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COMPOSITION OF THE PHOTOSYSTEMS AND CHLOROPLAST STRUCTURE IN EXTREME SHADE PLANTS

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SUMMARY

Chloroplasts were isolated from leaves of three species of tropical rainforest plants, *Alocasia macrorrhiza*, *Cordyline rubra* and *Lomandra longifolia*; these species are representative of extreme "shade" plants. It was found that shade plant chloroplasts contained 4–5 times more chlorophyll than spinach chloroplasts. Their chlorophyll *a*/chlorophyll *b* ratio was 2 3 compared with 2 8 for spinach. Electron micrographs of leaf sections showed that the shade plant chloroplasts contained very large grana stacks The total length of partitions relative to the total length of stroma lamellae was much higher in *Alocasia* than in spinach chloroplasts. Freeze-etching of isolated chloroplasts revealed both the small and large particles found in spinach chloroplasts.

Despite their increased chlorophyll content, low chlorophyll *a*/chlorophyll *b* ratio, and large grana, the shade plant chloroplasts were fragmented with digitonin to yield small fragments (D-144) highly enriched in Photosystem I, and large fragments (D-10) enriched in Photosystem II. The degree of fragmentation of the shade plant chloroplasts was remarkably similar to that of spinach chloroplasts, except that the subchloroplast fragments from the shade plants had lower chlorophyll *a*/chlorophyll *b* ratios than the corresponding fragments from spinach The D-10 fragments from the shade plants had chlorophyll *a*/chlorophyll *b* ratios of 1.78–2.00 and the D-144 fragments ratios of 3.54–4.07. We conclude that Photosystems I and II of the shade plants have lower proportions of chlorophyll *a* to chlorophyll *b* than the corresponding photosystems of spinach. The lower chlorophyll *a*/chlorophyll *b* ratio of shade plant chloroplasts is not due to a significant increase in the ratio of Photosystem II to Photosystem I in these chloroplasts.

The extent of grana formation in higher plant chloroplasts appears to be related to the total chlorophyll content of the chloroplast. Grana formation may simply be an means of achieving a higher density of light-harvesting assemblies and hence a more efficient collection of light quanta

Abbreviations DCIP, 2,6-dichlorophenolindophenol, DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DPC, 1,5-diphenylcarbazide, TCIP, 2,3′,6-trichlorophenolindophenol.

INTRODUCTION

The majority of studies on the fractionation of the photochemical systems of higher plants have been carried out with spinach chloroplasts with a chlorophyll *a*/chlorophyll *b* ratio of 2.7–3.0 (refs 1, 2). These studies show that Photosystem I of spinach chloroplasts has a chlorophyll *a*/chlorophyll *b* ratio of 5 7–6.0 and Photosystem II a ratio of 1.7. Immature spinach chloroplasts with a chlorophyll *a*/chlorophyll *b* ratio of 3.5 have been fractionated by passage through the French pressure cell A greater proportion of the chlorophyll of the immature chloroplast was released into the Photosystem I fraction, as compared with mature chloroplasts, but the chlorophyll composition of the Photosystem I fraction did not differ from that obtained from mature chloroplasts[3]. Goodchild and Park[3] found that immature spinach chloroplasts contained an increased ratio of stroma lamellae to grana lamellae. This result supported the earlier conclusion that stroma lamellae contain only Photosystem I and are fragmented more easily than grana[4].

Plants growing in extreme shade habitats have lower chlorophyll *a*/chlorophyll *b* ratios than "sun" species[5]. It was therefore of interest to attempt to fractionate the shade plant chloroplasts by digitonin incubation or by passage through the French press, and to examine the composition of the subchloroplast fragments. We also examined the structure of the shade plant chloroplasts and show that they have an increased ratio of total length of grana partitions to length of stroma lamellae. The degree of fractionation achieved with the shade plant chloroplasts on incubation with digitonin was similar to that obtained previously with spinach chloroplasts, but the Photosystem I and Photosystem II fractions of the shade plant chloroplasts had lower chlorophyll *a*/chlorophyll *b* ratios than those of spinach chloroplasts.

MATERIALS AND METHODS

Three species of rainforest plants were chosen for the present study, *Alocasia macrorrhiza* (L.) G. Don [*Araceae* family], *Cordyline rubra* Hueg. ex Kunth [*Liliaceae* and *Lomandra longifolia* Labill. [*Xanthorrhoeaceae*]. Leaves were harvested from plants growing in extreme shade habitats on the floor of the rainforest in Lamington National Park, Queensland[6]. This forest can be considered to be an extension of the Indo-Malasian tropical rainforest into the subtropics. The average daily radiation (in the waveband 400–700 nm) at the sites where the plants were growing was 21 μeinsteins $\cdot$ cm^{-2} $\cdot$ day^{-1}, compared with 5000 μeinsteins $\cdot$ cm^{-2} $\cdot$ day^{-1} above the forest canopy[6].

Leaves were collected early in the morning, packed on ice and flown to Canberra, where the chloroplasts were isolated on the same day However, chloroplasts isolated from leaves stored for one week at 0–4 °C showed no measurable decline in photosynthetic activity[7]. For chloroplast isolation, leaves were chopped with a razor blade into small pieces and blended in 0.05 M phosphate buffer, pH 7 2, containing 0.3 M sucrose and 0.01 M KCl (7 ml buffer/g fresh wt of leaves) in a Servall Omnimixer at 75 % line voltage for 10 s in the case of *Cordyline* and *Alocasia* and for 18 s for the much tougher *Lomandra* leaves. The breis were filtered through two layers of Miracloth and the chloroplasts sedimented by centrifugation at 1000 $\times$ *g* for 10 min. Spinach[8] and mutant pea plants[9] were grown as described previously and chloroplasts isolated in sucrose–phosphate medium.

Chloroplasts were fragmented by incubation with 0.5 % digitonin for 30 min at 0 °C (chlorophyll concentration 0.34 mg/ml) and the fragments separated by differential centrifugation, as described previously for spinach chloroplasts[8].

Chlorophyll *a* and chlorophyll *b* were determined after extraction into 80 % acetone[10]. Chlorophyll *b* of the mutant pea chloroplasts was determined in ethanol by the method of Boardman and Thorne[11]. Chloroplasts were counted in a Petroff–Hausser bacteria counter. The chlorophyll contents per chloroplast of the shade plants were compared with those of spinach and a chlorophyll-deficient pea mutant[9].

Fluorescence emission spectra at 77 °K were recorded on a fluorescence spectrophotometer incorporating automatic correction for photomultiplier and monochromator responses, and for variation in energy output of the light source[12]. The chloroplasts were suspended in 0.05 M phosphate buffer (pH 7.2)–glycerol (33 : 67, v/v) at a final absorbance of 0.1 *A* unit at 436 nm.

Photochemical activities

For $NADP^+$ reduction with water as the electron donor, the reaction mixture (final volume 0.6 ml) contained chloroplast fraction (5 μg chlorophyll) and 13.3 mM Tris–HCl buffer, pH 8.0, 23.3 mM NaCl, 3 3 mM $MgCl_2$ and 0.2 mM NADP together with saturating amounts of spinach enzymes, ferredoxin, ferredoxin–NADP reductase and plastocyanin. Illumination was for 2 min at saturating white light and $NADP^+$ reduction was calculated from the absorbance increase at 340 nm.

For the Photosystem I assay ascorbate + $DCIPH_2 \rightarrow NADP^+$, the reaction mixture (0.6 ml final volume) contained chloroplast fragments (5 μg chlorophyll) and 50 mM phosphate buffer, pH 6.8, 3.3 mM $MgCl_2$, 4 mM sodium ascorbate, 0.1 mM 2,6-dichlorophenolindophenol (DCIP), 0.4 mM $NADP^+$, 15 μM 3-(3′, 4′-dichlorophenyl)-1,1-dimethylurea (DCMU) and a final concentration 0.035 % Triton X-100, together with saturating amounts of the spinach enzymes, ferredoxin, ferredoxin–NADP reductase and plastocyanin.

For the Photosystem II assay $H_2O \rightarrow$ TCIP, the reaction mixture contained, in 3 ml, chloroplast fragments (10–15 μg chlorophyll) and 13 3 mM Tris–HCl buffer, pH 7.8, 23.3 mM NaCl and 0.02 mM 2,3′,6-trichlorophenolindophenol (TCIP). TCIP reduction was measured spectrophotometrically by the decrease in absorbance at 620 nm after 45 s of saturating white light.

Photosystem II was also assayed by the method of Vernon and Shaw[13], with 1,5-diphenylcarbazide (DPC) as electron donor and DCIP as acceptor. The reaction mixture contained in 3 ml, chloroplast fragments, (30 μg chlorophyll) and 50 mM phosphate buffer, pH 6.8, 0 5 mM DPC and 0.1 mM DCIP. For the D-144 fractions, 1 5 μM DCMU was also included. DCIP reduction was followed by the decrease in absorbance at 590 nm after 45 s of saturating white light.

Electron microscopy

Leaf tissue was fixed on the day that leaves arrived in Canberra by placing mm^2 leaf pieces in 3 % glutaraldehyde in 0.025 M phosphate buffer (pH 7.2) for 90 min at room temperature. The material was rinsed in buffer and post-fixed in OsO_4 in the phosphate buffer, then rinsed in buffer, dehydrated in alcohol, and embedded in an araldite–epon mixture. Sections were cut with a diamond knife and stained in uranyl acetate and lead citrate. For isolated chloroplasts and subchloroplast frag-

ments, preparations were centrifuged in a microfuge (Beckman Instruments) and the small pellets fixed in a similar manner to the leaf pieces except that the fixation times were reduced to 45 min at 4 °C.

For freeze-etching, chloroplasts and fragments were pelleted and a suspension of the pellet prepared with a minimal quantity of liquid. Drops of these suspensions were frozen on copper discs, without cryoprotection, by quenching in liquid freon 12 at −145 °C The freeze-etching was carried out in a Balzers BA 360 freeze-etch unit using platinum-carbon shadow of thickness 3.0 nm determined by a quartz crystal thin-film minitor. Replicas were cleaned with solutions of sulphuric acid and bleach All specimens were examined in a Philips EM 200 electron microscope

RESULTS

Chlorophyll content of chloroplasts

The leaves of shade plants generally have a higher chlorophyll content and a higher proportion of chlorophyll *b* to chlorophyll *a* than sun plants[5,14] In the present work, we have compared the chlorophyll contents per chloroplast of *Alocasia* and *Cordyline* with those of spinach and the chlorophyll-deficient mutant pea. The chlorophyll content of the mutant leaves were about one-half that of normal pea leaves. The shade plant chloroplasts contain 4–5 times more chlorophyll than spinach chloroplasts and about 10 times more chlorophyll than mutant pea chloroplasts (Table I). The molar ratio of chlorophyll *a*/chlorophyll *b* was 2 3 for *Alocasia* and *Cordyline* chloroplasts, as compared with 2.8 for spinach chloroplast The mutant pea has a much higher chlorophyll *a*/chlorophyll *b* ratio, which varies from 10 to 18 (ref 11).

TABLE I

CHLOROPHYLL CONTENT OF CHLOROPLASTS

Chloroplast	*moles chlorophyll per chloroplast*	*moles chlorophyll a per chloroplast*	*moles chlorophyll b per chloroplast*	*Chlorophyll a/ chlorophyll b molar ratio*
Alocasia	$21.9 \cdot 10^{-16}$	$15.3 \quad 10^{-16}$	$6\,6 \quad 10^{-16}$	2 31
Cordyline	$19.2 \quad 10^{-16}$	$13\,4 \cdot 10^{-16}$	$5\,8 \quad 10^{-16}$	2.30
Spinach	$4\,5 \quad 10^{-16}$	$3\,3 \quad 10^{-16}$	$1\,2 \quad 10^{-16}$	2 76
Pea mutant	$2\,0 \quad 10^{-16}$	$1\,85 \quad 10^{-16}$	$0\,15 \quad 10^{-16}$	12 4

Ultrastructure

Electron micrographs of leaf sections showed the chloroplasts of the three shade species studied to be relatively large and irregular in outline as illustrated by the *Alocasia* chloroplast (Fig. 1). The grana stacks are very large, irregularly arranged within chloroplasts and not orientated in one plane, so that some appear in surface view. As shown in Fig. 2, the number of thylakoids in a granum is huge and may reach one hundred or more. The number of grana thylakoids per chloroplast is greatly increased as compared to spinach chloroplasts.

It appears from an examination of a representative sample of electron micro-

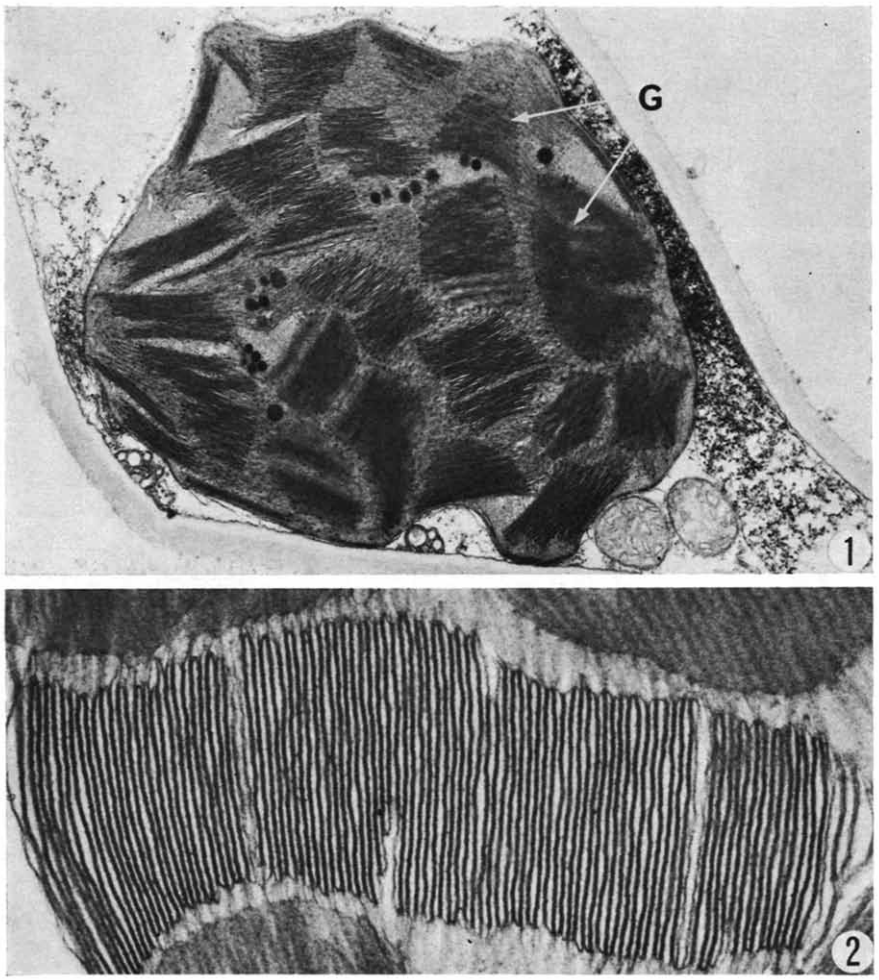


Fig 1 Section through an *Alocasia* chloroplast *in situ* showing large irregular grana (G) (15 600).

Fig 2 Section through a large granum of an *Alocasia* chloroplast *in situ* with more than 100 lamellae (46 000)

graphs that the number of stroma lamellae relative to the number of grana thylakoids is much the same in the shade plants as in spinach, but that the stroma lamellae in the shade plants are shorter than in spinach. Because of the variable orientation of the grana stacks in the shade plant chloroplasts, it is not possible to accurately determine the total length of grana thylakoids to stroma lamellae. However, we have made approximate estimates both of the relative number of grana partitions in relation to the number of stroma lamellae, and the total length of partitions relative to the total length of stroma lamellae for *Alocasia* and spinach. (Table II). Although the number of partitions, relative to the number of stroma lamellae, is smaller in *Alocasia* than in spinach, the total length of partitions in relation to the total length of stroma lamellae in *Alocasia* is double that of spinach.

TABLE II

ESTIMATES OF STROMA LAMELLAE AND GRANA PARTITIONS IN *ALOCASIA* AND SPINACH CHLOROPLASTS

Estimates obtained by making measurements on electron micrographs selected for structural clarity and suitable orientation of partitions and stroma lamellae

	Alocasia	*Spinach*
Average number of partitions per granum	43	8
Number of partitions/ Number of stroma lamellae	1.9	2.9
Total length of partitions/ Total length of stroma lamellae	3.2	1.4

Fractionation of shade plant chloroplasts

Incubation of chloroplasts from each of the three species of shade plants with 0.5 % digitonin for 30 min at 0 °C resulted in extensive fragmentation of the chloroplasts (Table III). The distribution of chlorophyll among the centrifugal fractions was similar to that obtained previously with spinach chloroplasts[8]. This was particularly so for *Alocasia*, where 62 % of the chlorophyll was recovered in the D-1 and D-10 fractions and 11 % in the D-144 fraction. There was a definitive fractionation of the chloroplast membranes in terms of the relative proportions of chlorophyll *a* and chlorophyll *b*. The chlorophyll *a*/chlorophyll *b* ratios of the D-1 and D-10 fractions were lower than the original chloroplast ratio, while the lighter fractions, D-50, D-144 and D-144S had higher ratios. The pattern of chlorophyll *a*/chlorophyll *b* ratios was similar to that observed previously for the fractions from spinach chloroplasts[8], except that all fractions from the shade plant chloroplasts had lower chlorophyll *a*/chlorophyll *b* ratios than the corresponding fractions from spinach. Thus the D-10 fractions from the shade plants had chlorophyll *a*/chlorophyll *b* ratios of 1.78–2.00 as compared with 2.27 for spinach, while the D-144 fractions had ratios of 3.54–4.07, compared with 5.34 for spinach.

Alocasia chloroplasts were also resuspended in 0.05 M Tricine buffer, pH 7.4, containing 0.15 M KCl and fragmented by passage through the French pressure cell according to the procedure of Sane *et al* [4]. The 160-K fraction had a chlorophyll *a*/chlorophyll *b* ratio of 3.48 and the 10-K fraction had a ratio of 2.08, indicating that these chloroplasts could also be partially fractionated by a mechanical procedure.

Fluorescence spectra

Fluorescence spectra at liquid N_2 temperature are useful in monitoring the fractionation of the photosystems. Spinach chloroplasts at 77 °K give a three-banded fluorescence emission spectrum with maxima at 683, 695 and 735 nm; the bands at 683 and 695 nm arise from the chlorophylls of Photosystem II and that at 735 nm mainly from the chlorophylls of Photosystem I (ref. 15). Table IV shows the ratios of the fluorescence energy emitted at the 735-nm band (ϕ_{735}) to the total fluorescence emission energy (ϕ_{total}). The ratios indicate that the D-10 fractions are enriched in the chlorophylls of Photosystem II as compared with chloroplasts, whereas the D-144 fractions consist mainly of Photosystem I. The spectra of the shade plant

TABLE III

COMPARISON OF THE CHLOROPHYLL COMPOSITIONS OF SUBCHLOROPLAST FRAGMENTS FROM SHADE PLANTS WITH THOSE OF SPINACH

Chloroplasts were incubated with 0.5 % digitonin for 30 min at 0 °C. The subchloroplast fragments were separated by differential centrifugation at 1000 × *g* for 10 min, 10 000 *g* for 30 min, 50 000 × *g* for 30 min and 144 000 × *g* for 60 min (designated D-1, D-10, D-50, D-144). The 144 000 *g* supernatant is designated D-144 S. Spinach values were determined previously[8].

Fraction	*Alocasia macrorrhiza*		*Cordyline rubra*		*Lomandra longifolia*		*Spinach*	
	Chlorophyll distribution (%)	*Chlorophyll a/ chlorophyll b*	*Chlorophyll* (%)	*Chlorophyll a/ chlorophyll b*	*Chlorophyll* (%)	*Chlorophyll a/ chlorophyll b*	*Chlorophyll* (%)	*Chlorophyll a/ chlorophyll b*
Chloroplasts	100	2.29	100	2.28	100	2.32	100	2.83
D-1	17	1.97	36	2.11	28	1.90	19	2.36
D-10	45	1.78	39	2.00	42	1.88	46	2.27
D-50	15	3.18	9	3.54	13	3.60	12	4.40
D-144	11	4.07	9	3.54	10	3.86	12	5.34
D-144 S	12	3.32	7	3.08	7	2.87	11	3.76
		2.28★		2.30★		2.23★		2.78★

★ The calculated ratio of chlorophyll *a*/chlorophyll *b* for chloroplasts obtained by individually summing the chlorophyll *a*/chlorophyll *b* ratios of the fractions.

TABLE IV

COMPARISON OF THE FLUORESCENCE EMISSIONS AT 77 °K OF SUBCHLOROPLAST FRAGMENTS FROM SHADE PLANT CHLOROPLASTS WITH THOSE OF SPINACH

The fluorescence energy emitted at the 735-nm band (ϕ_{735}) is determined as a percentage of the total fluorescence emission (ϕ_{total}). Excitation wavelength 436 nm. The values for spinach were determined previously[15]

Fraction	ϕ_{735}/ϕ_{total} (%)			
	Alocasia	*Cordyline*	*Lomandra*	*Spinach*
Chloroplast	73	75	70	75
D-10	56	56	57	60
D-144	93	92	92	97

fractions were comparable to those of spinach, although the shade plant fractions have somewhat lower fluorescence ratios than the corresponding fractions from spinach.

Photochemical activities

Photochemical activities of D-10 and D-144 fragments from *Alocasia* chloroplasts are compared with the activities of the chloroplasts before incubation with digitonin (Table V). The chloroplast activities are lower than those of spinach chloroplasts[8], but this is a feature of shade plants, and does not seem to be due to inactivation of the chloroplasts during isolation. Light-saturated photosynthetic rates *in vivo* of shade plants are low compared with those of sun species[16].

Rates of $NADP^+$ reduction by the D-10 fraction with water as the electron donor were about half those of chloroplasts. The D-144 fraction was unable to photoreduce $NADP^+$, unless supplied with an electron donor to Photosystem I. The high rates of $NADP^+$ reduction by the D-144 fragments in the presence of ascorbate *plus* DCIP confirm the earlier finding by Vernon and Shaw[13] with spinach Photosystem I fragments, and are probably due to the increased accessibility of the Photosystem I reaction centres to the reagents.

TABLE V

PHOTOCHEMICAL ACTIVITIES OF CHLOROPLASTS AND SUBCHLOROPLAST FRAGMENTS FROM *ALOCASIA*

Photochemical activities were assayed as described in Materials and Methods

Fraction	*Photochemical activities (μmole reduced/mg chlorophyll per h)*			
	$H_2O \rightarrow NADP^+$	$H_2O \rightarrow TCIP$	$DPC \rightarrow DCIP$	$Ascorbate + DCIPH_2 \rightarrow NADP^+$ (+0.035 % *Triton X-100*)
Chloroplasts	70	40	52	–
D-10	35	29	42	68
D-144	0	0	20(−DCMU) 12(+DCMU)	342

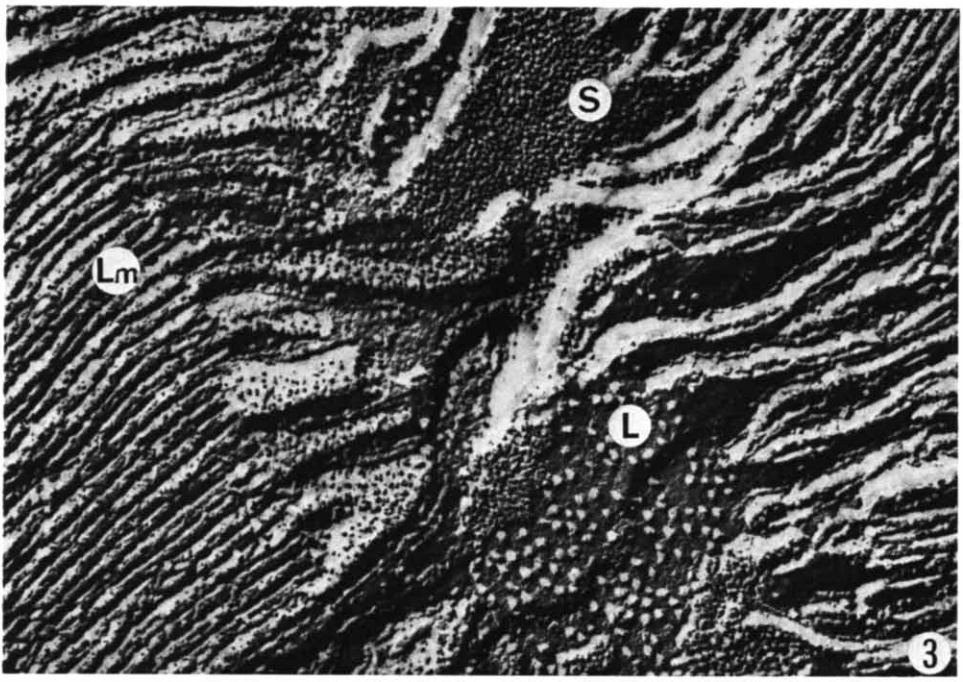
The rates of TCIP reduction by D-10 were somewhat lower than those by chloroplasts, but this may be explained by some inactivation of the water-splitting system during incubation with digitonin[8]. D-144 fragments showed no TCIP reduction. The use of diphenylcarbazide as an electron donor to Photosystem II permits the assay of Photosystem II in subchloroplast fragments which have lost the ability to evolve oxygen[13,17]. Photosystem II activity (DPC → DCIP) was found with chloroplasts and the D-10 fraction, and it was completely inhibited with 1.5 μM DCMU. Some DCIP reduction with DPC as donor was also observed with the D-144 fraction, but about half of this was resistant to inhibition by DCMU. A similar pattern of photochemical activities to those shown in Table V were found with the digitonin fractions from *Cordyline* and *Lomandra*

Freeze-etching

Freeze-etching of isolated *Alocasia* chloroplast preparations before digitonin fragmentation revealed two distinct fracture faces, one with large particles (L) and the other fracture face with small particles (S) as shown in Fig 3. This same pattern has been observed with spinach and other chloroplasts[2]. Grana stacks composed of many lamellae were also observed in cross fracture. When the digitonin fractions from *Alocasia* were freeze-etched, both small and large particle fracture faces were seen in the D-10 fraction and sections showed this fraction to be made up of grana fragments. The D-144 fraction, however, was made up of small vesicles that on freeze-etching revealed only small particles on their fracture faces (Fig 4). Arntzen *et al.*[18] have demonstrated that the small D-144 fraction from spinach shows only small particles as does the 160-K fraction obtained from French press fragmentation of spinach chloroplasts[4].

DISCUSSION

Despite the higher chlorophyll content and lower chlorophyll *a*/chlorophyll *b* ratio of the shade plant chloroplasts, their degree of fragmentation with 0.5 % digitonin is remarkly similar to that of spinach chloroplasts. The shade plant fractions, however, have lower chlorophyll *a*/chlorophyll *b* ratios than the corresponding spinach fractions The fluorescence spectra and the photochemical activity measurements show that the light fragments (D-144) are highly enriched in Photosystem I. The D-10 fragments are enriched in Photosystem II. We conclude, therefore, that both photosystems of the shade plant chloroplasts have higher proportions of chlorophyll *b* to chlorophyll *a* than the corresponding spinach photosystems, and that the lower chlorophyll *a*/chlorophyll *b* ratio of the shade plant chloroplasts is not due to a significant increase in the ratio of Photosystem II to Photosystem I. A similarity in the ratio of Photosystem II to Photosystem I in the shade plants and spinach is supported by our measurements[7] of chlorophyll/P-700 and chlorophyll/Q It seems that the proportions of chlorophyll *a* to chlorophyll *b* in the Photosystems depend on the chlorophyll *a*/chlorophyll *b* ratio of the chloroplasts, and that there are no universal chlorophyll *a*/chlorophyll *b* ratios for Photosystem I and Photosystem II. This conclusion is supported by fragmentation studies with chloroplasts with chlorophyll *a*/chlorophyll *b* ratios higher than that of spinach For example, maize mesophyll chloroplasts (chlorophyll *a*/chlorophyll *b* = 3.3) were fragmented with digitonin to



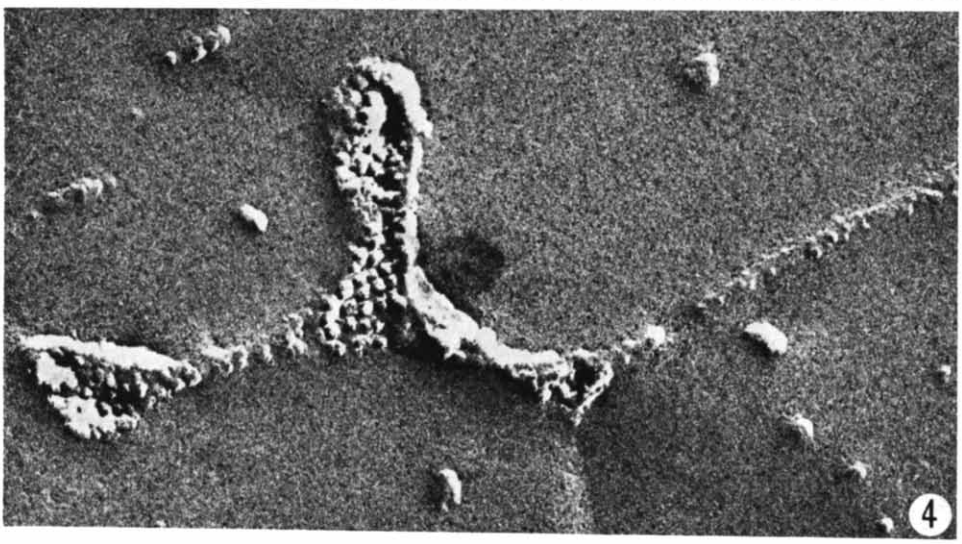


Fig. 3. Freeze–etch preparation of an isolated *Alocasia* chloroplast showing lamellae in cross fracture (Lm) and fracture faces with small (S) and large (L) particles (× 76 000)

Fig. 4 Freeze–etch image of D-144 subchloroplast fragments showing a vesicle with only small particles on the fracture face (× 132 000)

yield a D-144 fraction with a ratio of 6.4 and a D-10 fraction with a ratio of 2.8 (ref. 19), and more dramatically, pea mutant chloroplasts (chlorophyll *a*/chlorophyll *b* ratio of 14) on fragmentation with digitonin gave D-144 fragments with a ratio of 21 and D-10 fragments with a ratio of 11 (Boardman, N. K. and Thorne, S. W., unpublished observations)

Boardman[1] estimated that the D-10 fraction of spinach contained about 70 % of Photosystem II and 30 % of Photosystem I, and he calculated that pure Photosystem II fragments would have a chlorophyll *a*/chlorophyll *b* ratio of 1.7. Subsequently Arntzen *et al.*[20] obtained a near complete separation of Photosystem I and Photosystem II by incubating the 10-K fraction from a French press treatment of spinach chloroplasts with 2 % digitonin. The Photosystem II fragments from this treatment had a chlorophyll *a*/chlorophyll *b* ratio of 1.8. If the relative proportions of Photosystems I and II in the D-10 and D-144 fractions of *Alocasia* are similar to those of the corresponding fractions of spinach, we estimate that Photosystem II of *Alocasia* has a chlorophyll *a*/chlorophyll *b* ratio of 1.3, and Photosystem I a ratio of about 4.4 (*cf.* ref. 1)

The large grana stacks and higher ratio of total length of grana to stroma lamellae in the shade plant chloroplasts, relative to spinach, did not influence significantly the efficiency of the fractionation with digitonin. This supports the view that digitonin causes a fractionation of the photosystems of the grana[18,21], as well as fragmenting stroma lamellae[3].

The high chlorophyll content of the shade plant chloroplasts is no doubt a consequence of the need for these plants to capture all available light quanta reaching the leaves on the floor of the rainforest. The shade plants are also enriched in chlorophyll *b* relative to chlorophyll *a*, as compared with sun species. This increases further the light-harvesting capabilities of the shade plants by extending the wavelength range over which quanta are absorbed. The leaves of shade plants have much higher ratios of chlorophyll to soluble protein than those of sun plants, indicating that shade plants invest more of their synthetic capacity in the production of light-harvesting assemblies than in the synthesis of Fraction 1 protein (ribulose diphosphate carboxylase) and other soluble proteins[22].

With higher plant chloroplasts, there appears to be a correlation between the total amount of chlorophyll, the proportion of chlorophyll *b* to chlorophyll *a* and the extent of grana formation Thus, chlorophyll-deficient mutants have fewer grana per chloroplast as compared with their normal sibs. For example, a pea mutant which contained about half the total chlorophyll of normal pea leaves and had a chlorophyll *a*/chlorophyll *b* ratio of 10–18, was found to contain fewer grana per chloroplast, and fewer thylakoids per granum as well as fewer paired thylakoids in proportion to the total amount of lamellae in the chloroplast[9]. In the present study, we observe that the pea mutant chloroplasts contain only one-tenth of the chlorophyll of the shade plant chloroplasts. The chlorophyll-deficient mutants of Gaffron and Schmid (*cf.* ref. 2) were also found to have a high ratio of unpaired lamellae to paired grana thylakoids

In contrast, the shade plant chloroplasts contain more chlorophyll per chloroplast, an increased proportion of chlorophyll *b* to chlorophyll *a* and a big increase in the amount of grana thylakoids per chloroplast, as compared with spinach or *Atriplex patula*[23]. A correlation between chlorophyll content, proportion of

chlorophyll *b* to chlorophyll *a* and the extent of grana formation is observed on development of the higher plant chloroplasts during greening of dark-grown plants[2,24] Immature ivy chloroplasts in pale green leaves were also found to have less chlorophyll, a lower proportion of chlorophyll *b* to chlorophyll *a* and fewer paired thylakoids than did mature chloroplasts from dark-green leaves[25].

It seems, therefore, that a high content of chlorophyll per chloroplast favours a higher proportion of chlorophyll *b* to chlorophyll *a* and an increase in the ratio of total length of partitions to length of stroma lamellae. In view of the striking differences in chlorophyll *b* content of some chloroplasts (Table 1), it is pertinent to consider a possible role for chlorophyll *b* in grana formation. Chloroplasts with a high content of chlorophyll *b* tend to have more grana. Pyliotis *et al.*[26] observed an inverse correlation between chlorophyll *a*/chlorophyll *b* ratio and the total length of partitions per unit area of chloroplast for mesophyll and bundle sheath chloroplasts of five species of C_4 plants. But it is known from studies with a barley mutant devoid of chlorophyll *b*[27] that chlorophyll *b* is not essential for grana formation, although the number of grana per chloroplast and number of thylakoids per granum were much decreased in this mutant Goodchild *et al.*[27] suggested that chlorophyll *b* may facilitate the formation of grana. However, the large increase in the content of grana thylakoids in mature ivy chloroplasts as compared with the immature chloroplasts which have few partitions was not accompanied by as striking a decrease in chlorophyll *a*/chlorophyll *b* ratio as might have been expected; in some cases the immature chloroplasts had a chlorophyll *a*/chlorophyll *b* ratio of 3 6, compared with 2.7 for the mature chloroplasts[25]

It would appear more reasonable to relate the extent of grana formation to the total content of chlorophyll per chloroplast rather than to a low chlorophyll *a*/chlorophyll *b* ratio The increase in the proportion of chlorophyll *b* relative to chlorophyll *a* in chloroplasts with high values for the total partition length per area of chloroplast may be a reflection of the high chlorophyll content of those chloroplasts. Grana formation in the higher plant chloroplast may simply be the means of achieving a higher density of light-harvesting assemblies in the chloroplast, and hence a more efficient collection of light quanta.

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