of Botany, University of Sienna. Fresh pollen grains (1 mg ml^{-1}) were sown in a culture medium (described by Brewbaker & Kwack 1964) containing sucrose and incubated at 20°C.

Samples were prefixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4. After rinsing in buffer, samples were postfixed in 1% buffered osmium tetroxide. Following dehydration in ethanol, samples were embedded in ERL 4206 (Spurr 1969). Sections were cut on a LKB ultratome III using a diamond knife and stained with aqueous uranyl acetate and lead citrate. The specimens were examined with a Zeiss EM 9 electron microscope at 60 kV or with a JEOL Jem 100B at 80 kV.

Abbreviations: GC, generative cell; VC, vegetative cell; VN, vegetative nucleus; RER, rough endoplasmic reticulum.

For scanning electron microscopy the fresh pollen was mounted with tape on aluminium stubs, coated with gold, and examined with a Philips 501 B at 7.2 kV.

Immunofluorescence. Preparations were made essentially as reported by Wick *et al.* (1981). The primary antibody was a monoclonal mouse anti-tubulin (Amersham, Sydney); the second antibody was fluorescein isothiocyanate (FITC) conjugated with rabbit anti-mouse antibody (Cappel Worthington). Observations and micrographs were made using a Leitz fluorescence microscope with Tri-x Pan Kodak Film.

For DNA-specific fluorochrome, 4,6-di-amido, *p*-2-phenylindole (DAPI) was used (Heslop-Harrison *et al.* 1986).

RESULTS

External morphology

Linaria vulgaris pollen is binucleate and tricolpate (Figs. 1 and 2) (Faegri & Iversen 1964). The exine is faveolate (Fig. 3).

Internal morphology

Pollen wall. In cross-section the wall is formed by two distinct layers. The outer layer (exine), formed by sporopollenine, is semitectate; sometimes in the space between the bacula electron dense material can be observed (Fig. 4). The inner layer (intine), containing cellulosic material, is ultrastructurally similar to an ordinary cell wall (Fig. 4). An irregular plasma membrane delimits the intine from the pollen cytoplasm.

Generative cell (GC). The GC is lobed and delimited from the vegetative cytoplasm by a copiously lobed wall. It is located in the central part of the pollen grain, generally near the vegetative nucleus. In cross-section the GC has a round appearance (Fig. 5), while in longitudinal section it has a spindle shape (Fig. 6) and two opposite tail-like structures are sometimes observed. The nucleus is also lobed and assumes the shape of the cell; the chromatin is aggregated in electron dense masses. The generative cytoplasm occupies the external portion of the cell and contains ribosomes, mitochondria, cisterns of rough endoplasmic reticulum (RER) and bundles of microtubules generally oriented according to the longitudinal axis of the cell (Fig. 7). Immunofluorescence observations show the presence of a high quantity of tubulin in the GC (Fig. 8).

Vegetative cell (VC). The nucleus is very large, its shape is irregular and many invaginations can be observed. Many large stacks of RER can be observed in the cytoplasm, particularly in the central portion near the GC and VN (Fig. 5). Only occasionally one or two RER cisternae can be seen at the periphery of the cell. Plastids containing some lamellae and sometimes lined with a RER cistern were observed (Fig. 9). The dictyosomes are located in proximity to the wall but their structure is not well defined (Fig. 9). A number of spherical lipid bodies of varying sizes are randomly distributed in the cytoplasm. Generally, the lipid bodies are completely surrounded by a RER cistern (Fig. 9). The ribosomes are numerous and evenly distributed throughout the cytoplasm: free polyosomes seem to be absent. Mitochondria with many cristae can be observed in the cytoplasm, as can small vacuoles, the latter contain one or more electron dense inclusions. Masses that exist of fibrillar material and that are generally located near the GC and VN, are sometimes observed at very high magnification (Fig. 10).

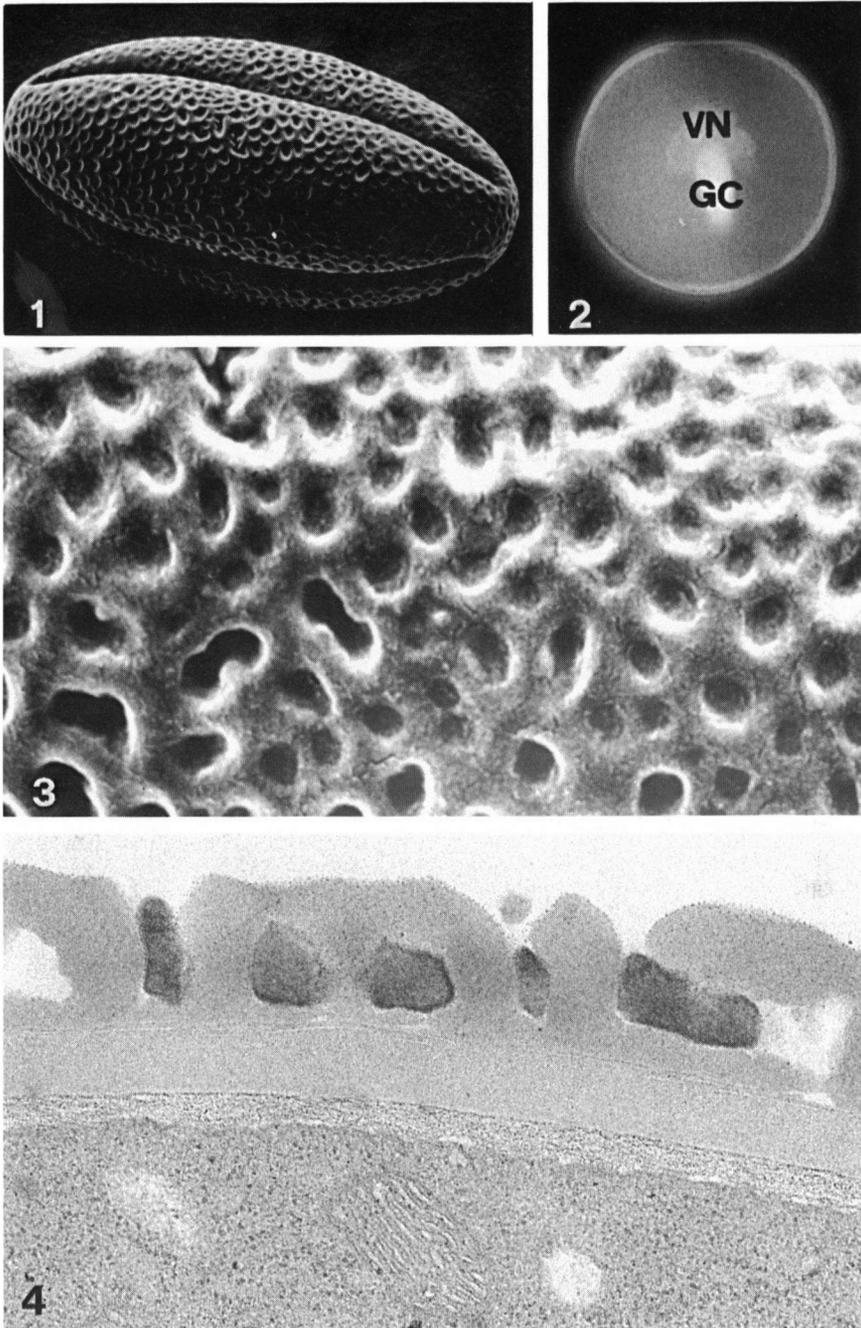


Fig. 1. *Linaria vulgaris* pollen grain (Scanning electron microscope $\times 3300$). **Fig. 2.** DAPI-staining: the generative cell (GC) and vegetative nucleus (VN) of *Linaria vulgaris* are clearly visible ($\times 1800$). **Fig. 3.** Pollen exine pattern in (Scanning electron microscopy $\times 14\ 700$). **Fig. 4.** Pollen wall ultrastructure ($\times 43\ 000$).

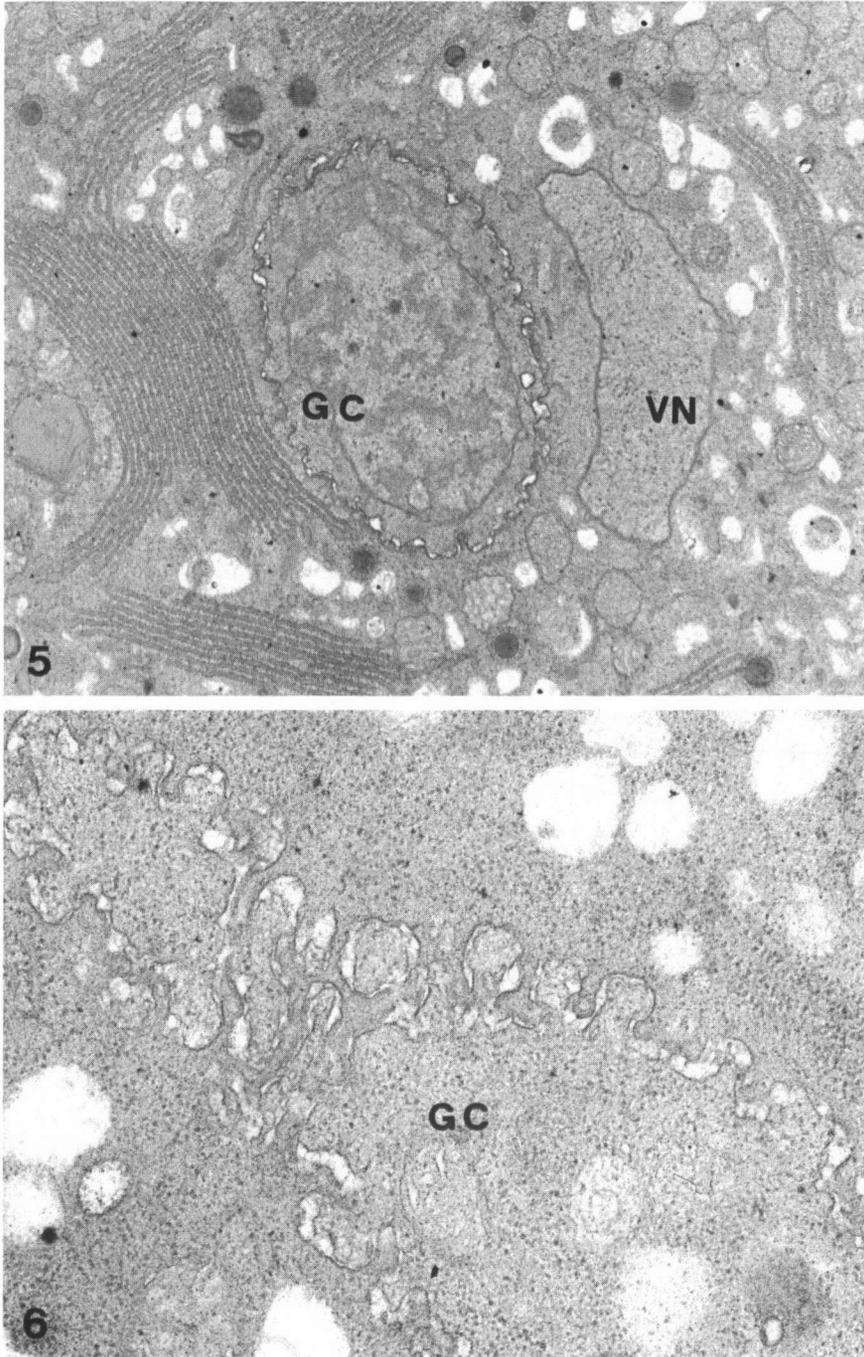


Fig. 5. Cross-section of the generative cell (GC) and vegetative nucleus (VN) in a pollen grain of *Linaria vulgaris*. Stacks of RER are present ($\times 15\,600$). **Fig. 6.** Portion of longitudinal section of the GC of *Linaria vulgaris*. The extremities form tail-like structures ($\times 43\,400$).

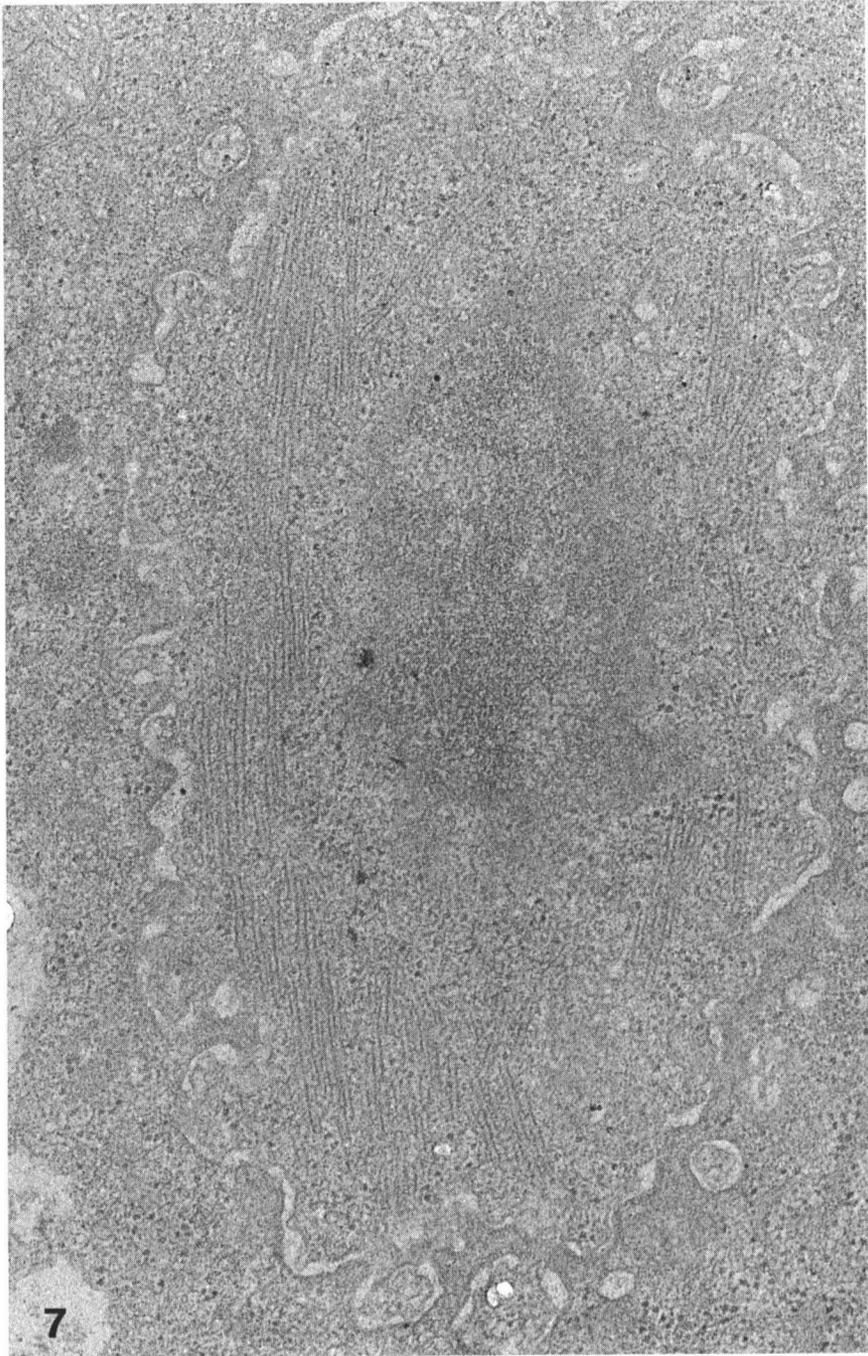


Fig. 7. Part of the GC in a pollen grain of *Linaria vulgaris* in longitudinal section containing microtubule bundles ($\times 53\ 500$).

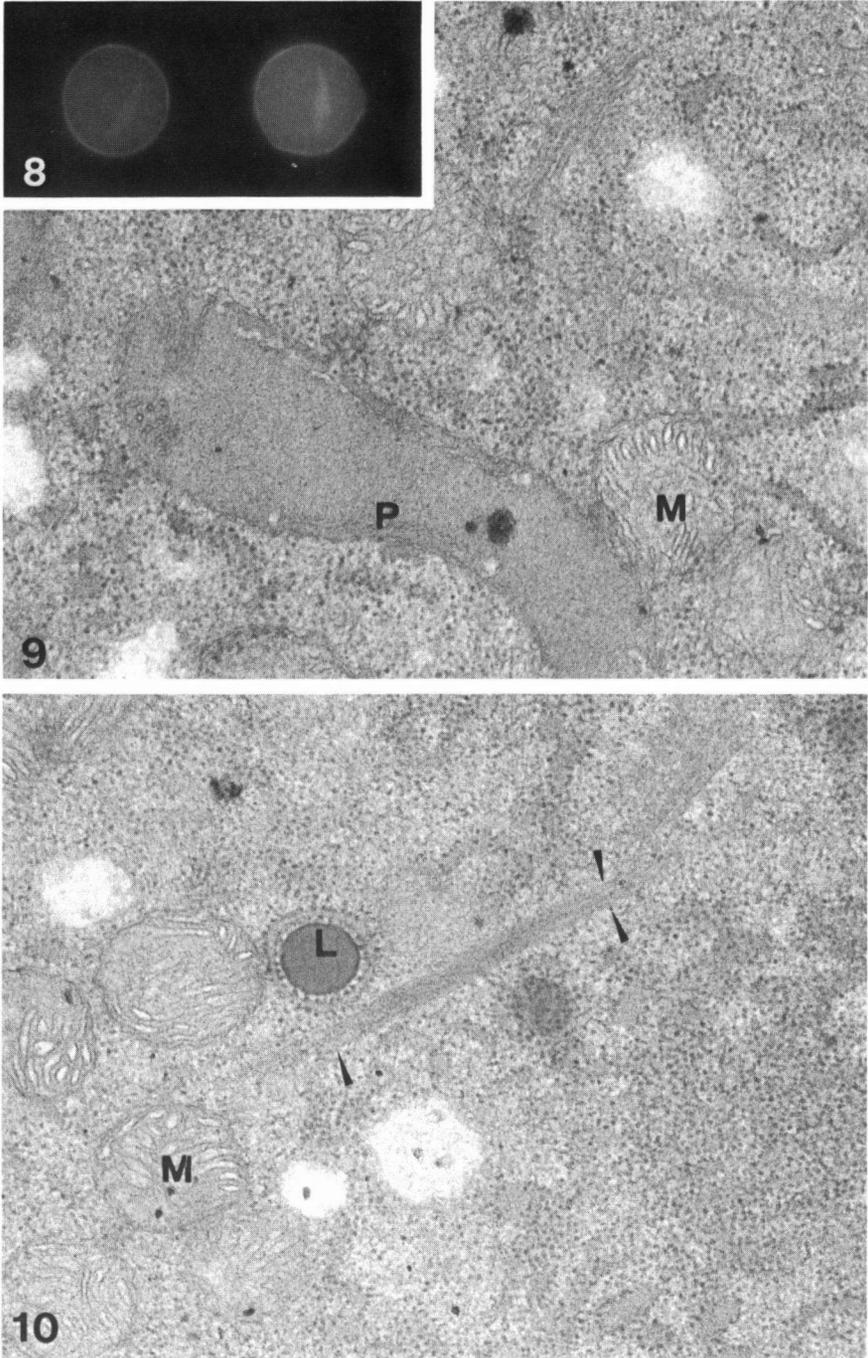


Fig. 8. Pollen of *Linaria vulgaris* treated with monoclonal antibody subunit. An intense staining is localized in the GC ($\times 820$). **Fig. 9.** Vegetative cytoplasm of *Linaria vulgaris* containing plastids (P) and mitochondria (M) ($\times 45\,300$). **Fig. 10.** Fibrillar masses (arrows) and lipid (L) surrounded by RER in the vegetative cytoplasm of *Linaria vulgaris* ($\times 43\,400$).

DISCUSSION

Linaria pollen does not show the paracrystalline bodies (probably formed by tubulin) already observed in *Linaria* styles and leaves (Ciampolini *et al.* 1980).

Linaria vulgaris pollen is binucleate and, generally, its internal morphology is comparable to that of other binucleate pollen (Sassen 1964; Kroh 1967; Fischer *et al.* 1968; Hoefert 1969; Sanger & Jackson 1971a,b; Dexheimer 1972; Echlin 1972; Jensen *et al.* 1974; Kozar 1974; Cresti *et al.* 1975, 1983, 1985).

One of the most significant aspects of *Linaria* pollen is the presence of a large quantity of stacked RER. This kind of RER configuration has been described in pollen of Solanaceae (Kroh 1967; Cresti *et al.* 1975, 1985) Scrophulariaceae (Jensen *et al.* 1974) and Euphorbiaceae (Murgia *et al.* 1986). The first reports on stacked RER are those in inactive tubers of *Solanum tuberosum* (Shih & Rappaport 1971) and in inactive buds of *Betula verrucosa* (Dereuddre 1971). These observations suggest that the metabolism of mature pollen, before pollination or activation, is in a phase of stasis; in fact the pollen becomes active only after hydration (see Heslop-Harrison 1987). It is also possible that stacked RER forms the storage site of nutrient materials, as suggested by Jensen *et al.* (1974). All these hypotheses are in agreement with the interpretation of the role of the vegetative cell as being a storage organ containing material indispensable for initial pollen tube growth (Jensen *et al.* 1974).

The ultrastructural aspect of the GC is another interesting feature of *Linaria* pollen. It is highly lobed and the wall is undulated at pollen development. The cytoplasm of the GC shows bundles of microtubules which are difficult to observe in other inactive ripe pollens. Also the immunofluorescence data confirm the large amount of tubulin. According to recent works, the microtubular bundles present in the GC might be utilized to maintain the shape of the cell but are probably also useful for GC motility during pollen tube growth (Cresti *et al.* 1984; Derksen *et al.* 1985; Tiezzi *et al.* 1986; Lancelle *et al.* 1987).

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