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The Homozygous and Heterozygous Effects of an *Aurea* Mutation on Plastid Development in Spruce (*Picea abies* (L.) Karst.)

De homozygota och heterozygota effekterna på plastidutvecklingen av en aurea-mutation hos gran (Picea abies (L.) Karst.)

by

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Introduction

Semi-dominant *aurea* mutations were described for the first time by BAUR (1907) in *Antirrhinum majus* and *Pelargonium zonale. Aurea* forms have later been found in several other plant species. They are characterised by a yellow-green phenotype and after self-fertilisation give an F_1 segregation of 1 green (wild type): 2 yellow-green (*aurea*): 1 lethal yellow or white (*xantha* or *albina*).

Aurea plants belong to that group of mutants—chloroplast or chlorophyll mutants—which have a disturbed chloroplast development. By using plants which are heterozygous and homozygous for an *aurea* factor, it is possible to study the effect on plastid morphogenesis of a semi-dominant gene in single and double doses. The investigation to be presented here (*cf.* WALLES, 1966 a) was made in an *aurea* type of spruce, the genetics of which has been described by LANGNER (1953). The heterozygous seedlings are yellow-green when they emerge, but can turn green with time, *i.e.* they are of a *virescens* (*cf.* GUSTAFSSON, 1940) phenotype. The new shoots on *aurea* trees are initially yellow with a greenish tint, and similarly delayed in becoming green. Seedlings homozygous for the *aurea* gene are of a yellow (*xantha*) phenotype. VON WETTSTEIN (1958) observed that plastids of heterozygous *aurea* seedlings in spruce developed small grana, consisting of a few layers of double discs, and from this fact he arrived at the important conclusion that photosynthesis seems possible even by such a simple chloroplast structure.

Material and Methods

Offspring from a single open-pollinated *aurea* tree (Th-2) were used. Among 162 seedlings were found 81 (50 per cent) normal green individuals, 74 (46 per cent) heterozygotes (*aurea*) and 7 (4 per cent) yellow homozygotes.

A preliminary study was made of seedlings grown in sand in the laboratory or in the greenhouse. Other plants which were grown in

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sand and given nutrient solution were kept in the phytotron of this college. The seedlings were given 16 hours of light per day (intensity of illumination: 40,000 Lux) or kept in total darkness. The temperature was 20° C during 16 hours and 15° C during eight hours.

From *aurea* and wild type individuals, samples were taken of the cotyledons and first needles of 10—37 days old seedlings and of needles of 10—18 months old plants. From homozygous *xantha* plants, samples were taken of the cotyledons of 10—21 days old seedlings. The collected material was (1) fixed in 6.5 per cent glutaraldehyde in 0.1 M phosphate buffer (pH = 7.0) for five hours, left in buffer over night and postfixed for two hours in buffered 2 per cent OsO_4 (in some cases 0.2 M sugar was added to the buffer) or (2) fixed in the glutaraldehyde solution for 24 hours, washed in buffer and postfixed for four hours in 5 per cent $KMnO_4$ in water or (3) fixed directly in 5 per cent $KMnO_4$ for two hours. In all cases the fixation and washing was performed at 4° C. The dark-grown seedlings were fixed in a dim green safe light. The objects were dehydrated in an acetone series followed by propylene oxide and embedded in Epon 812 (LUFT, 1961). The osmium-fixed material was stained after sectioning on the grids with 4 per cent uranyl acetate at 40° C and then with lead citrate (REYNOLDS, 1963). The sections were cut on an ultramicrotome constructed at this laboratory or on a LKB Ultrotome III and collected on formvar-coated grids. They were investigated in a Siemens Elmiskop I operated at 80 kV (Figs. 1—2, 5—11, 13—14) or in a Hitachi HS-7S electron microscope.

Chlorophyll determinations were made according to the method described previously (WALLES, 1963).

Observations

1. Wild type spruce

Chloroplasts of wild type spruce are shown in Figs. 1 and 4. They are lens-shaped bodies enclosed by a double envelope. The chloroplasts contain a lamellar system embedded in a matrix, the stroma, which after fixation appears granular and of moderate electron density. Fixation with glutaraldehyde, followed by OsO_4, reveals several different inclusions in the stroma (Fig. 1). Various amounts of so-called plastoglobuli (LICHTENTHALER and SPREY, 1966) occur regularly. Plastoglobuli are usually visible also after $KMnO_4$ fixation (*cf.* Fig. 2) although they are not well preserved. Nucleoplasm-like regions are seen as electron-transparent zones containing DNA fibrils (*cf.* Gunning, 1965; Kislev, Swift and Bogorad, 1965; Ris, 1962; Walles, 1966 b). Numerous electron-dense granules, representing ribosomes, occur all over the stroma where this is free from other inclusions. Starch grains are often seen both after glutaraldehyde — OsO_4 and $KMnO_4$ fixation (Fig. 4).

Spruce cotyledons can synthesize chlorophyll and develop chloroplasts, not only in the light, but also in the dark (von Wettstein, 1958; Laudi, 1964). The chloroplasts in cotyledons of dark-grown seedlings contain several prolamellar bodies in addition to a lamellar system with grana. Illumination of dark-grown seedlings causes a progressive disappearance of prolamellar bodies and a stimulation of lamellar growth and differentiation.

Figs. 1, 3 and 4 show how the lamellar system of chloroplasts from dark-grown or light-grown individuals consists of layers of thylakoids (discs) which in places are aggregated into grana. Prolamellar bodies (Fig. 2) are observed in the chloroplasts of dark-grown seedlings.

2. The *xantha* plants

The lethal *xantha* seedlings have a life span of about four weeks. They possess pure yellow cotyledons both in the dark and in the light. In acetone extracts of mutant seedlings no chlorophyll peaks could be observed in the spectrophotometer.

The plastids of *xantha* seedlings are of irregular shape and their internal organization remains at an early proplastid stage (Figs. 5—7). The structural material consists of vesicles and a few non-aggregating thylakoids. There is no formation of prolamellar bodies and there seem to be no structural differences between the plastids of dark-grown (Fig. 7) and of light-grown (Figs. 5—6) seedlings. There is a noticeable accumulation of plastoglobuli.

3. The *aurea (virescens)* plants

The amount of chlorophyll in dark-grown *aurea* seedlings is about 50 per cent of that in corresponding wild type seedlings (Table 1). In both genotypes illumination promotes chlorophyll synthesis. Light-grown 3—5 weeks old *aurea* seedlings contain 40 per cent of the amount of chlorophyll found in corresponding wild type seedlings. The figures given in Table 1 were obtained from plant material cultivated in the phytotron.

Table 1. Chlorophyll content $\left(\frac{E_{662}\ m\mu}{\text{gram fresh weight}}\right)$ in cotyledons of 21—37 days old spruce seedlings.

The figure for dark-grown *aurea* seedlings is based on 12 samples; in the other cases five samples were analysed.

	Dark	Light
Wild type	4.7 ± 0.37	9.1 ± 0.84
Aurea	2.4 ± 0.17	3.6 ± 0.28

The chloroplasts in cotyledons of dark-grown *aurea* seedlings contain several prolamellar bodies (Fig. 8). The lamellar system of these chloroplasts is reduced in comparison with corresponding organelles of the wild type. The grana contain a smaller number of thylakoids than in the wild type, often as few as two or three. The thylakoids may, however, be aggregated along a considerable part of their length and may thus obscure the differentiation between grana and intergrana regions in the lamellar system. The chloroplasts of young *aurea* seedlings growing in light have their lamellar system organised in the same way as have seedlings grown in the dark, but lack prolamellar bodies. They usually contain large starch grains. In their subsequent ontogeny the chloroplasts of the mutant deviate from the normal process of plastid morphogenesis. In seedlings which are three weeks old or older the lamellae are mis-shapen and bent in an odd way (Figs. 9—11). Many of the grana profiles appear in section as more or less semicircular or even circular. They are, however, of normal size, in contrast to the aberrant, cup-shaped grana of lethal mutants in barley (see WALLES, 1963). The structurally abnormal chloroplasts are observed in the *aurea* needles until they turn normally green. Old, dark-green needles were found to have normal chloroplasts (Figs. 12, 14). A few exceptional chloroplasts had features reminiscent of the sickle-shaped grana (Fig. 13) and might represent a transitional stage in the normalisation process.

Discussion

The majority of the mutations affecting chloroplasts are recessive. They interfere with the biosynthesis of chloroplast constituents (*cf.*

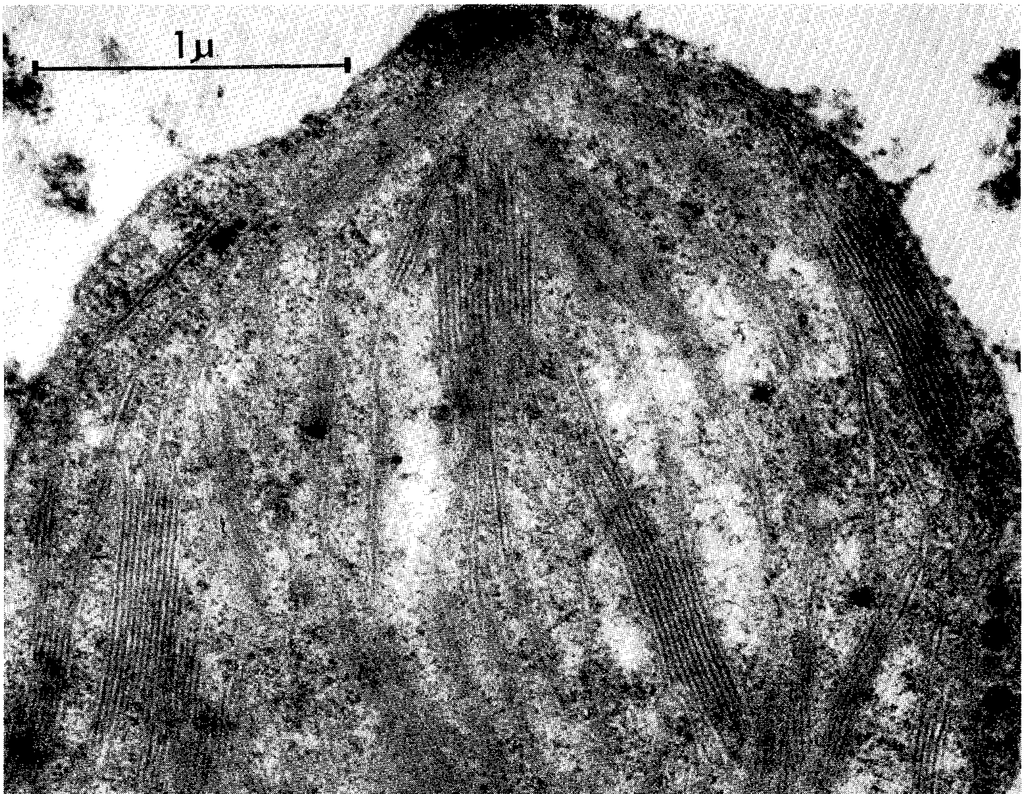
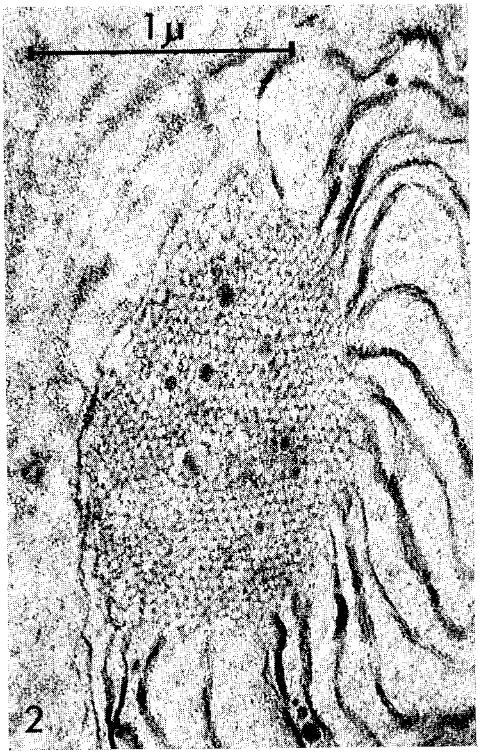


Fig. 1. Chloroplast in cotyledon of 27-day-old dark-grown wild type spruce. Fixed in glutaraldehyde—OsO_4.



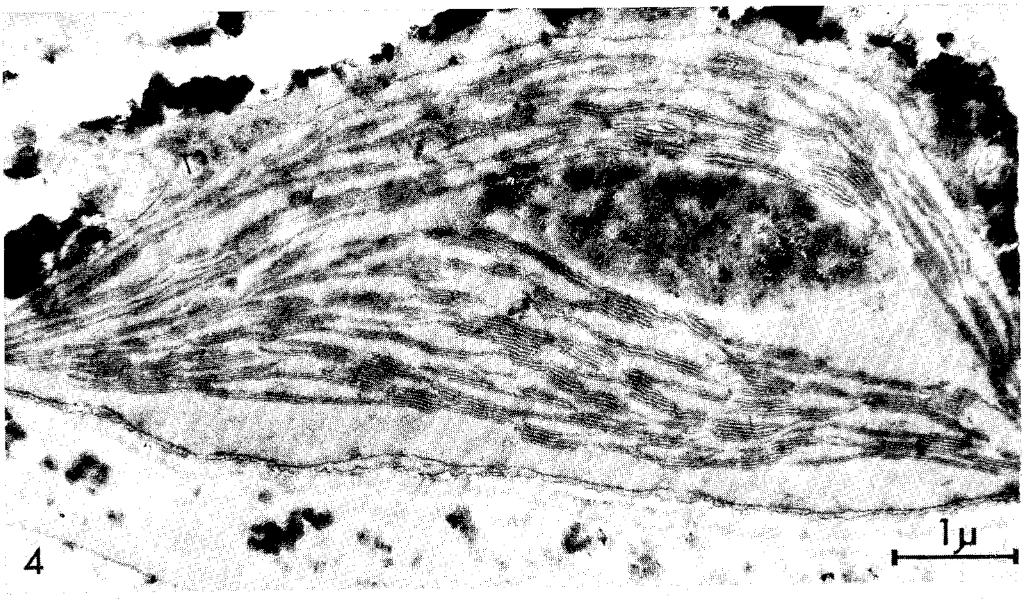


Fig. 2. Prolamellar body in chloroplast of 16-day-old dark-grown wild type spruce. Fixed in $KMnO_4$.

Fig. 3. Section through granum in chloroplast of 18-month-old wild type spruce. Fixed in glutaraldehyde—$KMnO_4$.

Fig. 4. Chloroplast from the same needle as illustrated in Fig. 3. Fixed in glutaraldehyde —$KMnO_4$.

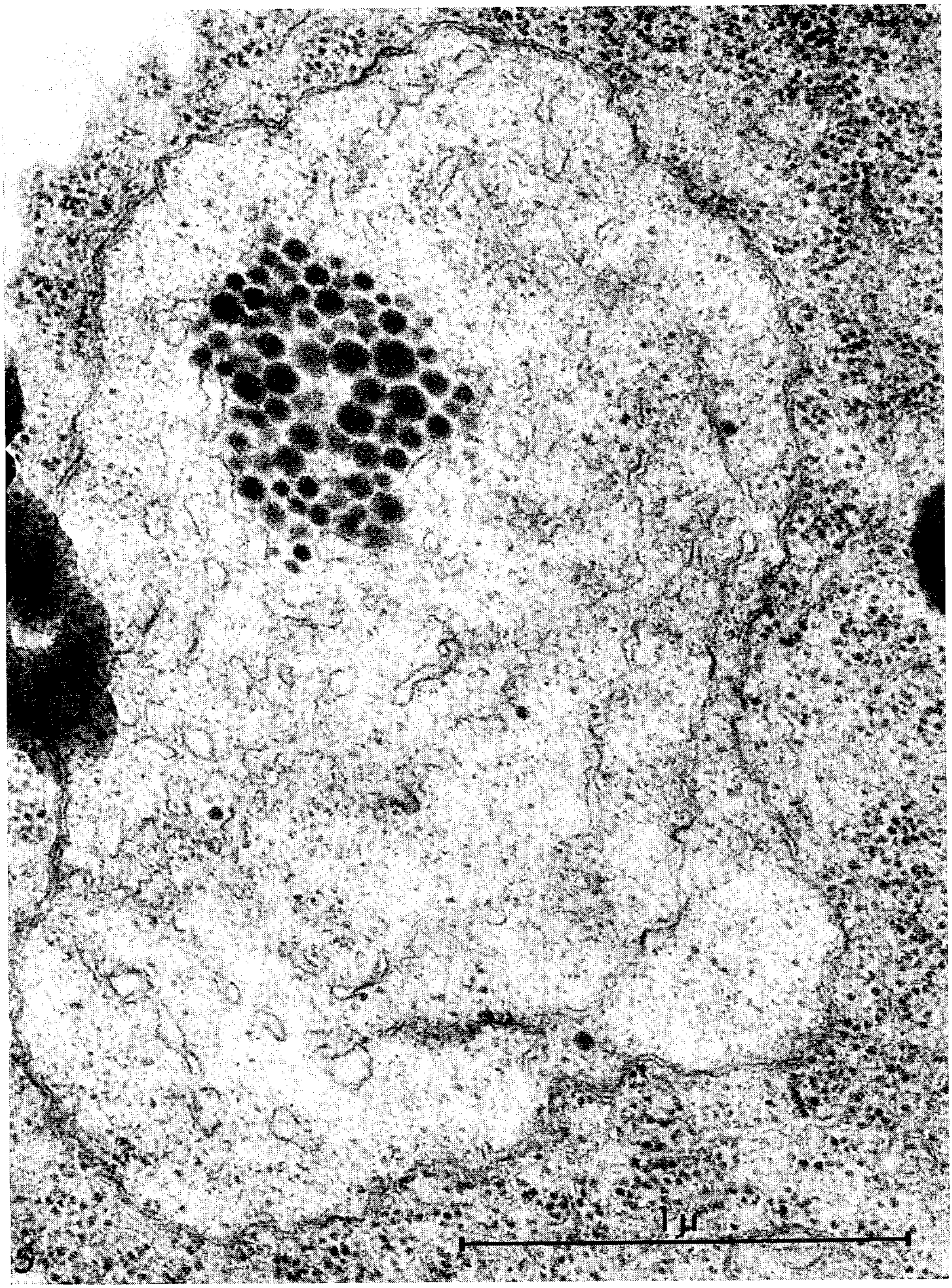
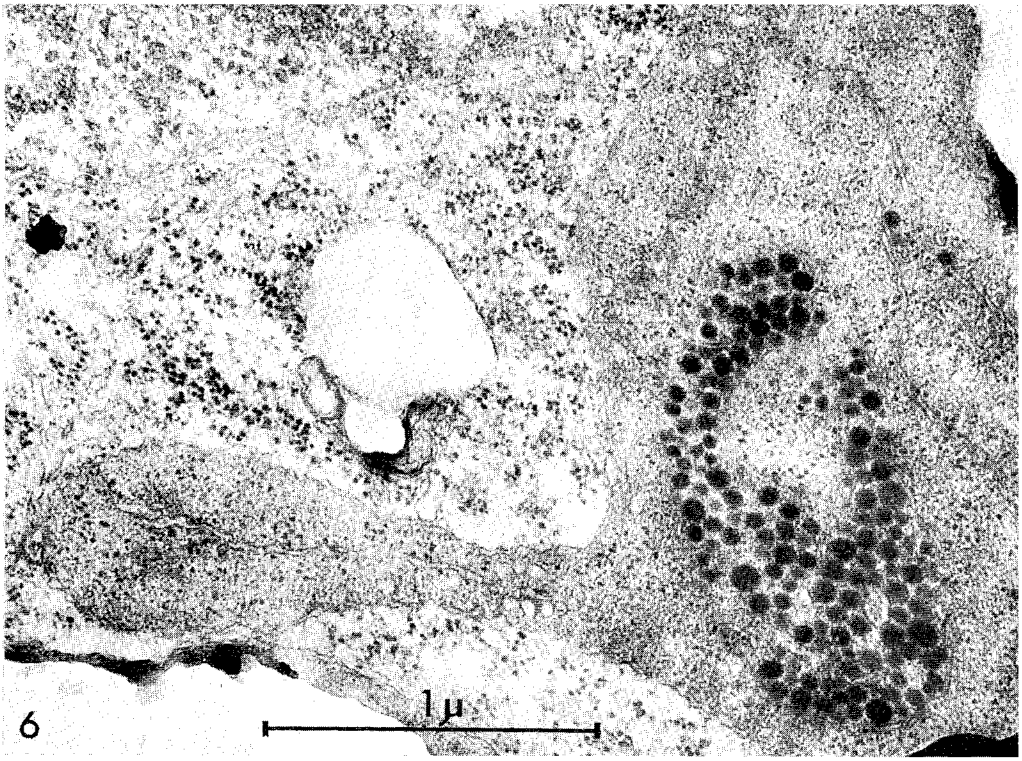


Fig. 5. Plastid in cotyledon of 14-day-old *xantha* seedling. Fixed in glutaraldehyde—OsO_4.



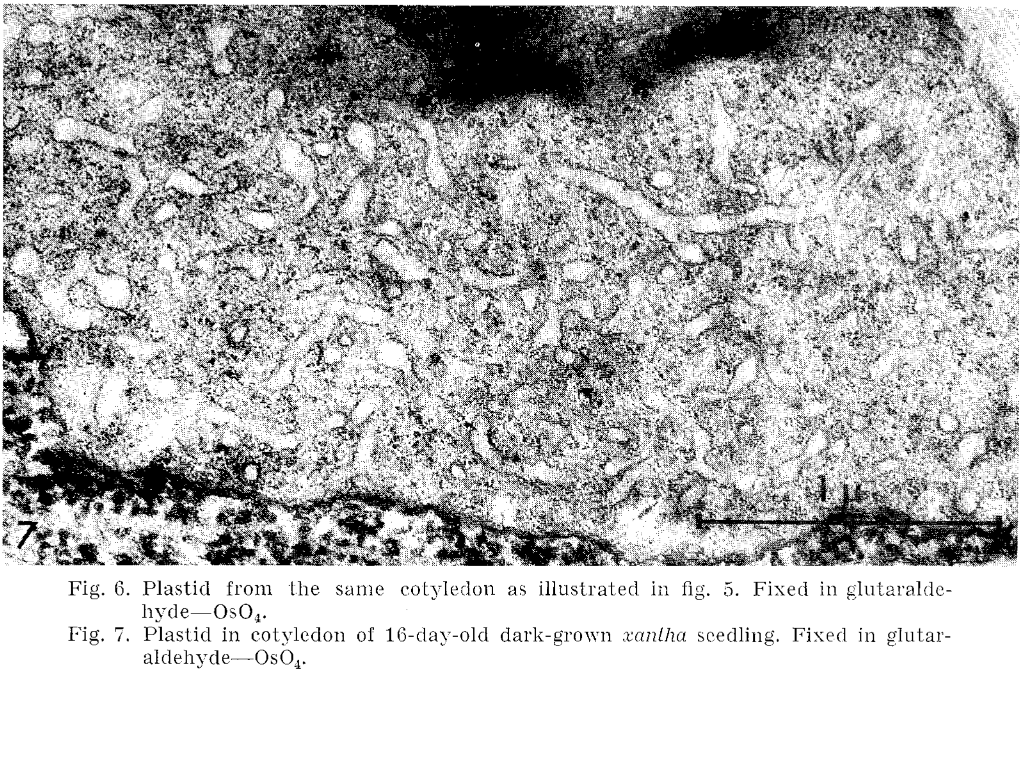
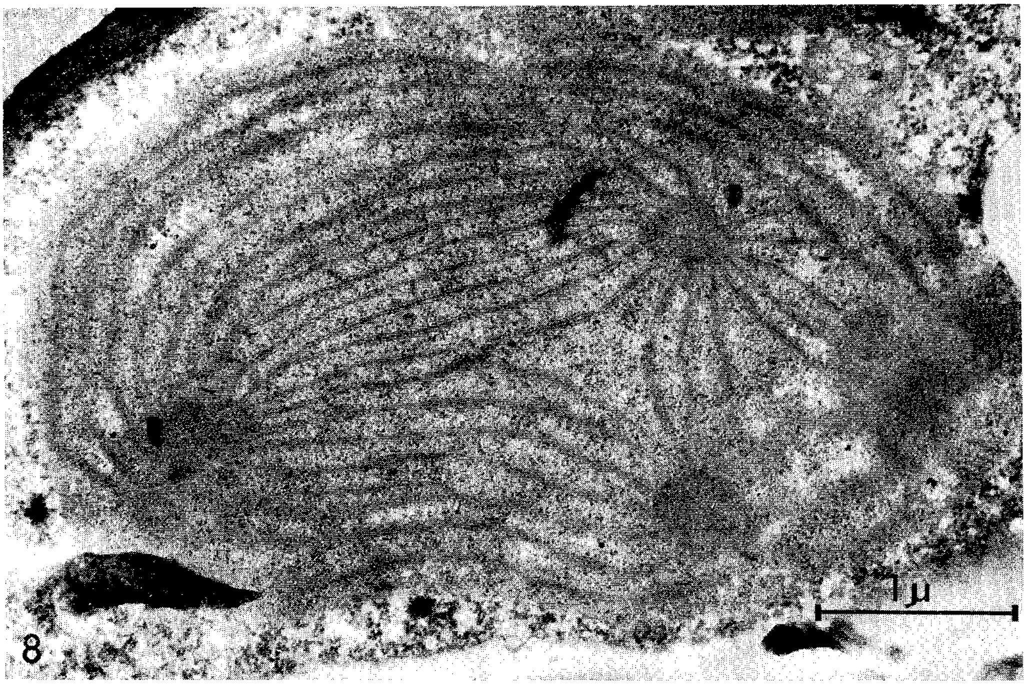


Fig. 6. Plastid from the same cotyledon as illustrated in fig. 5. Fixed in glutaraldehyde—OsO_4.

Fig. 7. Plastid in cotyledon of 16-day-old dark-grown *xantha* seedling. Fixed in glutaraldehyde—OsO_4.



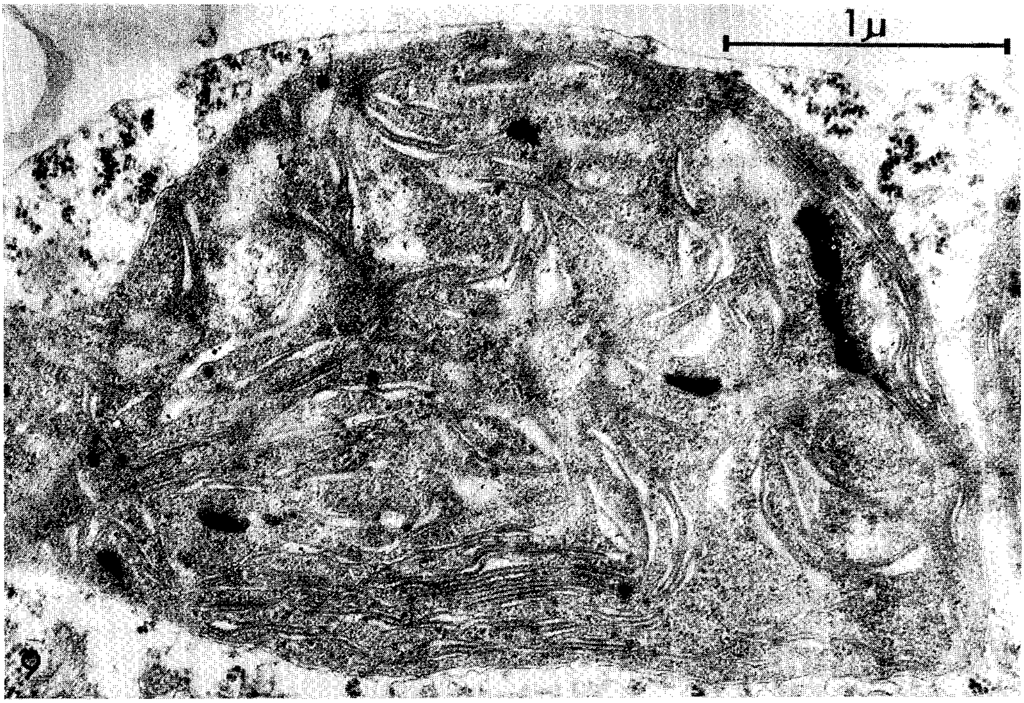
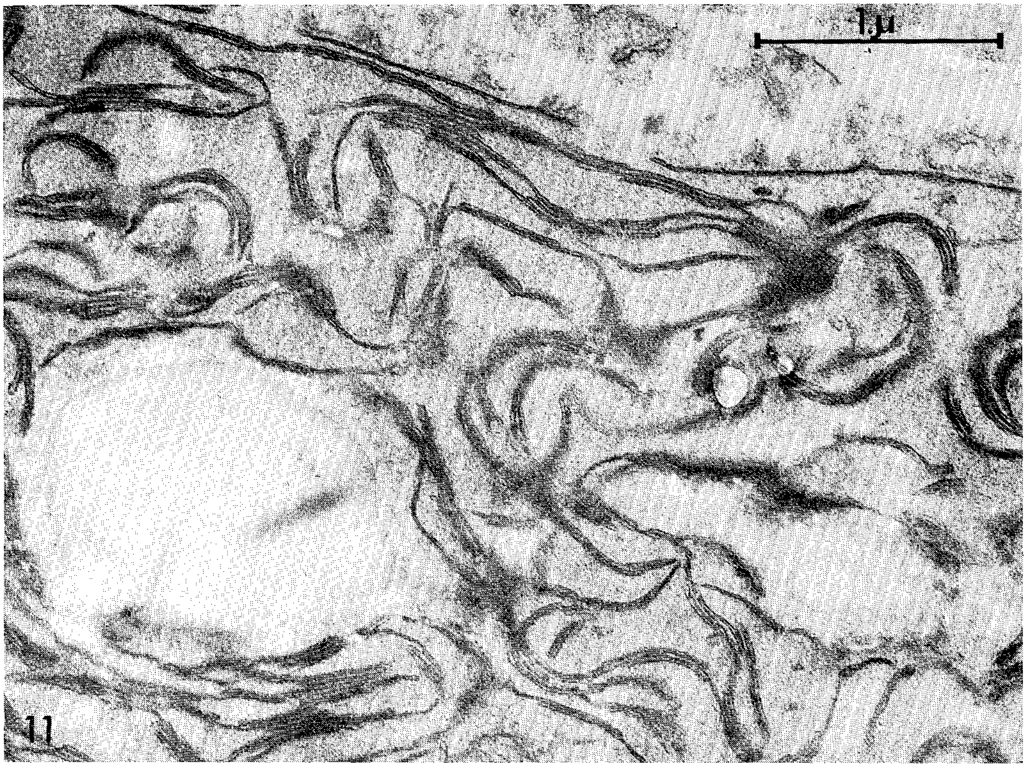


Fig. 8. Chloroplast in yellow-green cotyledon of 24-day-old dark-grown *virescens* (*aurea*) seedling. Fixed in glutaraldehyde—OsO_4.

Fig. 9. Chloroplast in yellow-green cotyledon of 3-week-old *virescens* seedling. Fixed in glutaraldehyde—OsO_4.

Fig. 10. Chloroplast in yellow-green cotyledon of 4-week-old *virescens* seedling. Fixed in $KMnO_4$.



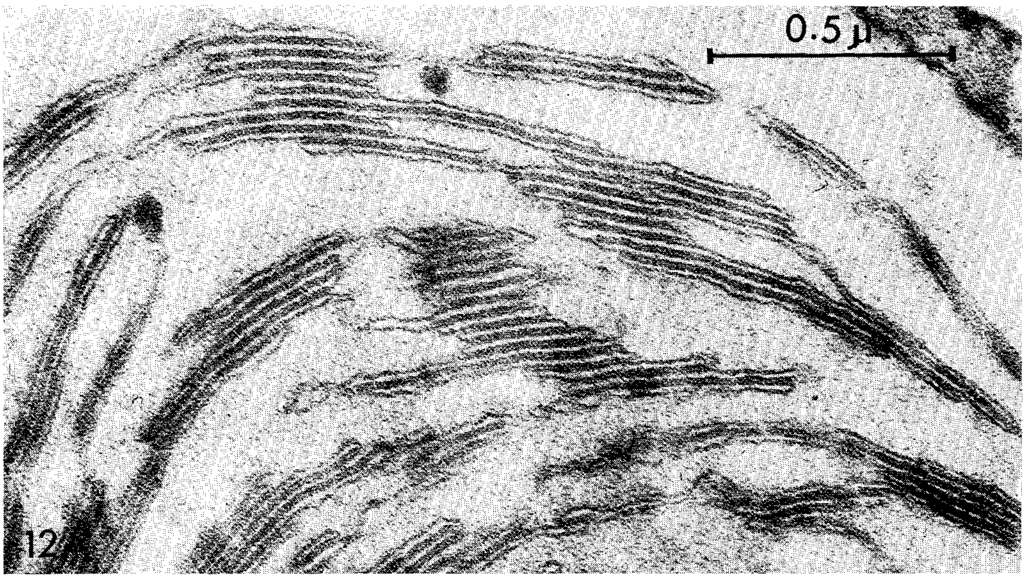


Fig. 11. Detail of chloroplast in yellow-green cotyledon of 3-week-old *virescens* seedling. Fixed in $KMnO_4$.

Fig. 12. Section through grana in green 2 cm long needle of 18-month-old *virescens* tree. Fixed in glutaraldehyde—$KNnO_4$.

Fig. 13. Chloroplast from the same needle as illustrated in Fig. 12. Fixed in glutaraldehyde—$KMnO_4$.

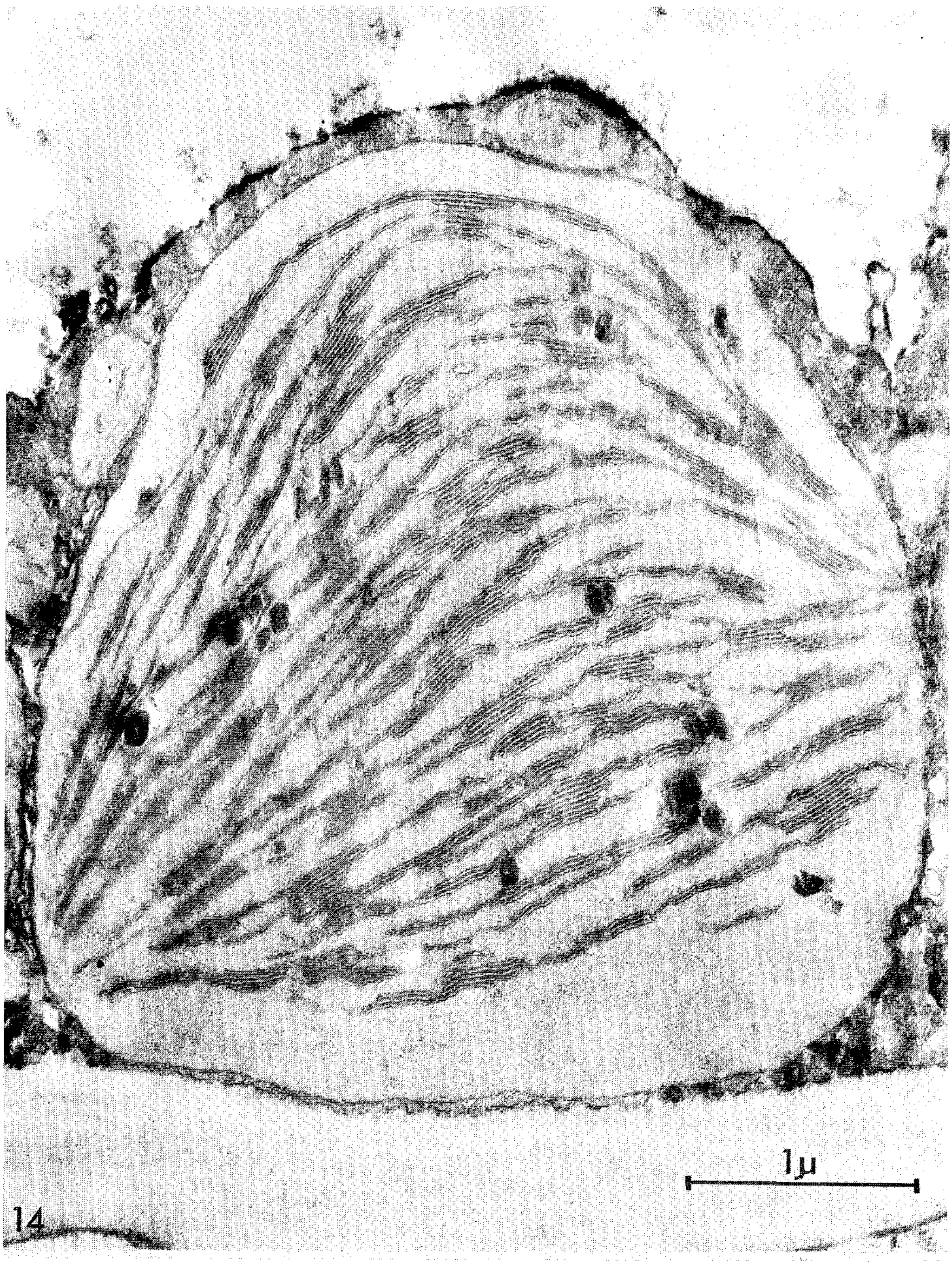


Fig. 14. Chloroplast from the same needle as illustrated in Fig. 12. Fixed in glutaraldehyde—$KMnO_4$.

WALLES, 1967) and in this way block the morphogenesis or function of the chloroplast. Plants which are heterozygous for one of these recessive mutations develop a normal phenotype, since one wild type allele is apparently sufficient for promoting synthesis of the choroplast constituent which is absent or reduced in amount in the mutant homozygote. For semi-dominant chloroplast mutations the biochemical mechanism has not yet been established, but some kind of suppressing or inhibitory action of the mutated gene might be expected.

Some reports provide information on how chloroplasts can be influenced by semi-dominant *aurea* mutations. In soybean (*Glycine max*) seedlings carrying the gene y_{11} the chlorophyll content, expressed as μg chlorophyll per gram fresh weight, was found to be 151 in $y_{11}y_{11}$ seedlings, 379 in $Y_{11}y_{11}$ seedlings and 738 in $Y_{11}Y_{11}$ (wild type) seedlings (WOLF, 1963). In all genotypes the amount of chlorophyll increases with the age of the plants and the figures cited are the highest obtained. According to an electron microscopical study by SUN (1963), the bright yellow, lethal homozygotes ($y_{11}y_{11}$) can produce some thylakoids and even a few grana in their plastids. In some plastids the thylakoids are large and extend through nearly the whole plastid or are bent and assembled into a concentric arrangement. The plastids contain also large vacuoles and groups of plastoglobuli. Heterozygous *aurea* plants in tobacco (BURK and MENSER, 1964) have been analysed by SCHMID and GAFFRON (SCHMID, PRICE and GAFFRON, 1966; SCHMID and GAFFRON, 1966, 1967). The plants are reported to have a chlorophyll content which is only one-eighth of that of the wild type. The photosynthetic capacity of the *aurea* plants at higher light intensities is superior to that of the wild type, and photosynthesis in the mutant becomes saturated at a considerably higher light intensity than that in green plants. The lamellar system in the *aurea* chloroplasts is much reduced. The grana consist of only two or three layers of thylakoids, whereas 20–40 layers are found in grana of the wild type. The organisation of the chloroplasts in this *aurea* mutant seems similar to that of the chloroplasts in a viable *viridis* (*chlorina*) mutant in *Arabidopsis thaliana* (WEHRMEYER and RÖBBELEN, 1965). This recessive *Arabidopsis* mutant has a chlorophyll content of about ten per cent of that of the wild type (RÖBBELEN and WEHRMEYER, 1965).

In the investigation presented here, an *aurea* factor was found to affect the plastid development in two ways, *viz.* by suppressing chlorophyll synthesis and by interfering with the organisation of the thylakoids. The studies on dark-grown seedlings show that the decrease in amount of chlorophyll in the mutant seedlings is not caused by bleaching (photo-oxidation) but must be due to impeded biosynthesis. In the homozygous *xantha* seedlings, no chlorophyll could be detected; the plastids are blocked in development prior to the formation of prolamellar bodies. In this connection it is of interest to note that the recessive chlorophyll-less mutant *xantha*-10 in barley can certainly form some discs but no prolamellar bodies in its plastids (VON WETTSTEIN, 1958; VON WETTSTEIN and ERIKSSON, 1965). The kind of aberrant lamellar structure seen in the chloroplasts of yellow-green needles of the *aurea* heterozygotes has so far not been reported for any other mutant. The needles are eventually normalised in regard to chlorophyll content and chloroplast structure. Spontaneous greening and normalisation of arrested plastids was also observed in a recessive *virescens* mutant of barley (MACLACHLAN and ZALIK, 1963).

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Summary

An electron microscopical investigation has been made of plastid development in spruce plants which are homozygous or heterozygous for a semi-dominant *aurea* mutation. The cotyledons of normal spruce seedlings synthesise chlorophyll and develop chloroplasts both in the dark and in the light. The chloroplasts in dark-grown seedlings have grana and several prolamellar bodies. No chlorophyll was detected in

acetone extracts of light-grown or dark-grown seedlings which were homozygous for the *aurea* factor. In these yellow lethal seedlings, plastid morphogenesis is blocked prior to the formation of prolamellar bodies. In the yellow-green heterozygous *aurea* seedlings, chlorophyll synthesis is reduced; when grown in the dark they have a chlorophyll content that is only 50 per cent of that in corresponding wild type individuals. The chloroplasts of these dark-grown *aurea* seedlings possess several prolamellar bodies and a poorly differentiated lamellar system, in which the grana usually contain only two or three discs. *Aurea* plants develop in the light an abnormal lamellar system with grana which appear sickle-shaped in cross-section. The yellow-green needles possess the ability of spontaneous greening. The chloroplasts in older green needles of the mutant are normalised and indistinguishable from wild type chloroplasts.

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Sammanfattning

De homozygota och heterozygota effekterna på plastidutvecklingen av en *aurea*-mutation hos gran (*Picea abies* (L.) Karst.)

En elektronmikroskopisk undersökning har utförts över plastidutvecklingen hos granplantor, som är homozygota eller heterozygota för en semidominant *aurea*-mutation. Hos normala groddplantor av gran bildar hjärtbladen klorofyll och kloroplaster såväl i mörker som i ljus. I mörkerodlade groddplantor innehåller kloroplasterna prolamellarkroppar samt s. k. grana. Grana består av skivlika bildningar (thylakoider), staplade på varandra. Klorofyll kunde inte påvisas spektrofotometriskt i acetonextrakt av groddplantor, som var homozygota för *aurea*-faktorn, oavsett om dessa hade vuxit i mörker eller i ljus. I dessa gula och letala plantor är plastidutvecklingen blockerad på ett tidigt stadium före bildningen av prolamellarkroppar. Hos de gulgröna heterozygota *aurea*-plantorna är klorofyllbildningen hämmad; på groddplantstadiet är klorofyllhalten i mörkerodlade *aurea*-plantor endast 50 % av klorofyllhalten i motsvarande vildtypsindivider. Kloroplasterna hos mörkerodlade *aurea*-plantor innehåller ett flertal prolamellarkroppar och ett föga utvecklat lamellsystem med grana, vilka vanligen består av endast två—tre thylakoider. I ljuset utvecklar *aurea*-plantorna ett defekt lamellsystem med grana, som i tvärsnitt är bågformigt böjda. De gulgröna barren kan med tiden bli gröna. Kloroplasterna i sådana gröna barr är normaliserade och liknar kloroplasterna hos normal gran.