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The ultrastructure of proplastids in the leaves of five species of Orchids

I. Organization of the inner membrane system and divisions of the proplastids*

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Abstract

The ultrastructure of proplastids in the leaves of five species of orchids was investigated: *Paphiopedilum*, *Stanhopea*, *Cymbidium*, *Coelogyne* and *Orchis*. The proplastids of the different species differ in the density of their stroma. The most electron dense is the stroma of *Paphiopedilum* proplastids and the lowest electron-density is shown by *Orchis* proplastids.

A characteristic feature of most of the investigated proplastids is the presence of prolamellar bodies. The frequency of their occurrence and their structure differ from species to species. The remaining membrane system is limited to single peripheral vesicles.

Dividing forms of the proplastids have been observed. The number of dividing proplastids averages 5 per cent. The proplastids have been observed to divide by constriction, invagination of the inner membrane or arrangement of vesicles in the division plane.

INTRODUCTION

Proplastids in the higher plants were first observed with the aid of a light microscope by S t r u g g e r (1950, 1953, 1954). Electron microscope studies conducted by a number of research workers in 1956—1974 confirmed their existence and dealt mostly with the presence of the prolamellar body, its structure and functions.

In spite of the large number of papers on the subject the factors determining the presence or absence of the prolamellar body in proplastids

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have not yet been finally resolved. Also obscure is what conditions the structure of these bodies.

Prolamellar bodies are regarded as a typical structure of the proplastids of plants growing in the dark or insufficient light conditions (Salema, Mesquita and Abreu 1972; Bachmann et al. 1973; Freeman 1973; Wrischer 1973; Bradbeer et al. 1974; Henningsen and Boynton 1974). They have, however, also been observed in the proplastids of plants growing in the light (Salema 1971; Cran and Possingham 1973). The youngest proplastids in meristematic cells are thought not to carry any prolamellar body, irrespective of the conditions in which a plant develops. This body may first appear in the proplastids of differentiating cells (Buvat 1958; Lance 1958; Caparoli 1959; Gerola et al. 1960; Murakami 1962; Döbel 1962) but also in this case the results of observations of one and the same material in the same light conditions were found to vary and even to be contradictory. The diversity of opinions met in the literature on the subject seems to confirm the theory advanced by Wettstein in 1959 that the presence or absence of the prolamellar body in the ontogenesis of a plastid is determined by a number of factors such as the physiological state of the cell, activity of the plastid, environment and genotype and not only by light as suggested by most authors.

MATERIAL AND METHODS

The observation material embraced cells of the youngest parts of the leaves of five species of orchids: *Paphiopedilum mastersianum* Pfitz, *Stanhopea tigrina* Batem., *Cymbidium insigne* Rolfe, *Coelogyne cristata* LDL, grown in the light in a glasshouse and *Orchis latifolia* grown in natural conditions. 1 mm^2 fragments of the bases of the leaves were fixed for 4 hours in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 at 4°C followed by 1 hour postfixation with 1% OsO_4 in the same buffer at 4°C. The material was dehydrated in ethanol series of increasing concentration and then embedded in Epon 812 after the method of Luft (1961).

Ultra-thin sections from LKB ultramicrotome were doubly stained: 1 hour in saturated aqueous solution of uranyl acetate and 20 min. in saturated solution of lead citrate (Reynolds 1963). The ultra-thin sections were photographed with the aid of a Tesla BS/513A electron microscope.

OBSERVATIONS AND DISCUSSION

The plastids in the cells of the basal part of the leaves in all five investigated orchid species are in the proplastid stage. They are surrounded by a double membrane. Their diameter is 2—3 μ though the length of dividing forms may exceed 6 μ.

The proplastids demonstrate a great diversity of shapes. The most frequent are those whose section is round oval or elongated (Plate I). In *Paphiopedilum* not infrequent are proplastids with protrusions or invaginations. In the latter case an appropriate section plane shows the proplastids to embrace part of the cytoplasm. This has also been observed in other species e.g. in *Stanhopea* (Plate II, fig. 6) and *Cymbidium* (Plate II, fig. 7). In *Stanhopea* and *Cymbidium* oval and spherical proplastids are accompanied by elongated, amoebiform and crescent-shaped forms (Plate II, figs. 8, 11).

The stroma of the proplastids is fine-grained and shows a varying degree of electron transparency. The most electron dense is the stroma of *Paphiopedilum* proplastids. (Plate I, fig. 1, Plate III, figs. 12, 13, Plate V, figs. 23, 24). Of average electron density is the stroma of the proplastids of *Stanhopea* and *Cymbidium* (Plate I, figs. 2, 3, Plate II, Plate IV, figs. 17, 19, Plate V, fig. 25, Plate VI, fig. 26). Less dense seems the stroma of *Coelogyne* (Plate I, fig. 4, Plate IV, figs. 18, 20, 22, Plate VI, fig. 27) and the most electron-transparent stroma of *Orchis* proplastids (Plate I, fig. 5, Plate VI, fig. 28).

In the stroma of the mentioned orchids, particularly *Stanhopea, Cymbidium* and *Coelogyne* small, dense ribosome-like particles are observed. These particles are distributed in the stroma at random, singly or in small groups.

A considerable number of the studied proplastids are featured by the presence of a prolamellar body. Most of the observed proplastids contain a single prolamellar body which takes up a considerable part of the section surface, located centrally or at one of the poles. Less often two prolamellar bodies occur, located at the opposite poles. The frequency of the occurrence of the prolamellar body in the proplastids differs, e.g. in *Stanhopea* almost half the observed proplastids were found to carry them whereas in other species they were less frequent, in *Orchis* being discerned but a limited number of times.

The structure of the prolamellar body visibly differs from species to species. In the proplastids of *Paphiopedilum* (Plate III, figs. 12, 13) the prolamellar body is seen as a regular lattice or irregular concentration of vesicles and saccules. Both these structures are featured by an electron-dense content inside the surrounding membranes. A similar content in the tubules of the prolamellar body and the thylakoids of plastids in the roots of *Vanda* sp. and etioplasts in the leaves of *Hedera helix* was observed by S a l e m a et al. (1972). The prolamellar body shown in Fig. 13 may represent the disorganization of the prolamellar body presented in Fig. 12.

Clear-cut regularities in the structure of the prolamellar body are seen at certain stages of the development of *Stanhopea* proplastids. As in the case of *Paphiopedilum* proplastids the structure of the prolamellar body is that of a regular crystalline lattice the basic unit of which is a

six-armed tubules star. (Pl. III, Fig. 14). In Fig. 15, the prolamellar body has been cut slantwise so the "fenestra" are not clearly seen. Fig. 16 shows the prolamellar body in the stage of disorganization in a *Stanhopea* proplastid. In *Cymbidium* proplastids the prolamellar body also shows the structure of a crystalline, electron-dense lattice, clearly visible against the stroma (Pl. IV, fig. 17). The compact arrangement of the membranes of this body renders the tubular structures forming the nodes and fenestra observed in *Paphiopedilum* and *Stanhopea* difficult to distinguish. At later developmental stages the body becomes a source of thylakoids, giving off in various directions either single lamellae or groups of lamellae.

According to Gunning and Jagoe (1967) an appropriate section of the crystalline prolamellar body reveals a lattice containing electron transparent fenestra. This is stroma entrapped during the formation of this structure. The fenestra are limited by tubular structures (giving a six-armed star) so connected that six tubules meet at each node. Sections usually reveal nodes in which only four tubules meet as the upper and lower tubules are not visible. The central part of the fenestrum is occupied by a ribosome. The authors suggest that the ribosome with surrounding region is a template on which complementary membranes are formed. The role of the ribosome is still, however, obscure. The prolamellar body is subjected to a rapid disorganization giving in effect a large number of irregularly distributed tubules and vesicles from which small tubes branch off in all directions.

In the proplastids of the remaining species — *Coelogyne* and *Orchis* — the prolamellar body, as mentioned, is seldom observed. The prolamellar body of these proplastids reveals an irregular structure which is difficult to distinguish in the stroma. They are formed by aggregations of different kinds of visibles. Although the light conditions for the growth of all the plants in the studies were the same as well the conditions in which the material was fixed in the proplastids of *Coelogyne* and *Orchis* no prolamellar bodies with crystalline structure were observed. Cran and Possingham (1973) in avacodo fruits grown in the light as well as Salema (1971) in proplastids of illuminated root of the rye seedlings observed vesicular prolamellar bodies. The ability of the plastids of plants to develop a crystalline prolamellar body seems to depend on a number of factors. Not always does the development of a plant in the dark ensure the formation of a crystalline prolamellar body. Sjolund and Weier (1971) cultivated callus cells of *Streptanthus tortuosus (Cruciferae)* in the dark and found that the proplastid never formed a crystalline prolamellar body but only a complex of loosely arranged membranes. Blackwell et al. (1968) observed in the stroma of etiolated plastids prolamellar bodies in the form of irregular tubular complexes which in the presence of light transformed into structures typical of a crystalline prolamellar body.

Literature on the subject brings many descriptions of prolamellar bodies whose regular structure disintegrates in the presence of light.

A number of papers show that in total darkness etioplasts produce a prolamellar body with regular crystalline structure. This has been observed by Salema, Mesquita and Abreu (1972) in the plastids of the colourless root tips of the aerial root of *Gongora* sp., *Vanda* sp. and the etioplasts of the leaves of *Hedera helix*. The existence of a prolamellar body with crystalline structure in the plastids of plants cultivated in the dark has also been determined by other authors: Bradbeer et al. (1973, parts VIII and IX) in the etioplasts of pea seedlings, Freeman (1973) in the etioplasts of epidermis and mesophyll of 13 day leaves of *Opuntia basilaris*. Wrischer (1973a) in etioplasts isolated from the leaves of *Zea mays*, Henningsen and Boynton (1974) in the etioplasts of young barley seedlings.

The crystalline structure of prolamellar bodies has also been observed in the case of plants grown in poor light conditions (Bachmann et al. 1973) or in the plastids of etiolated plants illuminated with low intensity light (Henningsen and Boynton 1974).

After illumination with high intensity light the crystalline structure of the prolamellar bodies of plants grown in the dark or etioplasts isolated in the dark undergo changes. First numerous vesicles and scattered tubules appear after which the body disintegrates giving rise to long lamellae (Salema, Mesquita and Abreu 1972; Bradbeer et al. 1973; Freeman 1973; Henningsen and Boynton 1973; Wrischer 1973a, b). This process depends on the intensity and duration of illumination and is much slower in poor light conditions (Salema, Mesquita and Abreu 1972; Bradbeer et al. 1973). In some cases even a short, several second illumination results in disorganization of the crystalline prolamellar body (Gunning and Jagoe 1967) whereas in others even a several hour exposure to light does not result in destruction of the prolamellar body (Henningsen and Boynton 1974). Chloroplasts with a well-developed lamellar system, carrying yet prolamellar body have been observed (Laetsch and Price 1969). Changes in the prolamellar body caused by illumination have been observed to occur faster in young plants than in older ones (Bradbeer et al. 1973, Henningsen and Boynton 1974). In some plants cultivation in the light does not affect the disappearance of the prolamellar bodies but results in changes in their crystallinity (Salema 1971; Cran and Possingham 1973). The observed differences in the structure of the prolamellar bodies in the cells of the investigated orchids seem to be typical for the different species.

The prolamellar bodies are a transitional form of membrane structures in the development of a plastid and one of the sources of a future internal lamellar system. The observed images suggest that in the presence

of prolamellar bodies with crystalline structure thylakoids form by elongation of tubules of the body (Salema et al. 1972). In the case of prolamellar bodies of the vesicular type thylakoids form as a result of the merging of vesicles.

The internal membrane system in the observed proplastids of orchids is limited, besides the prolamellar body, to single, peripherally located vesicles formed by invaginations of the inner surrounding membrane (Pl. IV, figs 20, 21, arrows). At further stages of proplastid development the vesicles merge giving thylakoids arranged parallel to the long axis of the plastid (Pl. IV, fig. 21). A similar way of the differentiation of the inner membranes system has been described by Mesquita (1971), Salema (1971), Salema and Abreu (1972), Salema et al. (1972), Freeman (1973), Cran and Possingham (1973).

Worth attention is the interesting phenomenon of the uneven rate of development of proplastids in a cell. Fig. 22 (Pl. IV) shows a fragment of a *Coelogyne* cell in which two plastids are visible of which one is in the stage of a typical proplastid whereas the second, with developed inner membranes, indicated an advanced stage in the differentiation of a proplastid into a chloroplast. The asynchronous development of plastids in tissue cultures of *Populus tremuloides* Michx. grown in the dark and various light conditions have been described by Blackwell et al. (1969).

In all the investigated species of orchids proplastids whose appearance indicated a stage of division were observed. The number of dividing proplastids averaged about 5 per cent (see Table)

Species	Total number of proplastids	Number of dividing forms	Per cent of dividing proplastids
Paphiopedilum mastersianum	319	18	5.6
Stanhopea tigrina	408	22	5.4
Coelogyne cristata	243	13	5.3
Cymbidium insigne	314	21	6.7
Orchis latifolia	174	5	2.9

average: 5.2%

In natural conditions divisions of proplastids are known to be asynchronous. However, by an artificial choice of specific light conditions the divisions of the plastids can be synchronized (Mache et al. 1973).

The organelles have been observed to divide in a number of ways. In *Paphiopedilum* cells, for instance, proplastids divide by either constriction (Pl. V, fig. 23) or invagination of the inner membrane of the proplastid (Pl. V, fig. 24). The formed partition may divide the proplastid symetrically

or not. Division of spinach chloroplasts by constriction or invagination of the membrane have been described by Cran and Possingham (1972). Diers (1966) and Mühlethaler (1958) after Cran and Possingham (1972) reported the presence of both types of division in the same tissue. In *Orchis* proplastids divide by the arrangement of a number of vesicles above each other in the stroma in the division plane (Pl. VI, fig. 28). Such division forms are observed very seldom. The proplastids of the other investigated species divide, by constriction (Pl. V, fig. 25, Pl. VI, figs. 26, 27).

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Ultrastruktura proplastydów liści pięciu gatunków storczyków

I. Organizacja wewnętrznego systemu membranowego i podziały proplastydów

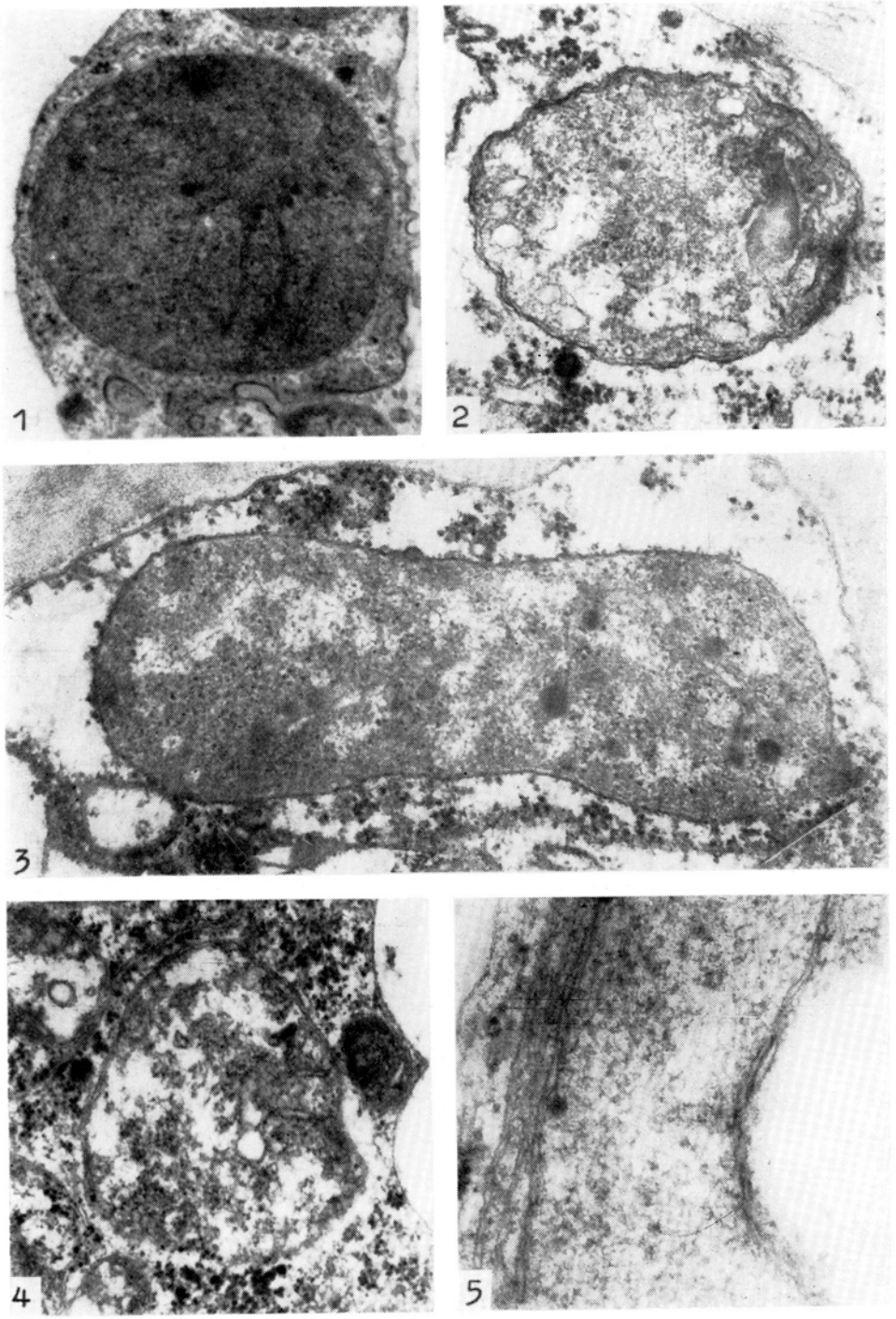
Streszczenie

Badano ultrastrukturę proplastydów liści pięciu gatunków storczyków: *Paphiopedilum mastersianum* Pfitz., *Stanhopea tigrina*, *Cymbidium insigne* Rolfe, *Coelogyne cristata* LDL i *Orchis latifolia*. Proplastydy poszczególnych gatunków różnią się gęstością stromy. Najbardziej elektronowo-gęsta jest stroma proplastydów *Paphiopedilum mastersianum* Pfitz., najmniej elektronowo-gęsta w proplastydach *Orchis latifolia*.

Pomimo tego, że obserwowane rośliny rosły na świetle, cechą charakterystyczną większości badanych proplastydów była obecność ciał prolamellarnych. Częstość występowania ciał prolamellarnych oraz ich struktura u poszczególnych gatunków różnią się. Pozost ały system membranowy ograniczony jest do pojedynczych pęcherzyków ułożonych peryferycznie.

U wszystkich badanych gatunków storczyków obserwuje się formy podziałowe ploplastydów. Ilość dzielących się proplastydów jak wynika z obliczeń wynosi ok. 5%. Obserwowane proplastydy dzielą się drogą przewężenia, przez wpuklanie wewnętrznej membrany lub przez układanie się pęcherzyków w płaszczyźnie podziałowej.

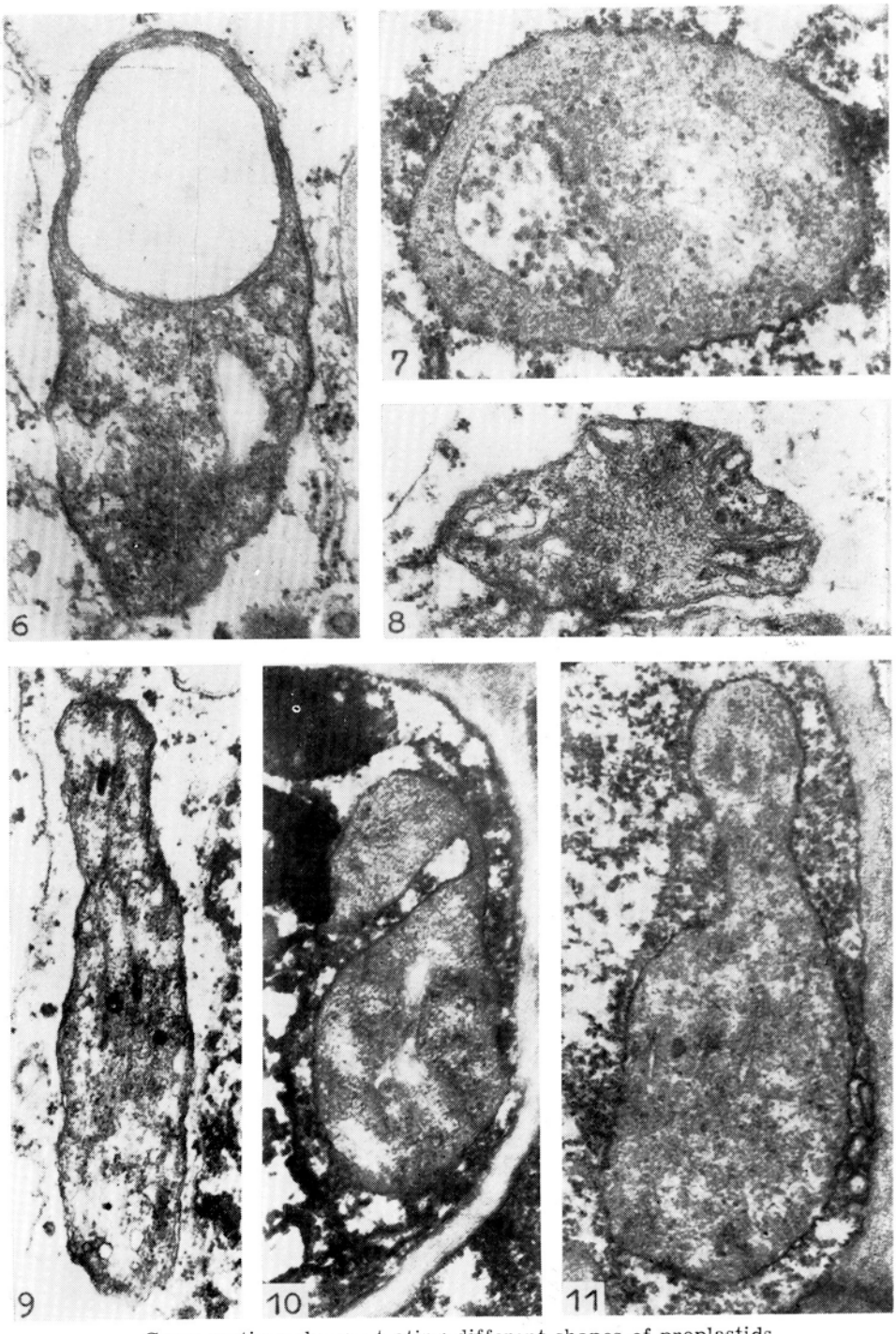
Plate I



Proplastids of the meristematic cells of leaves of orchids.

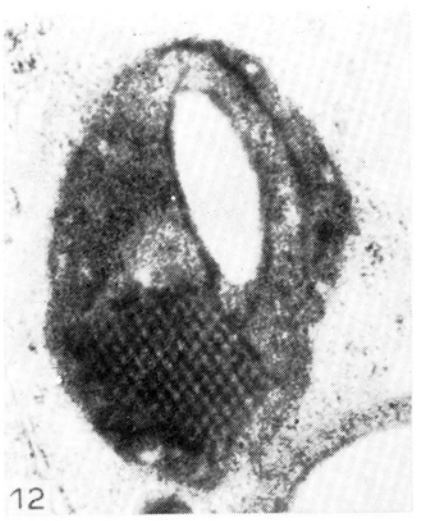
Fig. 1 — *Paphiopedilum mastersianum* (× 27.000) Fig. 2 — *Stanhopea tigrina*, (× × 45 500) Fig. 3 — *Cymbidium insigne* (× 44 000) Fig. 4 — *Coelogyne cristata* (× × 30 000) Fig. 5 — *Orchis latifolia* (× 75 000)

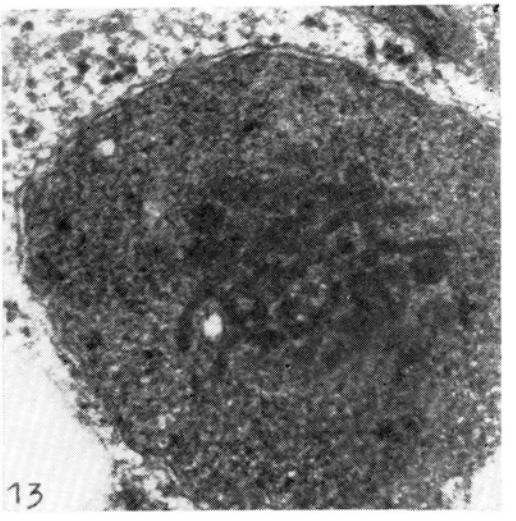
Plate II

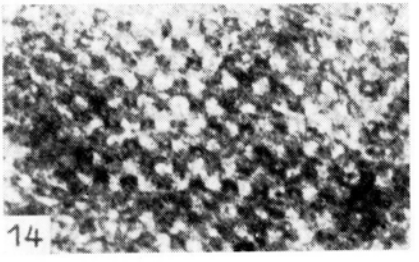


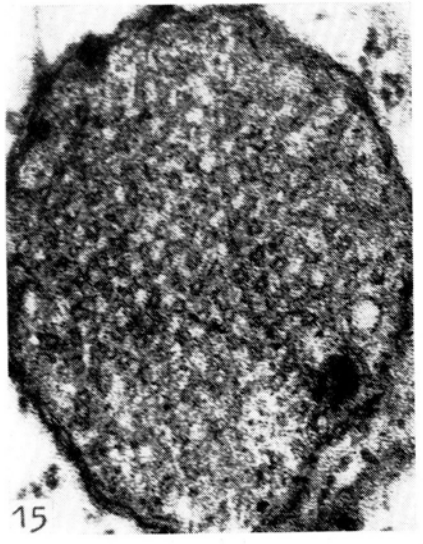
Cross-sections demonstrating different shapes of proplastids

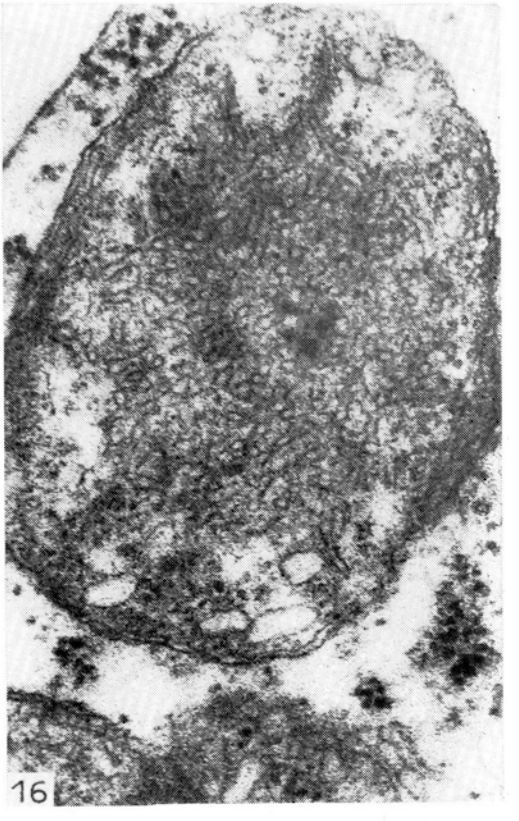
Figs. 6, 8, 9 — *Stanhopea tigrina* (6: × 27 000. 8: × 32 500. 9: × 45 000) Figs. 7, 10, 11 — *Cymbidium insigne* (7: ×70 000. 10: × 26 000. 11: × 43 000)



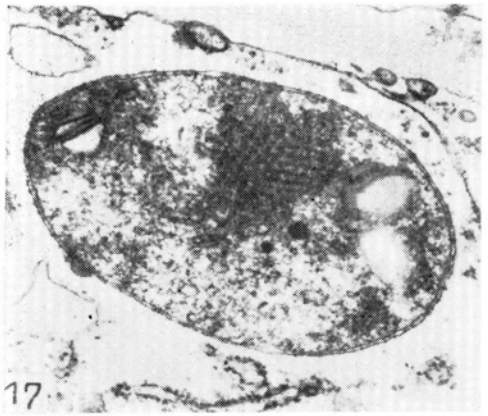


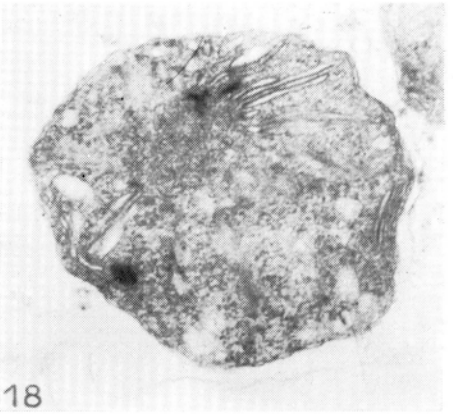


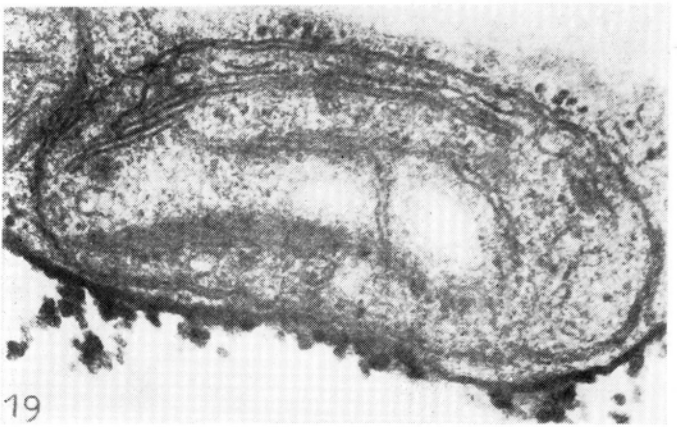


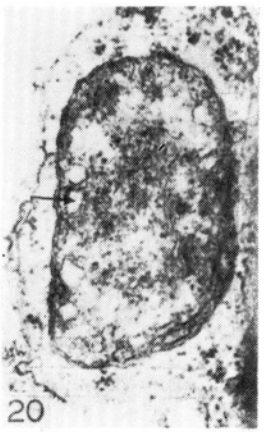


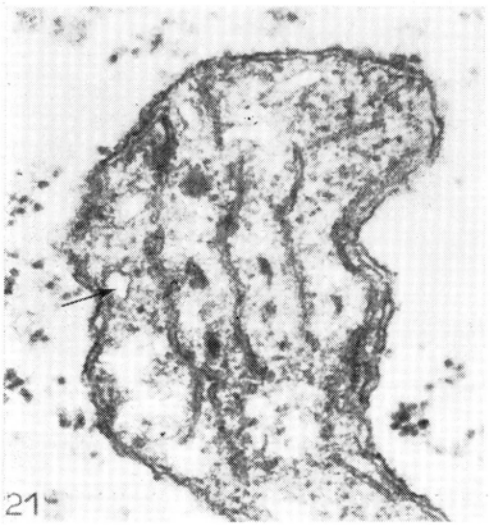
Figs. 12, 13. Prolamellar bodies in *Paphiopedilum mastersianum* proplastids. Tubules forming the prolamellar body filled with an electron-dense substance. 12 — crystalline prolamellar body (× 34 000) 13 — vesicular prolamellar body (× 59 000). Figs. 14—16. Prolamellar bodies in *Stanhopea tigrina* proplastids. 14, 15 — crystalline prolamellar bodies (14: × 93 000, 15: × 80 000), 16 — vesicular prolamellar body (× 70 000)











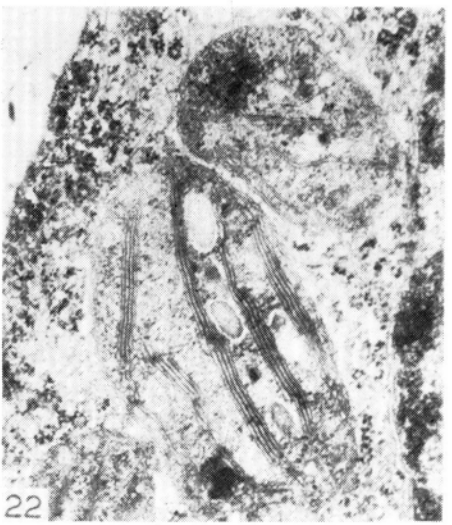
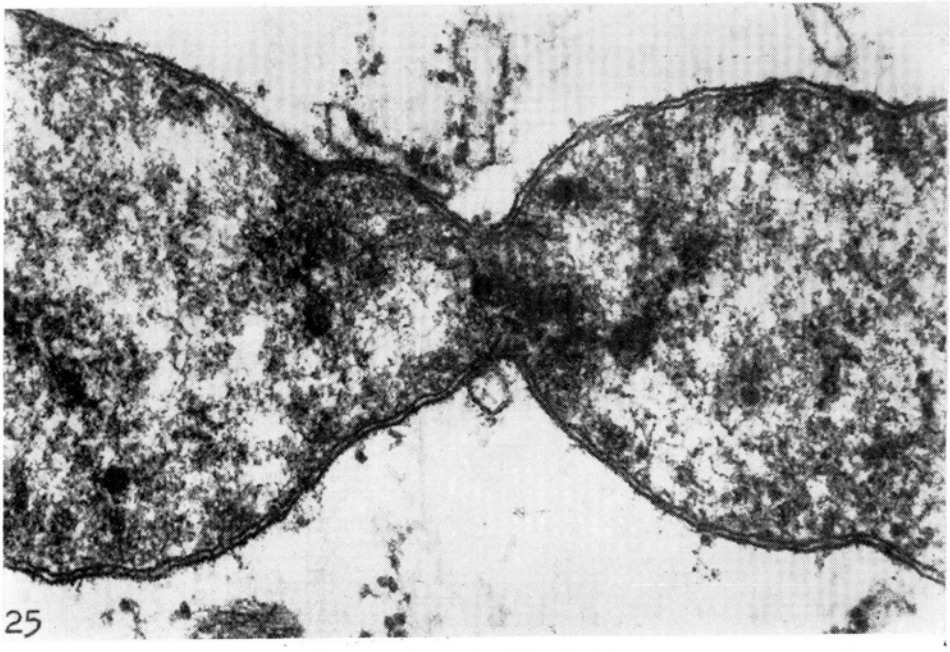
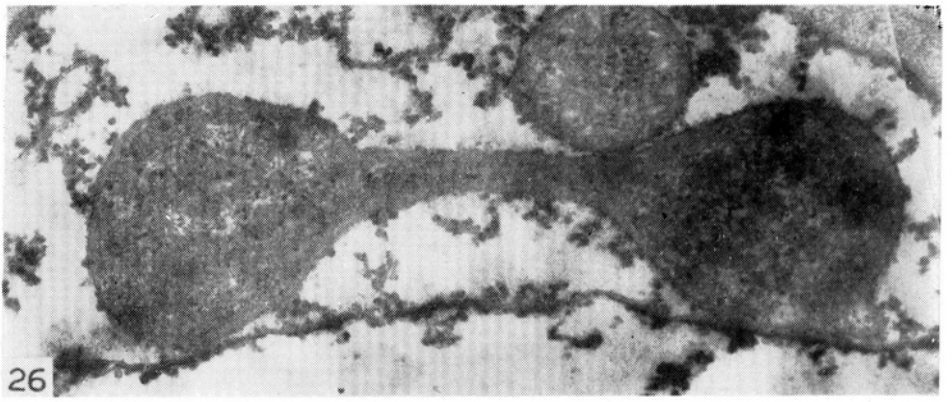


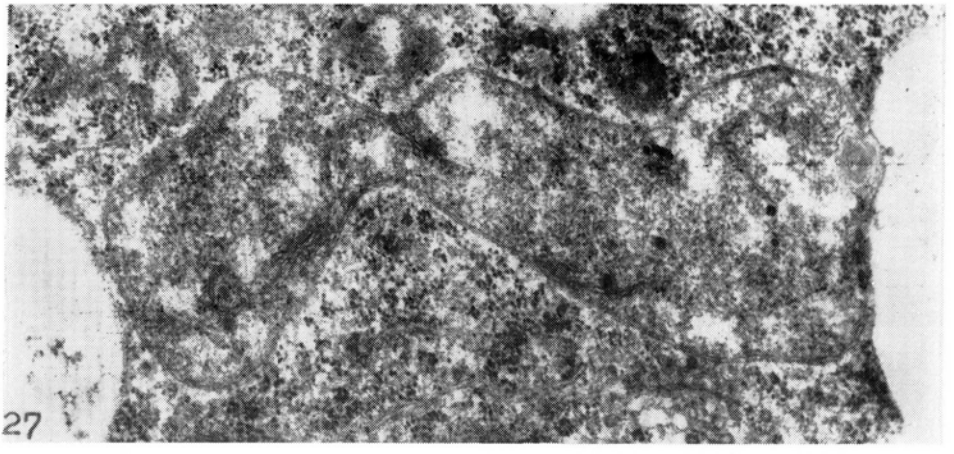
Fig. 17. *Cymbidium insigne* proplastid with crystalline prolamellar body (× 27 000). Fig. 18. *Coelogyne cristata* proplastid with vesicular prolamellar body (× 27 000). Figs. 19—21. Initial stages of the formation of a membrane system in proplastids. Vesicles formed by invaginations of the proplastid inner membrane can be seen (arrows). 19, 21. *Stanhopea tigrina* (19: × 87 500, 21: × 74 000), 20. *Coelogyne cristata* (× 26 000), Fig. 22. *Coelogyne cristata* proplastids in various stages of development (× 25 000)

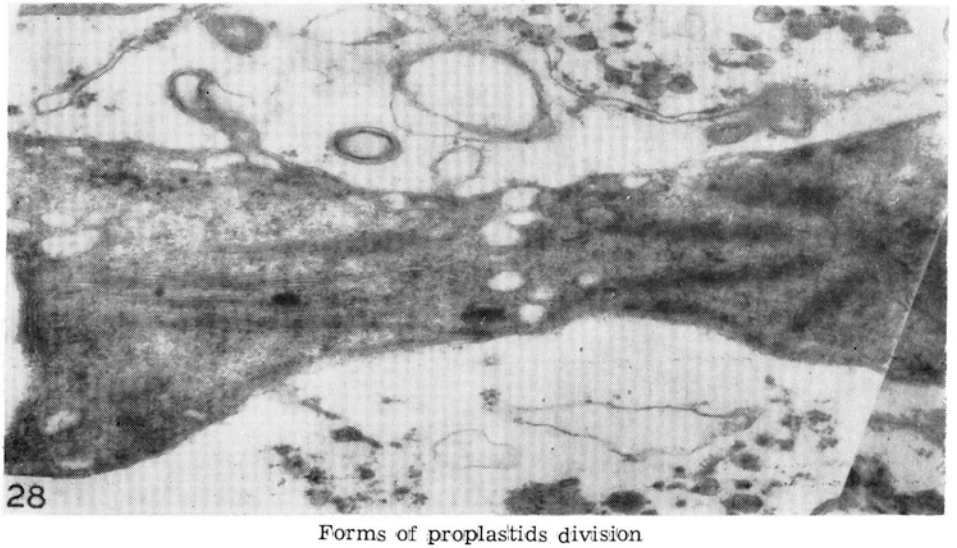


Forms of proplastids division

Figs. 23, 24. *Paphiopedilum mastersianum* proplastids. 23 — division by constriction, 24 — division by invagination of inner membrane of proplastid, (× 26 000) Fig. 25, *Stanhopea tigrina* proplastid, division by constriction (× 72 000).







Forms of proplastids division

Fig. 26. *Cymbidium insigne* proplastid — division by constriction (× 41 000) Fig. 27. *Coelogyne cristata* proplastid — division by asymmetric constriction (× 27 300). Fig. 28. *Orchis latifolia* proplastid showing arrangement of vesicles in division plane.