

# CHLOROPLAST DIVISION IN THE GAMETOPHYTE OF THE FERN *MATTEUCCIA STRUTHIOPTERIS* (L.) TODARO

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The mechanism by which fully developed chloroplasts divide has been reported to consist of an initial constriction around the entire chloroplast, which is followed by a pinching into two daughter chloroplasts. Constriction is accompanied by fusion of the inner and outer layers of the chloroplast membrane. Such a division has been documented by electron microscopy for the brown alga, *Fucus* (von Wettstein, 1), and the red alga, *Lomentaria* (Bouck, 2). In *Fucus* the axis of division is usually parallel to the longitudinal axis of the chloroplast lamellae. Light microscopic observations of mature chloroplasts dividing by constriction have been made in land plants ranging from bryophytes through the angiosperms (3, 4). Division of proplastids in higher plants (5) is similar to the condition reported for algal chloroplasts, in that pinching also is accompanied by fusion of all the layers of the chloroplast membrane. A second method of mature chloroplast division is described in this paper. The mature chloroplast is divided by the centripetal invagination of the inner-limiting membrane. This method of chloroplast division is here termed centralization.

## MATERIALS AND METHODS

Sterilized dormant spores of *Matteuccia struthiopteris*, which do not germinate without light, were sown in Petri dishes on 1 per cent mineral agar consisting of a modified Knop's solution (0.8 gm  $\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ , 0.2 gm  $\text{KNO}_3$ , 0.2 gm  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.2 gm  $\text{KH}_2\text{PO}_4$  in 1 liter distilled water). After sowing, a 48-hour period in the dark at about 25°C was allowed in order to synchronize germination as much as possible. This was followed by a 36-hour light period (150 ft-candles) at the same temperature. The germinated spores all in the two-celled stage (one primary prothallial and one rhizoid cell) were concentrated in one area and sandwiched between the agar on which they were grown and a thin pith-disk pre-soaked in melted agar. The spores thus prepared could be easily handled for fixation in 2 per cent aqueous  $\text{KMnO}_4$  for 1 hour at 4°C, followed by 10 per cent buffered formalin (pH 7.4) containing 2 per cent  $\text{CdCl}_2$  for 30 minutes. Dehydration through a graded alcohol series was followed by Epon 812 embedding. Sections were cut on an LKB ultratome and examined with a Hitachi HS-6 electron microscope.

## OBSERVATIONS AND DISCUSSION

The gametophyte of *Matteuccia struthiopteris* originates from a single haploid spore. After 36 hours

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FIGURE 1 Two daughter chloroplasts sharing the same outer-limiting membrane. Note that the outer-limiting membrane is single where it extends between the daughter chloroplasts (arrows). At this junction one can also see that the inner-limiting membrane is single. The chloroplast lamellae are not in the plane of division. The white areas are starch grains.  $\times 20,000$ .

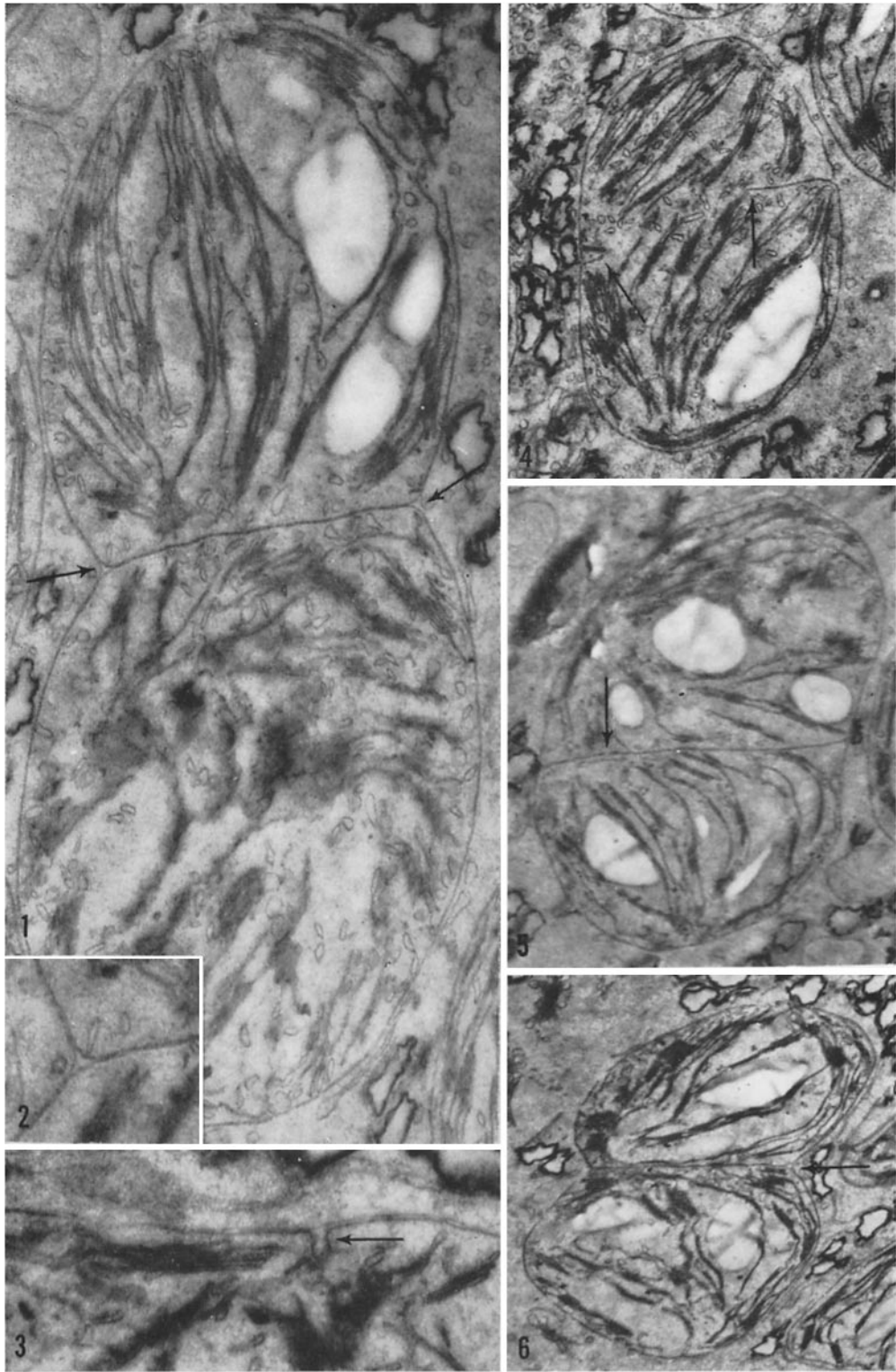
FIGURE 2 This is an enlarged area of Fig. 1 showing the junction between the daughter chloroplasts.  $\times 35,000$ .

FIGURE 3 Shown is the invaginating inner-limiting membrane.  $\times 49,000$ .

FIGURE 4 A mature chloroplast showing centralization. Invaginations (indicated by arrows) occur from both sides. The centralization does not appear to cause lamellar pinching.  $\times 15,500$ .

FIGURE 5 Incomplete division. Note discontinuity of invaginating membrane at arrow.  $\times 12,500$ .

FIGURE 6 Daughter chloroplasts sharing the same outer-limiting membrane (arrow). Chloroplast lamellae are parallel to the plane of division.  $\times 15,000$ .



of light the young gametophyte consists of an elongating rhizoid cell containing very few chloroplasts, and a primary prothallial cell which contains a minimum of one hundred mature chloroplasts. Division by centralization is believed to be the predominant, if not the only, method of chloroplast division at this stage of the developing gametophyte. This contention is supported by the fact that out of 100 centrally sectioned chloroplasts 11 dividing chloroplasts showed partial or completed centralization of the inner-limiting membrane. No chloroplasts undergoing constriction were found; nor were any recognizable proplastids present in these cells.

At this stage of development division of chloroplasts (as opposed to development from proplastids) appears to be responsible for maintaining and increasing the chloroplast number. In *Matteuccia struthiopteris* chloroplast division occurs by centralization of the inner-limiting membrane (Figs. 1 to 6). This membrane invaginates more or less equally around the periphery of the chloroplast roughly perpendicular to the long axis of the chloroplast (Figs. 4, 5). The invaginating inner-membrane, regardless of whether it is parallel, diagonal, or perpendicular to the lamellae does not pinch them. Figs. 1 and 6 show results of centralization; each daughter chloroplast has an individual inner-membrane but both are enclosed by a common outer-membrane. Fig. 2 is an enlarged view of the junction between the daughter chloroplasts shown in Fig. 1. The manner in which the daughter chloroplasts separate is not known. Perhaps it occurs simply by the rupture of the outer-limiting membrane during, or after, invagination.

Centralization division differs from constriction division (reported in *Fucus* (1) and *Lomentaria* (2)) by the invagination of the inner-limiting membrane, the non-fusion of the outer-limiting membrane, and the absence of lamellar pinching.

It has been generally accepted that the inner-limiting membrane of proplastids gives rise to lamellae of developing chloroplasts by internal proliferation (6). Figs. 3 and 4 show that this ability to proliferate is not restricted to lamellae formation in immature chloroplasts, but is also operative in division of mature chloroplasts.

According to Kaja (7), proplastids, which develop into chloroplasts at a later stage, are present in the apical meristem of the sporophyte of *Matteuccia struthiopteris*. The lack of recognizable proplastids in this young gametophytic stage does not preclude the possibility that proplastids are present in the older gametophyte, and are not restricted to the sporophyte.

#### SUMMARY

The young gametophyte of *Matteuccia struthiopteris* shows a method of chloroplast division, here termed centralization, which differs from the previously reported constriction-type. The salient features are:

1. The chloroplast is divided by the ingrowth of the inner-limiting chloroplast membrane.
2. During invagination, the inner-membrane does not cause a lamellar pinching.
3. The lamellae of the daughter chloroplasts are not necessarily in the same plane as is the division.
4. Although the separation of the daughter chloroplasts has not yet been observed, centralization appears to be the major if not the only method of chloroplast division at this stage of development.

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