VARIATION IN THE CHLOROPLAST TO CELL AREA INDEX IN *DESCHAMPSIA ANTARCTICA* ALONG A 16° LATITUDINAL GRADIENT

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ABSTRACT. Samples from leaves of *Deschampsia antarctica* Desv. taken from sites along a 16° latitudinal gradient from the Falkland Islands to the Refuge Islands exhibit a chloroplast to cell area index cline ranging from 0.38 to 0.96. This was the result of variation in cell size, chloroplast size, and to a lesser extent the number of chloroplasts, between samples taken from different sites. This cline was not related to the mean daily hours of sunshine along the transect at the time of collection, but did show an inverse relationship with the temperature at each site.

INTRODUCTION

The chloroplast to cell area index for any piece of leaf tissue is dependent on the number of chloroplasts in each cell, the size of the individual chloroplasts, and the size of each cell. The index provides a useful measure of the amount of the cell surface area covered by chloroplasts, which in turn indicates the potential for light interception by the leaf.

Most studies of environmental effects on chloroplast and leaf characters use plants grown under controlled high and low light conditions (e.g. Longstreth and others, 1981), compare sun and shade leaves from the same plant (e.g. Lichtenthaler and others, 1981), or compare species adapted to contrasting conditions (e.g. Bjorkman and others, 1976). There are few examples of studies using ecotypes of the same species from a broad range of natural conditions (e.g. Bjorkman and Holmgren, 1963), particularly where the ecotypes are also in reproductive isolation from each other.

The collection of material from a single species (*Deschampsia antarctica* Desv.) along a latitudinal gradient gave us the opportunity to examine the chloroplast to cell area index in isolated populations adapted to a natural range of environmental conditions.

MATERIALS AND METHODS

Sampling

Samples were taken from a long, irregular transect running from near Stanley, Falkland Islands, in the north to the Refuge Islands in the south. Details of the sites are included in Table I, and the transect is shown in Fig. 1.

In general samples were collected from six or more plants in the population. Mature leaves, usually of plants in flower, were selected from plants which had been brought back to a laboratory (either at one of the British Antarctic Survey research stations, or on board RRS *Bransfield*). The centre of the blade was cut into transverse slices of approximately 1 mm thickness with a razor blade, and the slices fixed in 3.5% glutaraldehyde in 0.1 M phosphate buffer for 2 h, then transferred to 0.1 M NaEDTA pH 9.0 for storage and transport, and to initiate cell separation.

Sites

The collection site on East Falkland Island was only a few metres above sea level in a zone subjected to sea spray. *D. antarctica* was growing in cracks between rocks in

Table I Localities where *Deschampsia antarctica* samples were collected, and the dates of collection Latitude and longitude are given for the collection site, and hence may differ slightly from the Gazetteer of the British Antarctic Territory (HMSO, 1977).

Site no.	*Locality*	*Latitude, longitude*	*Date of collection*
1	Nr Rookery Bay, East Falkland Island	51° 43′ S, 57° 48′ W	24 Dec 80
2	Bird Island, South Georgia	54° 00′ S, 38° 03′ W	30 Dec 80
3	Grytviken, South Georgia	54° 17′ S, 36° 30′ W	10 Jan 81
4	Lynch Island, South Orkney Islands	60° 39′ S, 45° 36′ W	25 Feb 81
5	Signy Island, South Orkney Islands	60° 43′ S, 45° 36′ W	25 Feb 81
6	Livingston Island, South Shetland Islands	62° 41′ S, 61° 06′ W	28 Mar 81
7	Andrée Island, Charlotte Bay	64° 31′ S, 61° 30′ W	23 Mar 81
8	Cuverville Island, Errera Channel	64° 41′ S, 62° 37′ W	21 Mar 81
9	Galindez Island, Argentine Islands	65° 15′ S, 64° 16′ W	5 Mar 81
10	Jenny Island, Marguerite Bay	67° 44′ S, 68° 24′ W	14 Mar 81
11	Refuge Islands, Marguerite Bay	68° 21′ S, 67° 08′ W	11 Mar 81

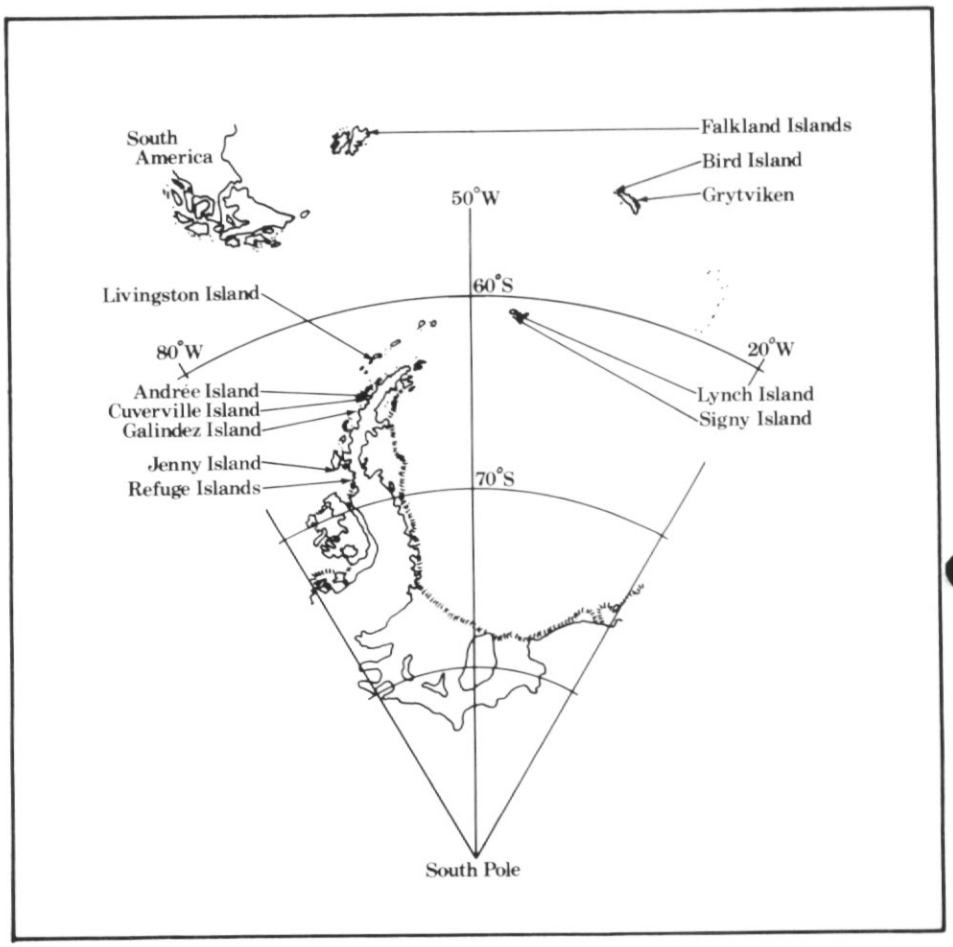

Fig. 1. A map showing the location of the eleven collection sites listed in Table I.

an otherwise open community, and was in full flower. On Bird Island, South Georgia, *D. antarctica* was forming small 'lawns', about 60–80 m above sea level and above the zone of tussock grass, in the valley behind the station. Although these lawns were virtually a monoculture of *D. antarctica*, there was no evidence of flowering at the time of collection. The other South Georgian collection was made at Grytviken, where *D. antarctica* was growing abundantly on the tracks and level areas associated with the disused whaling station, at an elevation of 1 or 2 m above sea level. The plants were flowering at the time of collection.

Six samples from north of the Antarctic Circle were analysed. Two of the samples came from the South Orkney Islands. Lynch Island, a small island which is a Specially Protected Area (visited by permit) close to Coronation Island, has small areas dominated by *D. antarctica* on cliff tops on the north-west coast. The collection was made from one of these areas, at an altitude of about 10 m above sea level. Near the BAS station on Signy Island, *D. antarctica* grows as scattered plants at the base of Factory Bluffs. The site is only about 5 m above sea level, and at the time of collection the plants were in full flower. The Livingston Island population was a relatively dense community, growing on Sealer's Hill on the south coast of Byers Peninsula, at an altitude of about 20 m above sea level. Flowering had finished by the time of collection. On both Andrée and Cuverville Islands *D. antarctica* was growing in open communities towards the north of the islands on sites with generally northern aspects. It was in flower when collected from both sites. On Galindez Island, in the Argentine Islands, a few plants of *D. antarctica* were growing near the BAS station buildings. These were slightly trampled, but were in flower at the time of collection. On all three of these latter islands the collection site was within 10 m above sea level.

A further two sites are south of the Antarctic Circle. On Jenny Island both *D. antarctica* and *Colobanthus quitensis* (Kunth) Bartl. are scattered but abundant at about 20–30 m above sea level on the north-east side of the island. *D. antarctica* was flowering at the time of collection. The Refuge Islands population was discovered in 1981 (Smith, 1982) and represents the southernmost known population of any flowering plant on Earth. It was growing at about 10 m above sea level, on the north-facing slopes of the largest island, and often was restricted to cracks between the rocks where it obtained some shelter. The plants were smaller than those from any other habitat included in Table I, were rather olive-green in colour, but were in full flower at the time of sampling.

Climate

Climatic data are not available for the majority of the sites, but data for a number of British stations have been published by Pepper (1954) and subsequently in reports of the British Antarctic Survey (kindly made available by Dr R. I. Lewis Smith). A summary of mean daily temperature and mean daily number of hours of sunshine is shown in Fig. 2. The sites chosen are at Port Stanley in the Falkland Islands, at Grytviken in South Georgia (105 km from Bird Island, which is known to have less sunshine than Grytviken), Signy Island, the Argentine Islands, and an estimate made for Marguerite Bay which is based on the weighted average of data from Stonington Island, Horseshoe Island and Adelaide Island. The data shown therefore represent, as closely as possible, five of the collection localities listed in Table I, and they also span the complete north to south range.

The sunshine data (Fig. 2) show strong seasonal variation. Marguerite Bay, within the Antarctic Circle, has no sun during the austral winter, but is the sunniest area in the Antarctic during the austral summer (December and January). Both Signy Island and the Argentine Islands have virtually no sun during the winter (May to July), but

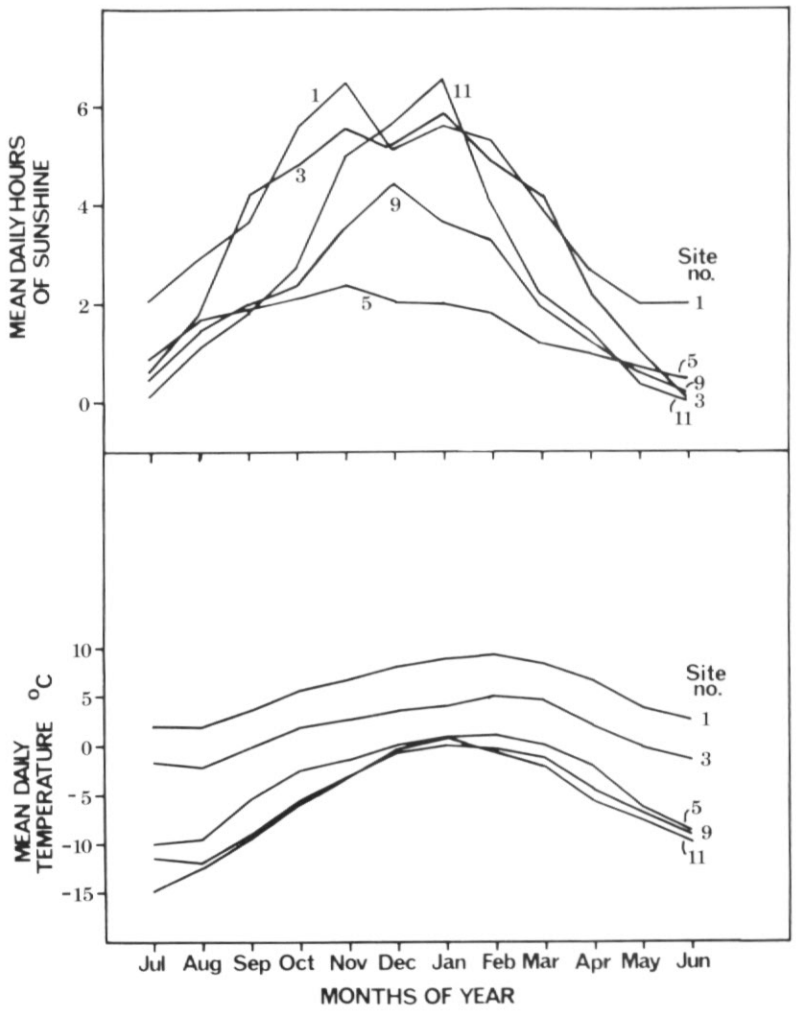

Fig. 2. The mean daily hours of sunshine and the mean daily temperature for five of the sites marked in Fig. 1, with site numbers given in Table I. The data for Marguerite Bay are a weighted average of data collected at Stonington, Horseshoe and Adelaide islands.

they differ in the summer. The Argentine Islands data, which are similar to data collected at Admiralty Bay in the South Shetland Islands, indicate a relatively sunny summer with a mean of about $4\,h\,d^{-1}$, whilst at Signy Island the dull, overcast weather gives a mean of only about $2\,h\,d^{-1}$. Stanley in the Falkland Islands does not have such a clearly defined winter period, the average number of hours of sunshine being $2\,h\,d^{-1}$. In many respects South Georgia is intermediate between the Falkland Islands and the Antarctic: the summer resembles the former and the winter is particularly sunless like the Antarctic sites. More detail is given about South Georgia in another paper (Jellings and others, 1983).

The temperature data (Fig. 2) show a gradation from north to south as would be expected. The mean daily temperature is above 0°C for all months of the year in

Stanley and for half the year in South Georgia. These figures drop to only three months at Signy, and one month in both the Argentine Islands and Marguerite Bay. Although there is greater difference in latitude between Signy Island and Marguerite Bay (over 7°) than between the three northern sites, the temperatures of the Antarctic sites are generally very similar.

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

The cells were separated by shaking the leaf slices in 0.1 M NaEDTA pH 9.0 at 60°C for 3 h (based on Possingham and Smith, 1972), and slide preparations made using 0.1 M NaEDTA as the mountant. The numbers of chloroplasts in 15 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 by means of a 986A Hewlett Packard digitizer linked to a Model 30 Hewlett Packard computer. Cell areas were determined from each of the 15 cells per site used to count chloroplast numbers. In order to ensure that each sample was representative of the general cell population a larger sample of cells ($n = 50$) were photographed at low power and their areas measured, the mean cell area of the smaller sample could then be compared with that of the larger sample. For five sites (nos. 1, 3, 4, 5 and 10), chloroplast areas were determined for each of the 15 cells used above, for the other six sites only five of the cells were used to determine mean chloroplast area; these are referred to as the chloroplast area large sample and the chloroplast area small sample respectively.

The chloroplast to cell area index was calculated for each cell as the ratio of the product of the number of chloroplasts and the mean chloroplast size to the cell area. These data were used to calculate the mean chloroplast to cell area index for each site.

Results and Discussion

Mesophyll cell size distribution at each of the sites is shown in Fig. 3, where cell size was measured as cell plan area. Mesophyll cell plan area (including data from all sites) showed a range from 450 to 1600 μm^2 and the site means for cell area varied between 800 and 1030 μm^2 (from Bird Island and Livingston Island respectively). Analysis of variance for cell area showed significant variation between sites ($P = 0.01$), and the general trend was for the larger mean cell areas to be found in material from mid-transect sites and the smaller mean cell areas from sites at the extremes of the transect (Table II).

The mean cell size for a species is closely correlated with nuclear DNA content (Martin, 1966). *D. antarctica* sampled from Galindez Island has a C-value of about 5 pg (Bennett and others, 1982). Polyploidization of a species involving an increase in nuclear DNA amount results in a concomitant increase in cell size but the variation in cell size found between *D. antarctica* from different sites in this study is insufficient to invoke the existence of sub-populations at more than one ploidy level. Intra-specific nuclear DNA variation (within a single ploidy class) has occasionally been reported, and there is a possibility that it exists among these isolated sub-populations of *D. antarctica:* this is currently the subject of a separate study (M. D. Bennett, pers. comm.). The importance of the size of the nuclear genome regarding the relationship of such characters as minimum generation time to the demands of the harsh Antarctic environment is discussed by Bennett and others (1982).

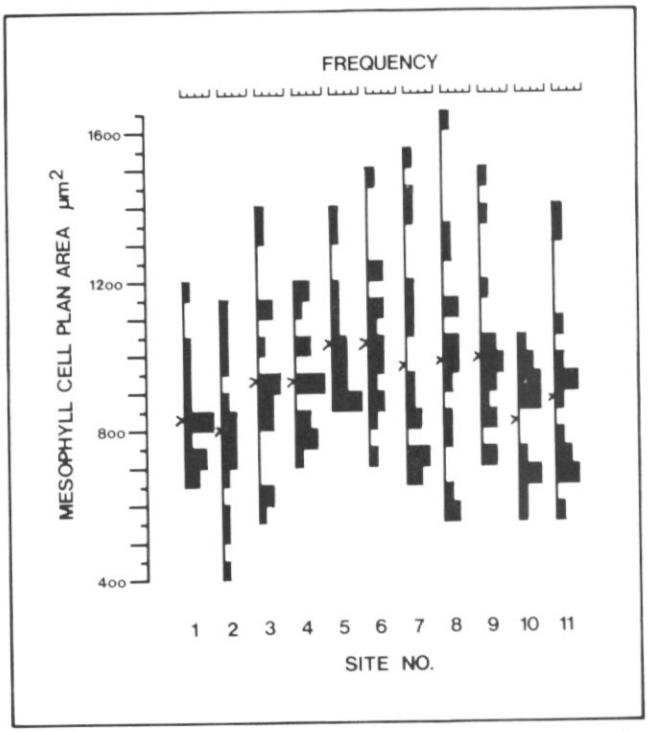

Fig. 3. The frequency of distribution of mesophyll cell size in *D. antarctica*, measured as cell plan area, at each collection site ($n = 15$ for each site). Arrows indicate mean cell area.

The number of chloroplasts per cell, and chloroplast sizes measured as plan areas, for each site are given in Table II. It is usual for the number of chloroplasts in a cell to be correlated with the size of the cell in samples taken from a single tissue type within a species (Leech, unpublished data), and this was true within each site sample in the

Table II Mean data for each site, including mesophyll cell plan area ($n = 15$), number of chloroplasts per cell ($n = 15$), mean chloroplast area per cell ($n = 15$ except for those figures in brackets for which only five cells were sampled for chloroplast area), and the calculated chloroplast to cell area index.

Site no.	*Latitude (°S)*	*Mesophyll cell plan area (μm²)*	*Number of chloroplasts per cell*	*Chloroplast area (μm²)*	*Chloroplast to cell area index*
1	51.72	833	38	8.3	0.38
2	54.00	799	35	(17)	0.74
3	54.28	930	34	13.1	0.49
4	60.65	931	33	19.1	0.69
5	60.72	1 027	35	21.5	0.71
6	62.68	1 033	35	(20)	0.68
7	64.52	974	38	(21)	0.82
8	64.68	982	43	(19)	0.83
9	65.25	991	36	(18)	0.65
10	67.73	822	39	20.1	0.96
11	68.35	878	46	(16)	0.84

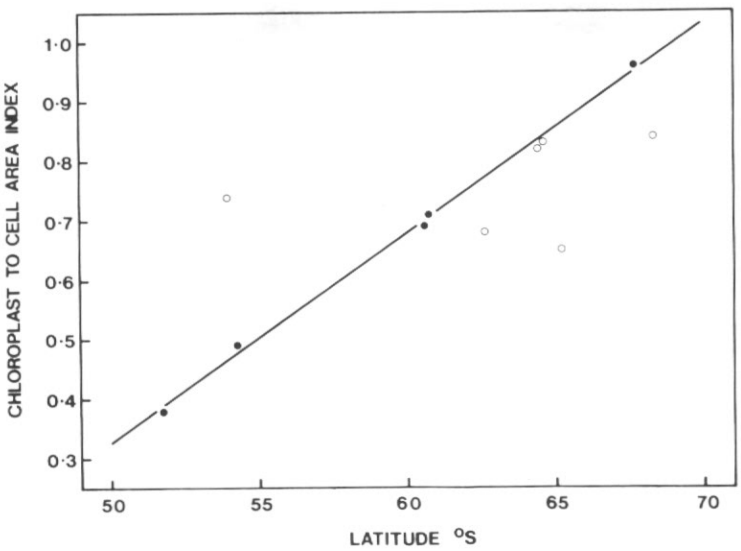

Fig. 4. The relationship between chloroplast to cell area index of *D. antarctica* and the latitude of a series of sites. Closed circles signify chloroplast area large samples, and open circles signify chloroplast area small samples. The correlation for the five large samples is $r = 0.99$ (***) (regression line shown), and for all eleven samples is $r = 0.80$ (**).

present study. There was no correlation between the number of chloroplasts per cell and cell size across sites however. The range for number of chloroplasts per cell within samples (e.g. 24–51 for Falkland Islands sample) and between sample sites (33–46, Lynch Island and Refuge Island respectively) is similar to that found for other species.

Mean chloroplast size measured as plan area varied between 8.3 μm^2 in the Falkland Islands sample to 21.5 μm^2 in the Signy Island sample (Table II). Chloroplast size is a much more flexible character than the number of chloroplasts per cell (Butterfass, 1979) and has been shown to be affected by environmental factors such as nutrient levels (Jellings and others, 1983). Chloroplast size was significantly lower in material sampled at two sites between 50 and 55° S than in that sampled from sites at latitudes greater than 60° S.

The chloroplast to cell area index was calculated from the measured data (Jellings and Leech, in press) and is given in Table II. This index is an indicator of the capacity for light interception at the cellular level. The variation in the index with latitude is shown graphically in Fig. 4. When data from each of the 11 sites is included the chloroplast to cell area index is significantly correlated with latitude ($P = 0.01$). In other words, at lower latitudes the chloroplast to cell area index is low relative to those at higher latitudes. Some interaction between environmental variables and the date of collection in determining the index cline cannot be excluded. The variation in the index between sites is greater than we have found for any species grown under standard growth room conditions.

Thus it appears that a chloroplast to cell area index cline exists in *D. antarctica* growing along a 16° latitudinal gradient in the South Atlantic/Antarctic Peninsula region. Variation in cell size, chloroplast size, and to a lesser extent number of chloroplasts, contributes to the formation of this cline. Examination of the sunshine data given in Fig. 2 shows that the latitudinal cline for the chloroplast to cell area

index is not related to the hours of sunshine along the transect at the time of collection (December–March). However, chloroplast to cell area indices along the transect are negatively correlated ($P = 0.01$) with both the mean daily temperature at the time of collection of the samples (Fig. 2), and with the mean annual temperature at each site. Thus it seems that changes in temperature along the transect may be reponsible for establishing the latitudinal cline of the chloroplast to cell area index for *D. antarctica* found in this study.

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