CHLOROPLAST SIZE IN TALL AND SHORT PHENOTYPES OF *POA FLABELLATA* ON SOUTH GEORGIA

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ABSTRACT. The size of the chloroplast compartment is determined by the size of the individual chloroplasts, by the number of chloroplasts per mesophyll cell, and by the number of mesophyll cells per unit leaf area. Studies on two phenotypes of *Poa flabellata* from South Georgia indicate that the presence of seal excreta is associated with much larger individual chloroplasts and fewer mesophyll cells per unit leaf area. The number of chloroplasts per cell is similar in both phenotypes so that overall the chloroplast compartment is 18% larger in those plants growing in elephant seal wallows.

Both the size of the chloroplast compartment relative to leaf area and the distribution of the chloroplasts within the thickness of the leaf are of considerable significance in photosynthesis, and thus to the growth and production of plants. Little is known about the quantitative effects of nutrient supply on the component characters of chloroplast compartment size (Butterfass, 1979). These component characters are the size of the individual chloroplasts, the number of chloroplasts per mesophyll cell, and the number of mesophyll cells per unit leaf area.

On the sub-Antarctic island of South Georgia, coastal vegetation is generally dominated by tussock grass or tussac, *Poa flabellata* (Lam.) Hook.f. (Greene, 1964), a species which can reach 2 m in height on South Georgia and over 3 m in the Falkland Islands. This winter-green grass grows profusely in a wide range of habitats from relatively wet coastal flats to dry, often exposed, hillsides and screes. Stands close to the shore are often strongly influenced by seals and sea-birds, and it is here that the grass grows most luxuriantly, the animal presence being readily distinguished from a distance by the dark green coloration of the foliage. By contrast, plants growing in dry non-nitrogen enriched habitats are relatively short and the leaves pale or yellowish-green. The occurrence of two strikingly different phenotypes of *Poa flabellata* in close proximity to each other in South Georgia, one tall in the wallows of elephant seals (*Mirounga leonina*) and with abundant seal excreta, and the other short and without the seal excreta, gave the opportunity to examine chloroplast compartment size in what is thought to be a single genotype with natural variation in nutrient supply. It is possible that a similar abundance of fur seal (*Arctocephalus gazella*) excreta would have a similar effect on *P. flabellata*, since the contrasting phenotypes were seen on Bird Island near to the main breeding colony of seals (M.B.U., personal observation). Penguin excreta, for example around the breeding colonies of gentoo penguins (*Pygoscelis papua*) at Maiviken, Papua Beach or on Jason Island, appeared to have no noticeable effect on the phenotype of *P. flabellata* (M.B.U., personal observation).

MATERIALS AND METHODS

Site

Two samples of *P. flabellata* were collected from the population growing in the vicinity of the British Antarctic Survey's research station at King Edward Point, South Georgia (54° 16′ S, 36° 30′ W). The habitat is part of a system of raised beaches

(Clapperton, 1971), is more or less level at only a few metres above sea level, and is backed by a steep SSW-facing slope. The climate of South Georgia is summarized in Smith and Walton (1975). It is cool oceanic with a mean air temperature of −1.5°C for August, the coldest month, and a mean of 5.3°C for February, the warmest month. During the summer months the temperature generally does not fall below −5°C, whilst in the winter the minimum usually does not fall below −15°C. The mean annual precipitation is very variable, averaging about 1400 mm. There is strong seasonal variation in the amount of sunshine, with virtually none in June and an average of over 5 h d^{-1} in summer. (The winter data are an underestimate due to shading of the meteorological instruments by Mt Duse.)

The two samples of *P. flabellata* were collected as close together as possible. One, termed the 'tall tussock' (phenotype T), was collected in an area of elephant seal (*Mirounga leonina*) wallows, where tussocks were 1.0–1.5 m high, and where individual leaves were often at least 1 m long. The other sample, the 'short tussock' (phenotype S), was collected from the landward edge of the population, as the ground rises to Hope Point, away from the seal wallows. Here tussocks were appreciably shorter, often not exceeding 0.5 m in height, with individual leaves being 0.3–0.4 m long. Both samples, therefore, came from the same population of this wind-pollinated species. The only obvious difference in the field was the presence of elephant seals, with their associated excreta, in the zone of more vigorous tussock growth. It is unlikely that the small spatial separation between the two samples, less than 100 m, would give rise to appreciably less nutrients being deposited in wind-blown sea spray: the habitat is exposed to strong winds throughout the year with a mean annual wind speed of 4.3 m s^{-1}.

Sampling

Transverse leaf slices of approximately 1 mm thickness were taken from near the centre of fully expanded, green leaves of *P. flabellata* in late January 1981. Samples were fixed immediately in 3.5% glutaraldehyde in 0.1M phosphate buffer for 2 hours, then transferred to 0.1 M NaEDTA pH 9.0 for storage and transport, and to initiate cell separation.

Estimation of number of chloroplasts, cell area and chloroplast area per cell

The cells were separated by shaking the leaf slices in 0.1M NaEDTA at 60°C for 3 hours (based on Possingham and Smith, 1972), and slide preparations made using 0.1M EDTA as the mountant. The numbers of chloroplasts in at least ten mesophyll cells were counted using Nomarski differential interference optics fitted to a Zeiss photomicroscope. Photographs of these cells were taken (×40) using Nomarski optics and bright field illumination. Chloroplast and cell plan areas were determined from these photographs using a 986A Hewlett-Packard digitizer linked to a Model 30 Hewlett-Packard computer. In order to ensure that the cell sample used was representative of the general cell population, low power (×6.3) photographs of the cell preparation were taken and at least 100 cell areas determined. The number of chloroplasts per cell was found to be correlated ($r = 0.60$ and 0.73 for phenotype S and T respectively) with cell area in the small sample ($n \geq 10$). The regression equation from these data was used to estimate what is termed here the 'adjusted mean number of chloroplasts per cell' from the mean cell area from the large sample ($n \geq 100$). Chloroplast area was not correlated with cell size and was therefore not adjusted from the measured value.

Estimation of number of mesophyll cells per unit leaf area

Three transverse leaf slices for each phenotype were washed in distilled water and their widths measured. The number of mesophyll cells was determined from chromium trioxide digests using a 0.2-mm depth haemocytometer as described by Jellings and Leech (1982).

Results and Discussion

The results of the analysis of phenotypes S ('short tussock') and T ('tall tussock') of *P. flabellata* are shown in Table I.

Mesophyll cell size was very similar in the two phenotypes. Since it is generally accepted that cell size is closely linked to nuclear DNA amount (Martin, 1966; Price, Sparrow and Nauman, 1973) and that the haploid nuclear DNA amount is constant and characteristic for a particular species (Van't Hof and Sparrow, 1963; Bennett and Smith, 1976), the similarity of the cell sizes in Table I excludes the possibility that phenotype T is a polyploid of phenotype S.

Since the number of chloroplasts per cell was approximately the same in both phenotypes, it is unlikely that differences in nutrient status affect the number of chloroplasts in *P. flabellata*. Butterfass (1979) concluded from experiments using sugar beet that fertilizer application alone had no effect on chloroplast number per cell despite its considerable effect on dry weight accumulation in the whole plant.

The mean chloroplast area in the two phenotypes was significantly different, that in phenotype T being about twice that in phenotype S. This is demonstrated clearly in Fig. 1 which shows two representative cells from each phenotype. It is therefore concluded at the presence or absence of elephant seal excreta has a profound effect on chloroplast size. Nitrogen application in particular would be expected to increase chloroplast size by way of stimulation of protein synthesis, but the influence of other elements cannot be excluded. The minor elements copper, manganese, boron, cobalt, nickel and zinc have all been shown to increase chloroplast size in both lettuce (Godnev and Leshina, 1961) and sugar beet (Lipskaya, 1961), although the number of chloroplasts was also affected in these instances.

The bigger chloroplasts of phenotype T resulted in a larger chloroplast to cell area index than that of phenotype S; i.e. a larger part of the cell volume of phenotype T was made up of chloroplast material (see Fig. 1). The index was 0.55 in phenotype S

Table I. Measured and calculated characters in phenotypes S and T of *Poa flabellata* with standard deviations (in parentheses).

Character	*Phenotype S*	*Phenotype T*
Leaf width (mm) ($n = 3$)	10.0 (0.9)	15.7 (0.6)
Mesophyll cell plan area (μm^2) ($n \geq 10$)	962 (90)	837 (76)
Estimated number chloroplasts per mesophyll cell ($n \geq 10$)	35 (3)	32 (2)
General mesophyll cell plan area (μm^2) ($n \geq 140$)	928 (25)	1024 (34)
Adjusted number chloroplasts per mesophyll cell	34	35
Chloroplast plan area (μm^2) ($n = 50$)	15 (1)	29 (2)
Chloroplast to cell area index	0.55	0.99
Mesophyll cell number per unit leaf area	91×10^4	54×10^4
Chloroplast number per unit leaf area	31×10^6	19×10^6
Chloroplast to leaf area index	4.65	5.51

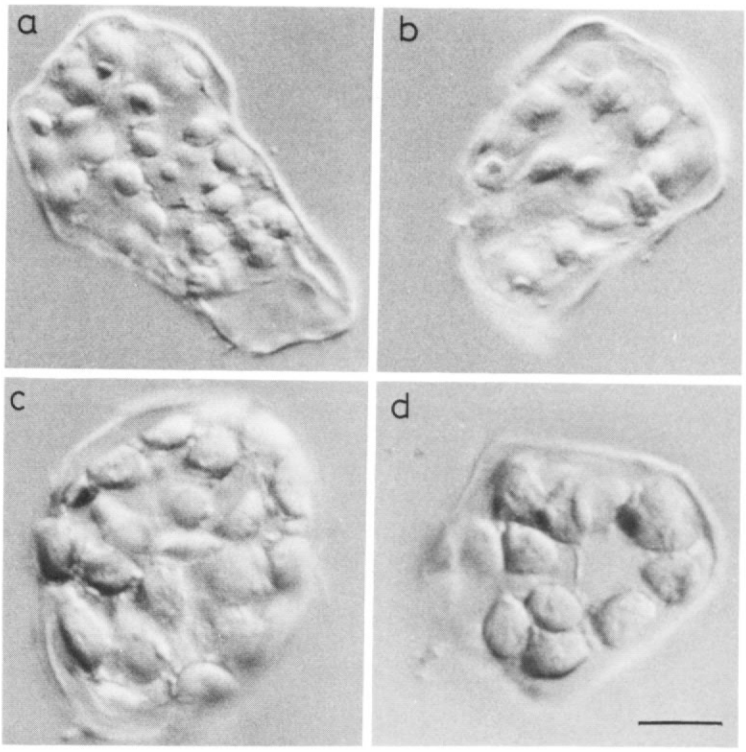

Fig. 1. Separated cells of *Poa flabellata* viewed with Nomarski optics to show individual chloroplasts. a/b phenotype S, c/d phenotype T. Magnification is the same in each case. The bar represents 10 μm.

and 0.99 in phenotype T. Most *Triticum* (wild and cultivated wheats) genotypes have an index in the range 1.0–1.5 (Jellings and Leech, unpublished data). The magnitude of the index clearly reflects the characteristics of the density and distribution of the chloroplasts within the leaf tissue and may relate to the efficiency of diffusion of carbon dioxide to the carboxylation sites: its functional importance with respect to light interception will be modified by the number of cells under unit leaf area in each phenotype.

The number of mesophyll cells under unit leaf area was greater in phenotype S than in phenotype T despite phenotype T leaves being thicker (see Fig. 2). There are two explanations for this seeming contradiction. Firstly, more leaf volume was composed of vascular cells and sclerenchyma in phenotype T and, secondly, regions midway between the vascular bundles lacked functional material in phenotype T though phenotype S appeared normal in this respect (see Fig. 2). These empty regions contained cell wall traces but no cytoplasm or chloroplasts. This study cannot determine the cause of this phenomenon.

Since the number of chloroplasts per cell was the same in both phenotypes, the greater number of cells per unit leaf area in phenotype S resulted in a greater number of chloroplasts per unit leaf area. This compensated to some extent for the smaller chloroplast area in this phenotype so that the chloroplast to leaf area index was closer in the two phenotypes than would have been expected from a chloroplast size comparison alone. Thus the size of the chloroplast compartment relative to leaf area was only about 18% greater in phenotype T than in phenotype S.

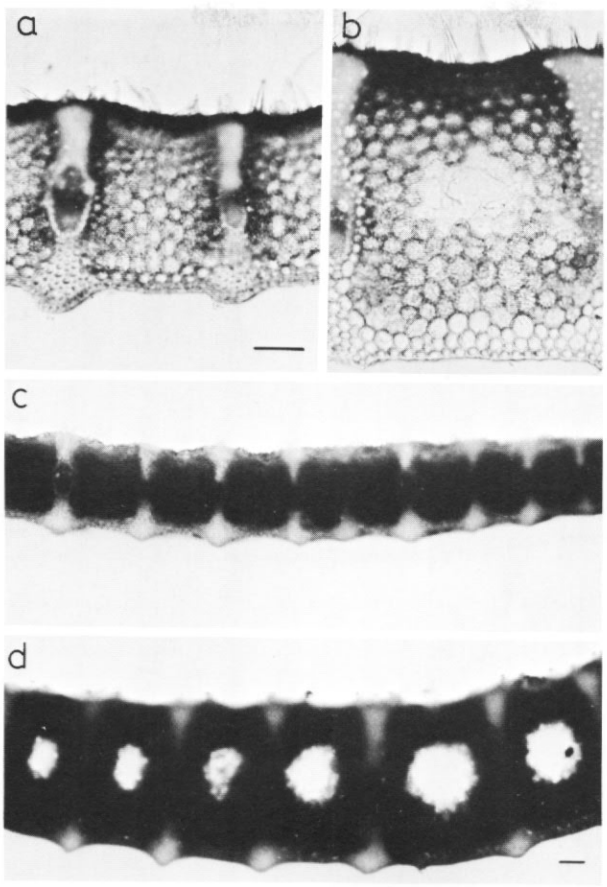

Fig. 2. Hand-cut transverse sections of glutaraldehyde-fixed *Poa flabellata*. a/c phenotype S, b/d phenotype T. Bars represent 100 μm.

It is concluded that the presence of elephant seal excreta is linked to larger chloroplasts, fewer mesophyll cells under unit leaf area, and a greater chloroplast to leaf area index in *Poa flabellata*. The greater chloroplast to leaf area index in the presence of the seal excreta may be sufficient to account for the greater size of plants of phenotype T compared to those of phenotype S.

The chemical composition of *Poa flabellata* samples similar to those used in this study has been analysed previously (Walton and Smith, 1979). Their samples, taken from tussocks growing at a margin of elephant seal wallows, contained three times (on a per cent dry weight basis) the amount of calcium, nearly twice the amount of magnesium, and over twice the amount of phosphorus and nitrogen as samples taken from nearby tussocks growing on a steep well-drained hillside uninfluenced by animals. This information leads to the tentative conclusion that one or more of these elements (i.e. calcium, magnesium, phosphorus and nitrogen), may be responsible for the differences found between phenotypes S and T of *P. flabellata*.

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