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Genesis of grana and stroma thylakoids in leaf chloroplasts of four orchid species

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Abstract

In the chloroplasts of orchid leaves (*Paphiopedilum mastersianum* Pfitz., *Stanhopea tigrina* Batem., *Coelogyne cristata LDL* and *Cymbidium insigne* Rolfe) grana stacks differentiate on the base of primary thylakoids. This process occurs by stratification due to overlapping of thylakoids, by their bending and by invagination of the membrane into the thylakoid. There also may form two membranes ending blindly at both ends, called "central contact zone" ("Kontaktzone") in the interior of the mother thylakoid. Thylakoid multiplication in the grana stacks takes place by the same processes, and also by the "overgrowth" of thylakoids over the stroma localized between the closely overlaid grana. The increase in the number of stroma thylakoids usually occurs by fusion of the flattend vesicles lying in rows in the stroma or by elongation of the grana thylakoids.

INTRODUCTION

One of the most important stages in the development of chloroplasts is the arrangement of primary thylakoids into grana stacks (Izawa, Good, 1966; Paolillo, 1970; Wellburn, Wellburn, 1971; Argyroudi-Akoyunoglou et al., 1976). Further grana formation requires the synthesis of new photosynthetic membranes.

The final stage of chloroplast differentation is the increase in the number of both the grana and stroma thylakoids. Various modes of thylakoid multiplication were described in the literature. As the basic way of this process, Menke (1960, 1961) considers the division of thylakoids by invagination of the membrane in parallel to the surface. This opinion was shared also by Döbel (1962), Wilsenach (1963) and Schötz (1965). Wehrmeyer (1963, 1964a, b) has shown that formation of thylakoid stacks in spinach chloroplasts occurs in the process of stratification due to superimposition of thylakoids ("Stapelbildung durch Überschiebungsprocesse"). Weier et al. (1965) report that the increase in the number of thylakoids within the same grana zone occurs by longitudinal splitting of the thylakoids along their whole length. The invagination of the thylakoid membrane or bending of the thylakoids may be single or multiple. These ways of thylakoid miltiplication may occur, according to Schötz (1965), in the same granum simultaneously on several immediately neighbouring thylakoids. Accorling to Wehrmeyer (1964a), the increase in the height of the new grana stack may occur by adhesion of stroma thylakoids to the upper or lower surface of the grana. Argyroudi-Akoyunoglou et al. (1976) suggest that the growth of the grana occurs by the incorporation of the newly synthesised membrane material into preexisting grana thylakoids, which in effect spread and then become folded.

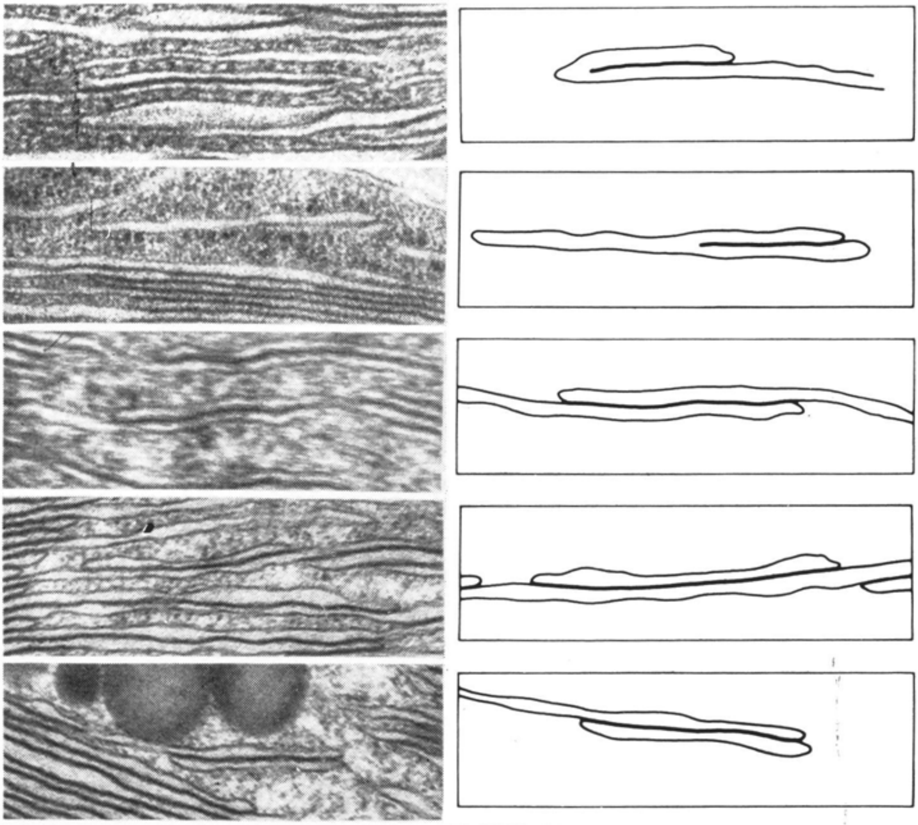
The investigations presented in this paper deal with the genesis of grana and stroma thylakoids in leaf chloroplasts of four orchid species.

MATERIAL AND METHODS

The studies were performed on the leaf plastids of four orchid species: *Paphiopedilum mastersianum* Pfitz., *Stanhopea tigrina* Batem., *Coelogyne cristata* LDL and *Cymbidium insigne* Rolfe. The preparation of material for electron-microscopic examination has been described in the preceding paper (Damsz, 1979).

RESULTS

Cross sections of chloroplasts from leaves of *Paphiopedilum, Stanhopea, Cymbidium* and *Coelogyne* suggest that the first grana arise on the base of stroma thylakoids. Grana formation occurs by bending of thylakoids (Photo 1a), by invagination of the membrane into the mother thylakoid (Photo 1b) or by elongation and arrangement of thylakoids immediately above one another (Photo 1c, d, e). The grana thylakoids formed in this way adhere to each other and are not separated by stroma. Further thylakoids are built onto the preceding ones, thus forming not very high grana. The above patterns of grana formation occured both in early and later stages of development of the examined chloroplasts. Very infrequently a picture was seen of differentiating membranes within the closed thylakoid, which separate it into two newly organized thylakoids (Photo 2). The internal membranes formed in this way do not reach the ends of the mother thylakoid.



Ia

Ib

Ic

Id

Ie

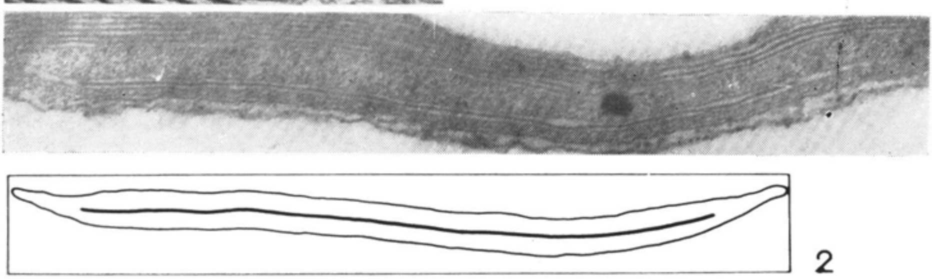


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Photo 1 a-e. First stage of granum differentiation in chloroplasts. × 80 500
Photo 2. Division of thylakoid by the formation of two inner membranes. × 25 000

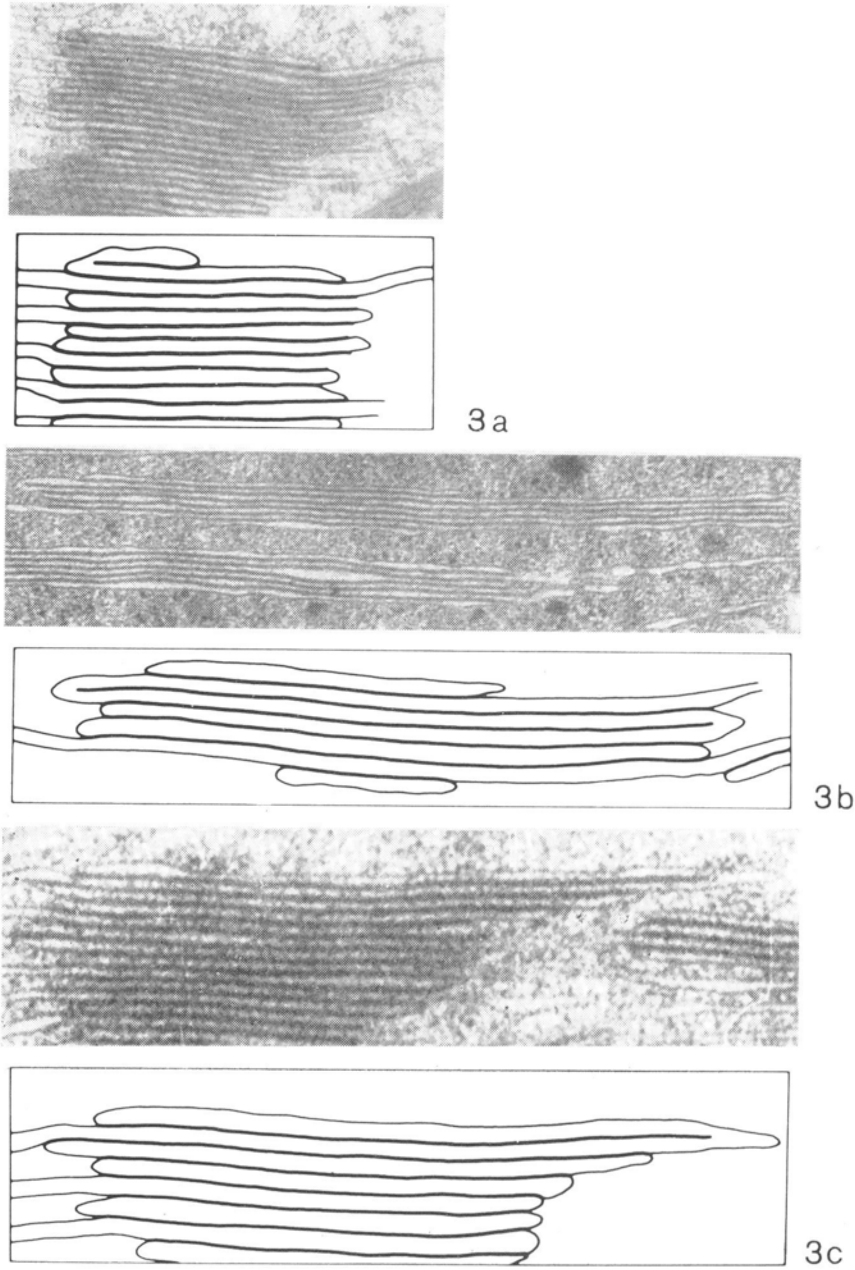
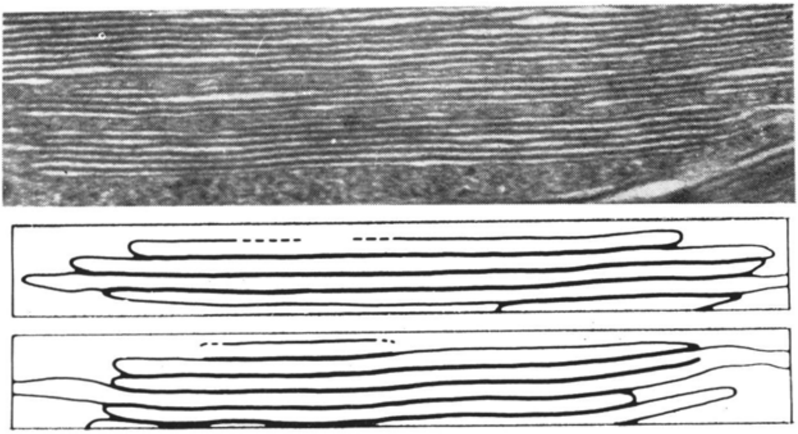
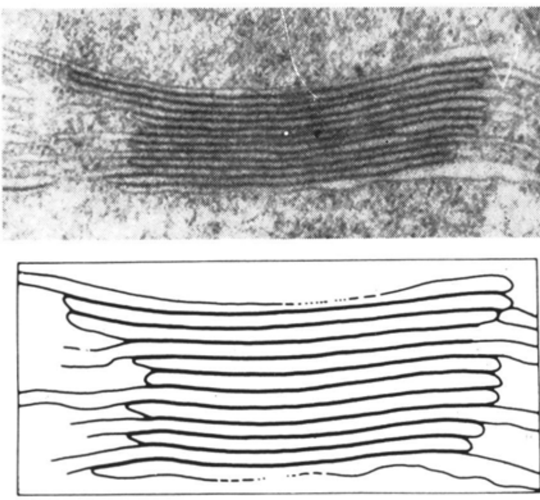


Photo 3 a-c. Possible processes involved in multiplication of chloroplast grana thylakoids. × 80 500

PLATE III



4a



4b

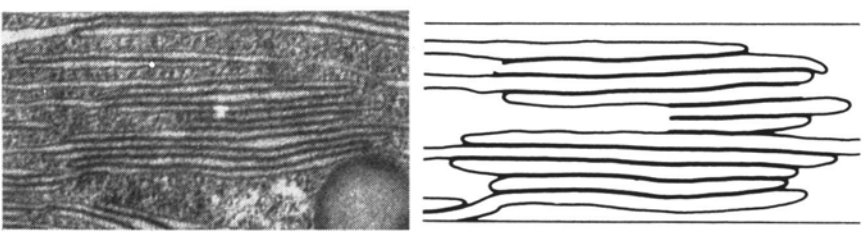


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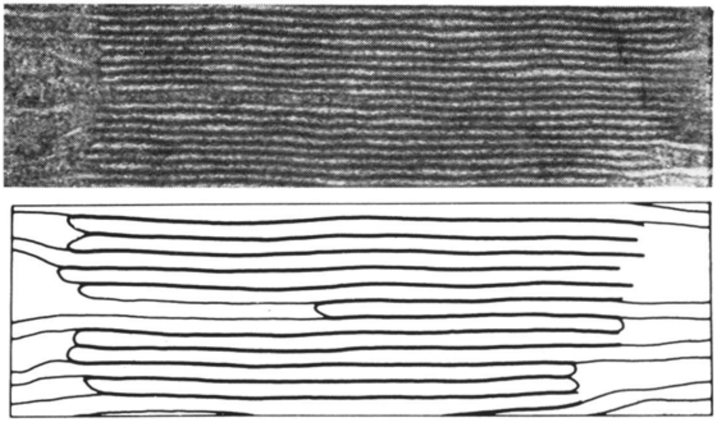
Photo 4 a, b. Formation of new thylakoid membranes onto the upper or lower surface of grana. × 80 500

Photo 5. Overlapping of stroma thylakoids on external grana thylakoids. × 80 500

PLATE IV



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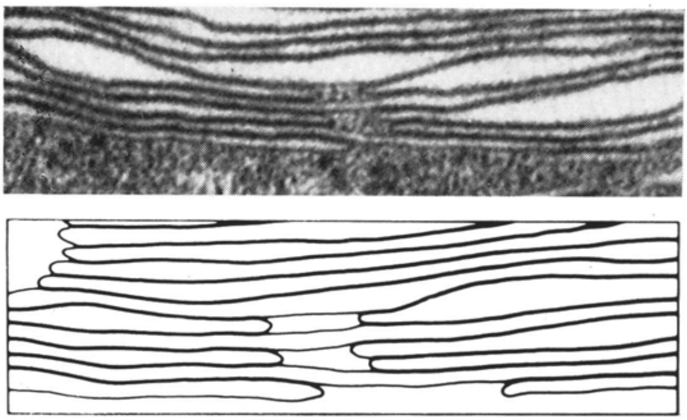

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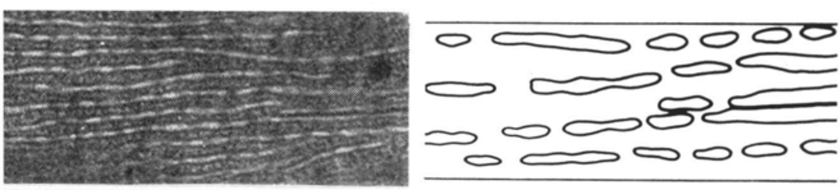
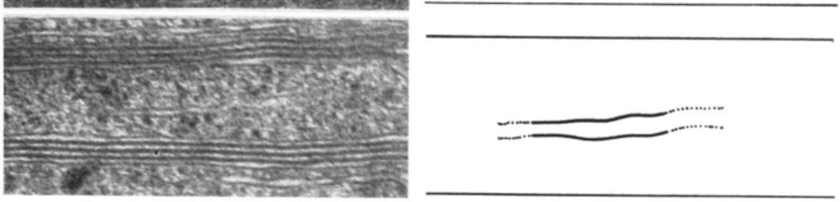


8a



8b

Photo 6. Formation of one high granum by intrusion of stroma thylakoid between two closely lying grana above one other. × 80 500.

Photo 7. Increase of granum height by overgrowth of thylakoids. × 80 500.

Photo 8 a, b. Formation of stroma thylakoids (a) by merging of flattend vesicles, (b) by joining of granules in rows. × 80 500.

PLATE V

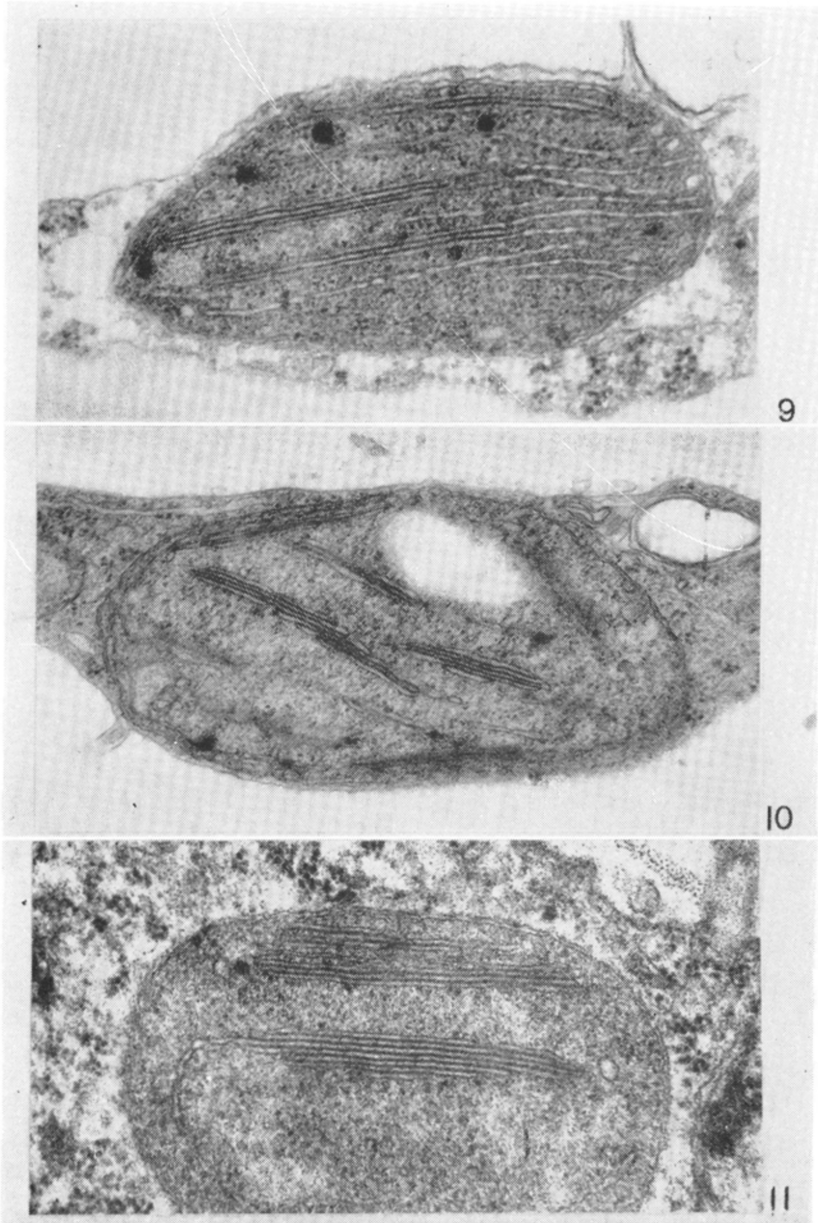
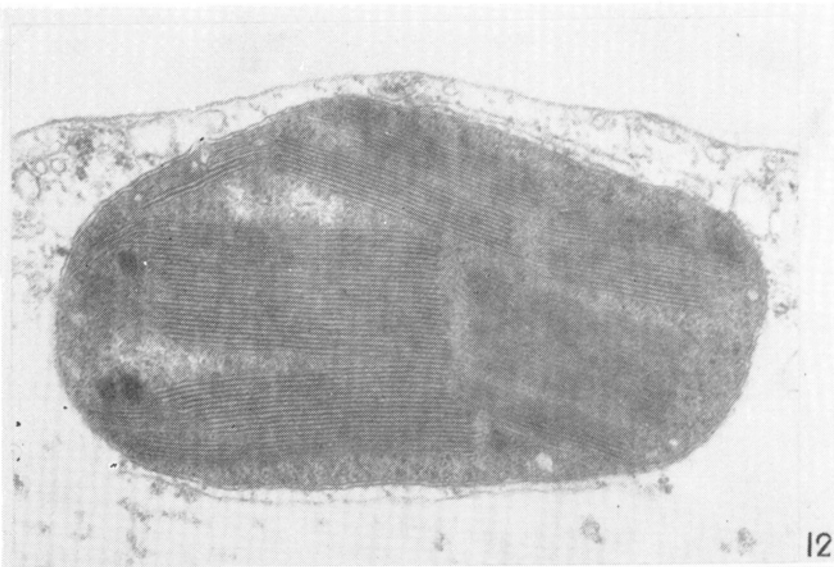


Photo 9-11. Arrangement of grana in differentiating chloroplasts of orchid leaves; 9 — *Cymbidium*, × 30 000, 10 — *Stanhopea*, × 30 000, 11 — *Coelogyne*, × 30 000



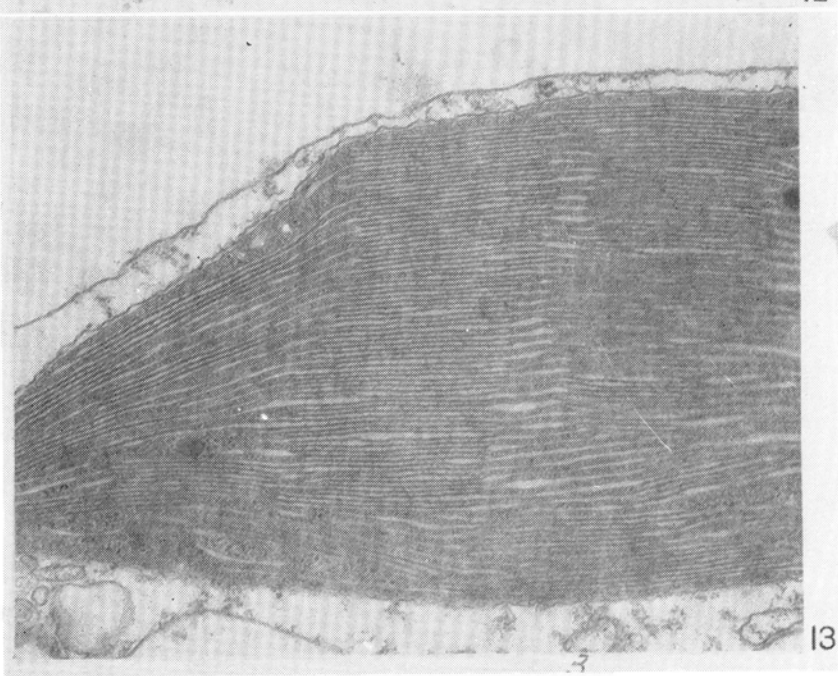


Photo 12. Young chloroplast of *Cymbidium* leaf. × 30 000
Photo 13. Mature chloropast of *Coelogyne* leaf. × 30 000

The increase in the number of grana thylakoids occurs in many different ways. Most frequently the new grana thylakoids arise by bending of grana thylakoids lying externally in the stack. Thylakoids may bend once (Photo 3a) or twice (Photo 3b). The number of thylakoids may also increase by invagination of the mother thylakoid membrane (Photo 3c). This invagination of the membrane begins at one end and progresses to the other. The two thylakoids formed in this way adhere to each other on their whole length. The protrusion of membranes, however, do not always separate the two newly organized thylakoids along all their lengths. The presence of minute grains arranged in rows over the external surface of the grana stacks suggest that the formation of new grana thylakoid membranes may occur by their fusion (Photo 4a, b). Large contribution to the increase of the granum height, particularly in order chloroplasts is made by stroma thylakoids. These thylakoids may be superimposed on the external thylakoids of grana (Photo 5). They may also intrude into the spaces between the grana, which lie close above one another (Photo 6) thus, adjoining them into one hign granum. The process of "overgrowth" of grana by thylakoids has also been sporadically observed (Photo 7). It is manifested by bilateral penetration of thylakoids between the loosely arranged stroma thylakoids close to the grana stacks. The number of stroma thylakoids in the successive stages of chloroplast development gradually increases, but more slowly however that of grana thylakoids. The increase in the number of stroma thylakoids occurs usually by fusion of the flattened vesicles lying in rows in the stroma (Photo 8a). The stroma thylakoids formed in this way are slightly folded. Their number may also increase by elongation of the grana thylakoids. The thylakoids joining neighbouring grana arise in this way. Formation of stroma thylakoid membranes by fusion of rows of grains has been found only very rarely (Photo 8b).

In all developmental stages of chloroplasts the grana are clearly visible and distinctly outlined against the background of the stroma. The arrangement of the differentiating grana in early developmental stages of the chloroplasts is usually regular, parallel to the axis of the plastid cross section (Photo 9). However, a tendency is observed, particularly in *Stanhopea* (Photo 10) and *Paphiopedilum*, to an irregular arrangement of the grana (for instance skewed in various directions relative to the plastid long axis). In the chloroplasts of *Stanhopea* which sometimes are nearly triangular in cross sections the grana are arranged in parallel to the sides. In young chloroplasts of the orchids a regular arrangement parallel to the long axis of grana and stroma thylakoids is observed most frequently, whereas in mature chloroplasts numerous and fully developed grana are arranged regularly only in *Cymbidium* and *Coelogyne*. In *Paphiopedilum* as well as in a great part of *Stanhopea* mature chloroplasts the grana are arranged irregularly in various directions.

The length of grana thylakoids in the chloroplasts of the studied orchids varies, both within one granum and within the grana in the given plastid. Owing to this, the diameter of cross sectioned chloroplast grana varies in length. During development of the chloroplasts the grana diameter changes. In mature ones a tendency is noted to a diminution of the granum diameter as compared to that in earlier stages. In the latter long thylakoids arranged in stacks are seen (Photo 11). Division of such stacks has never been observed. On the cross section of plastid there are as few as 2-3 stacks. In mature chloroplasts the grana are numerous and their diameter is much smaller. One may suppose that during plastid growth the arrangement of long thylakoids packed in stacks becomes looser. On some segments these thylakoids may bend or split and in this way they would form new grana with diameter typical for mature chloroplasts.

With the development and growth of chloroplasts the area occupied by thylakoids increases, whereas the stroma is markedly reduced to about 25 per cent of the cross section area (Photo 12). In mature chloroplasts the system of thylakoids occupies almost the entire area of the plastid cross section (Photo 13) while the stroma may occupy only as little as 10 per cent (Damsz, 1978).

DISCUSSION

The arrangement of thylakoids in grana stacks is the most characteristic and stable feature of a typical thylakoid system in the chloroplasts of higher plants. As seen from the electron micrographs of chloroplast cross sections from the examined species of orchids grana in early development stages are formed by the aggregation of primary thylakoids in stacks. The micrographs of plastid cross sections indicate various ways in which the thylakoids become superimposed so that grana with several disks arise. Similar observations were made by Argyroudi-Akoyunoglou et al. (1976) in the plastids of young pea leaves. The arrangement of thylakoids in stacks in orchid plastids occurs by invagination and superimposition of thylakoids. The investigations of Menke (1961) and Manton (1962) on the differentiation of chloroplasts of *Anthoceros* as well as the results obtained by Wehrmeyer and Röbbelen (1965) allowed these authors to distinguish two types of thylakoid arrangement in grana. Type I, called "disjunctive" is manifested by spatial local separation of interconnected thylakoid areas, owing to the formation of a gap in the membrane. This type was noted in the chloroplast of spinach leaves (Wehrmeyer, 1964a, b). Type II called "conjunctive" resulting from the transformation of a single thylakoid into a double one by forming a y-shaped branching, with the inner edge of the contact border formed enclosed within the branching thylakoid. In *Anthoceros* mutants both these types occur in various intercombinations. The results presented in this paper seem to indicate that both these types occur in the studied orchids as well. The schemes of grana formation in Photo 1a, d, e drawn on the basis of thylakoid cross sections from the chloroplasts of the four orchids correspond to the three-dimensional "conjunctive" models, while the scheme in Photo 1c corresponds to the "disjunctive" model.

Grana formation, increase in number of thylakoids within the grana as well as stroma thylakoid formation result, according to the author's observations, from the operation of several mechanisms. These may be: invagination of the membranes in the thylakoids, superimposition of thylakoids, their bending (one or several times within one granum) or formation inside the thylakoids of a "central contact zone" ending blindly at both ends. The latter mode is observed least frequently. Electron micrographs presented in this paper are in agreement with the data in the literature (Menke, 1961; Manton, 1962; Wehrmeyer, Röbbelen, 1965). The membranes of the new grana thylakoids lying inside the stack and the stroma thylakoids may arise by fusion of the stroma grains arranged in rows. A similar way of stroma thylakoid membrane formation from granular substance in the chloroplast stroma of *Clivia miniata* has been suggested by Mikulska (1964). Biochemical studies indicate (Argyroudi-Akoyunoglou, Akoyunoglou, 1970, 1973; Kirk, 1971; Bishop, Senger, 1972; Beck, Levine, 1974), that thylakoids may be formed in the process of gradual accumulation of membrane components. Initially, the thylakoid membranes include only a minimal number of components, later on other elements are acquired when the leaves become green. The stroma thylakoids greatly contribute to the increase of the grana stacks which may be superimposed on the external thylakoids in the grana or intrude into the spaces between two grana lying close one above the other. New stroma thylakoids in the orchid leaf plastids are also formed in the process of fusion of the flattend vesicles arranged in rows in the stroma as described earlier for the chloroplasts of *Clivia miniata* and *Billbergia* sp. leaves by Mikulska (1964).

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Geneza tylakoidów granowych i tylakoidów stromy w chloroplastach liści czterech gatunków storczyków

Streszczenie

W chloroplastach liści storczyków (*Paphiopedilum mastersianum* Pfitz., *Stanhopea tigrina* Batem., *Coelogyne cristata* LDL i *Cymbidium insigne* Rolfe) stosy granowe wyróżnicowują się na bazie tylakoidów pierwotnych. Proces ten zachodzi przez nawarstwianie drogą nasuwania się tylakoidów na siebie, przez zaginanie tylakoidów a także przez wpuklanie membrany do wnętrza tylakoidu. Mogą też we wnętrzu tylakoidu macierzystego powstawać dwie membrany obustronnie ślepo zakończone zwane „centralną strefą kontaktową". Pomnażanie liczby tylokoidów w stosach granowych zachodzi w ten sam sposób, a także przez tak zwane „zarastanie" tylakoidami stromy znajdującej się między leżącymi blisko nad sobą granami. Wzrost liczby tylakoidów stromy odbywa się najczęściej przez zlewanie się spłaszczonych pęcherzyków leżących w stromie szeregowo lub drogą wydłużania się tylakoidów granowych.