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Variation in Hypothallus Width and the Growth of the Lichen *Rhizocarpon geographicum* (L.) DC.

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Abstract

Variations in hypothallus width were studied in relation to radial growth in the lichen *Rhizocarpon geographicum* (L.) DC. in South Gwynedd, Wales, UK. Variations were present both within and between thalli and in successive three-month growth periods, but there was no significant variation associated with thallus size. In individual thalli, there were increases and reductions in hypothallus width in successive three-month growth periods attributable to hypothallus growth and changes at the margin of the areolae. Total radial growth over 18 months was positively correlated with initial hypothallus width. These results suggest: 1) individual thalli of similar size vary considerably in hypothallus width, 2) fluctuations in the location of the margin of the areolae in successive three month periods is an important factor determining this variability, 3) hypothallus width predicts subsequent radial growth over 18 months, and 4) variation in hypothallus width is a factor determining between thallus variability in radial growth rates in yellow-green species of *Rhizocarpon*.

Keywords: Lichen, *Rhizocarpon geographicum* (L.) DC., hypothallus width, radial growth, areolae, lichenometry

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1. Introduction

The yellow-green species of the crustose lichen genus *Rhizocarpon* comprise discreet areolae, which contain the alga *Trebouxia*, on a black prothallus or hypothallus composed largely of fungal hyphae. The hypothallus extends beyond the areolae to form a marginal ring up to 2 mm in width. The marginal hypothallus grows particularly slowly with annual recorded radial growth rates (RGR) which range from 0.02 to 2 mm yr^{-1} (Hale, 1983; Armstrong, 1983; Innes, 1985; Matthews, 1994). As the hypothallus extends radially, new areolae develop at the margin. The development of new areolae or the growth of existing areolae results in the advance of the areolar margin behind that of the hypothallus. In addition, areolae may also disappear from the margin resulting in 'retreat' of the areolae and exposure of the black hypothallus (Armstrong and Smith, 1987).

The slow growth of the yellow-green *Rhizocarpon* species has made them an ideal group for use in dating the age of exposure of rock surfaces (lichenometry) (Beschel, 1973; Innes, 1985). The growth curve of *Rhizocarpon* species derived from lichenometric studies, i.e., where RGR is calculated from a graph of the diameter of the largest thallus versus substratum age, suggests an initial 'lag' phase leading to a phase of accelerating growth. The period of relatively rapid growth is followed by a phase of declining growth with large thalli growing at a slower more constant rate (Beschel, 1973; Mottershead and White, 1972; Miller and Andrews, 1972; Miller, 1973). This growth curve, however, differs significantly from that reported for many foliose and placodiod lichen species which show a phase of slow but increasing RGR succeeded by a phase of faster but more constant RGR (Aplin and Hill, 1979; Armstrong, 1974a; 1976; 1996; Hale, 1983; Proctor, 1977).

Relatively few studies have attempted to determine the growth curve of the yellow-green *Rhizocarpon* species by direct measurement in the field. Armstrong (1983) studied 39 thalli of *Rhizocarpon geographicum* (L.) DC. in South Gwynedd, Wales, UK and demonstrated a phase of increasing RGR up to a diameter of approximately 1.5 cm, a phase of more constant RGR until 4.5–5 cm diameter, and a declining phase in thalli greater than 4.5 cm in diameter. In most other studies, however, the variability in RGR between thalli was too great to establish the different growth phases with any certainty (Proctor, 1983; Haworth et al., 1986; Mathews, 1994). In previous studies of *Rhizocarpon geographicum* (L.) DC. (Armstrong, 1983; Armstrong and Smith, 1987; 1996a; 1996b), considerable variation in the width of the marginal hypothallus was observed both within and between thalli which could be a factor determining the variability in RGR (Proctor 1983). The present study had the following objectives: 1) to establish the sources of variability in hypothallus width, i.e., within and between thalli of different size and in successive three-month growth periods, 2) to investigate the factors, such as radial growth of the hypothallus and changes at the margin of the areolae, which may contribute to this variability, and 3) to discuss the implications of variation in hypothallus width for studies of lichenometry.

2. Materials and Methods

Site and thalli

The study was conducted in the field in an area of Ordovician slate rock in south Gwynedd, Wales (Nat. Grid Ref. SN 6196) (Armstrong, 1974b). Unless otherwise stated, all experiments were made using thalli of *Rhizocarpon geographicum* (L.) DC., each on a piece of slate, which were collected from several south-facing rock surfaces. Identification to species can be difficult in this group and thalli are often identified to the subgenus level using the criteria of Poelt (1988). Using the broadly circumscribed criteria of Purvis et al. (1992), however, all thalli used in the study were identified as *R. geographicum*. The collected thalli were placed on horizontal boards in the field and allowed to equilibrate with the environment for a year before the start of each experiment.

Variation in hypothallus width

The objective was to determine the degree of variation in hypothallus width both within and between thalli. Four thalli were selected from each of eight diameter size classes, viz., 1–5 mm, 6–10 mm, and then increasing in 5 mm increments to the 36–60 mm size class; the maximum size of thallus observed on rocks in South Gwynedd. Hypothallus width was measured at four randomly chosen locations around each thallus using Vernier callipers. The data were analysed by analysis of variance (ANOVA) (SuperANOVA software, Abacus Concepts Inc., Berkeley, CA, USA) of a nested classification with the total variance partitioned into variance components, i.e., the variance in hypothallus width attributable to variation within thalli, between thalli of the same size and between thalli of different size (Ridgman, 1975; Armstrong and Smith, 1992).

Variation in hypothallus width and RGR in successive growth periods

Variation in hypothallus width and in the radial growth of the hypothallus was studied in successive three-month growth periods in five thalli from October 1 1995 until March 1 1997. Four locations were selected at random around each thallus and hypothallus width and the radial growth of the hypothallus measured at the beginning of each three-month period using previously described methods (Armstrong, 1973). Measurements were made at the same locations in each time interval. Variations in hypothallus width and in radial growth were analysed for each thallus separately using a two-way (location x growth periods) ANOVA, with the total variance partitioned into components, i.e., the proportion of the total variance attributable to different locations, growth periods and locations x growth periods (Armstrong and Smith, 1992). The location x months variance measures the degree to which differences in hypothallus width or radial growth at various locations around the thallus were maintained in successive growth periods. If this component of variance was large, it would indicate that hypothallus width and radial growth at a specific location was independent of the other locations within the thallus (Armstrong and Smith, 1992).

Radial growth and hypothallus width

The objective was to determine whether variations in the RGR of the hypothallus could account for variations in hypothallus width. On January 1 1996, 32 thalli were selected from the population on the boards. Radial growth of the hypothallus and hypothallus width were measured at five randomly located positions around each thallus using previously described methods (Armstrong, 1973) at three-month intervals until June 1997. The data from the different locations were averaged for each thallus. The correlations between total radial growth over 18 months, the time elapsed in months before measurable growth of the hypothallus was detected, the radial growth of the hypothallus during the first measurable growth period and hypothallus width were tested using Pearson's correlation coefficient ('r'). In addition, the data were analysed using a stepwise multiple regression to determine whether differences in thallus diameter could account for the relationship between radial growth and hypothallus width.

Hypothallus width and changes at the margin of the hypothallus and areolae

The objective was to determine the relative effect of hypothallus growth and changes at the margin of the areolae on changes in hypothallus width in successive three-month growth periods. Four locations were selected at random around each of five thalli. Hypothallus width and the radial growth of the hypothallus were measured at the beginning of each three-month period from October 1 1997 until March 1999 using previously described methods (Armstrong, 1973). In addition, the distance from markers on the rock surface to the edge of the areolae was also recorded at each location. Data were averaged for each thallus. The relationships between change in hypothallus width, radial growth of hypothallus and the degree of advance or retreat of the areolae in a three-month period were studied using stepwise multiple regression to

Table 1. Analysis of variance of hypothallus width and radial growth measured at various locations in five individual thalli of *Rhizocarpon geographicum* (L.) DC. over successive three-month growth periods.

Thallus	F, location	F, periods	s^2L	s^2G	s^2LGP
A) Hypothallus width					
1	5.83**	7.20**	0.30	0.58	0.38
2	4.60*	12.35***	0.12	0.86	0.23
3	0.85 ns	21.94***	neg	0.42	0.04
4	0.73 ns	1.76 ns	neg	0.03	0.07
5	3.82 ns	26.52**	0.02	0.47	0.04
B) Radial growth					
1	0.80 ns	3.24*	3×10^{-6}	4.5×10^{-5}	8.3×10^{-5}
2	0.64 ns	0.80 ns	5×10^{-3}	4.0×10^{-3}	1.0×10^{-3}
3	1.36 ns	13.64**	neg	3.0×10^{-4}	6.0×10^{-5}
4	0.87 ns	0.39 ns	neg	neg	1.1×10^{-3}
5	2.61 ns	1.01 ns	3.1×10^{-4}	0	7.0×10^{-5}

F = Variance ratio, L = Location, GP = Growth periods, neg = negligible, s^2L = Component of variance for location within thalli, s^2GP = Component of variance for months, s^2LGP = Component of variance associated with differences between locations between months. ***P<0.001, **P<0.01, *P<0.05, ns = not significant, i.e., P>0.05.

Table 2. Stepwise multiple regression analyses of the data. A: hypothallus width versus hypothallus growth and thallus diameter, B: hypothallus width versus hypothallus growth and changes at the margin of the areolae.

Y variable	X selected	R	R^2	F	SS_{Ex}
Hypothallus width (A)	Radial growth (X_1)	0.43	0.18	6.58*	18%
	Thallus diameter (X_2)	ns			ns
Hypothallus width (B)	Areolae growth or retreat (X_2)	0.95	0.91	223.06***	91%
	Hypothallus growth (X_1)	0.96	0.93	141.10***	2%

R = Multiple regression coefficient, F = Variance ratio, SS_{Ex} = Percentage of variance attributable to each X, ns = not significant.

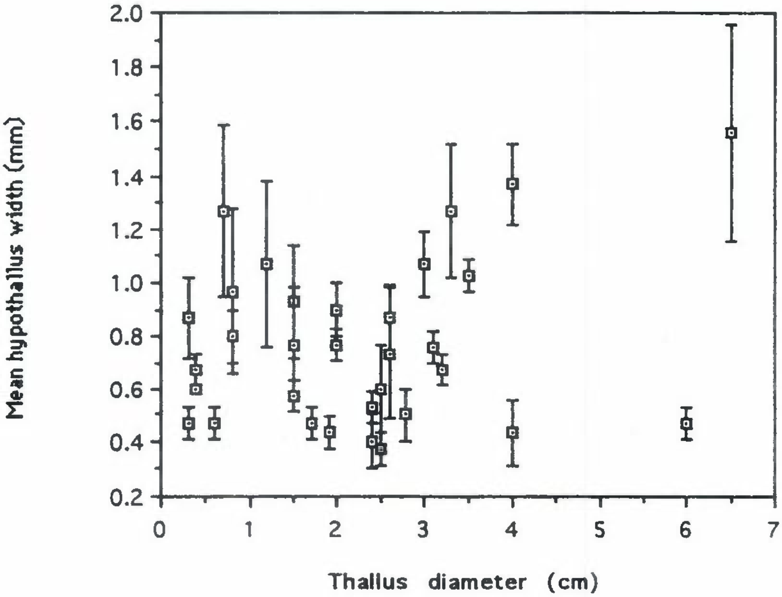

Figure 1. Variation in hypothallus width (error bars indicate 1 standard deviation of the mean) at different locations around individual thalli of different size the lichen *Rhizocarpon geographicum* (L.) DC. (Components of variance: within thalli 2.7×10^{-2}, between thalli within size classes 8.4×10^{-2}, between size classes 5.0×10^{-3}).

determine whether changes at the margin of the areolae or in hypothallus growth was the most important factor determining change in hypothallus width in a three-month period.

3. Results

Variations in hypothallus width in individual thalli of *R. geographicum* of different size are shown in Fig. 1. The standard deviations of hypothallus width suggest variations within the majority of thalli studied but with some thalli showing greater variability than others. The ANOVA suggested no significant variation in hypothallus width between thalli in the different size classes ($F=1.22$, $P>0.05$) but significant differences were present between thalli within a size class ($F=10.12$, $P<0.001$). In addition, the largest component of variance was between individual thalli within a size class; variation in hypothallus width within an individual thallus being of secondary importance.

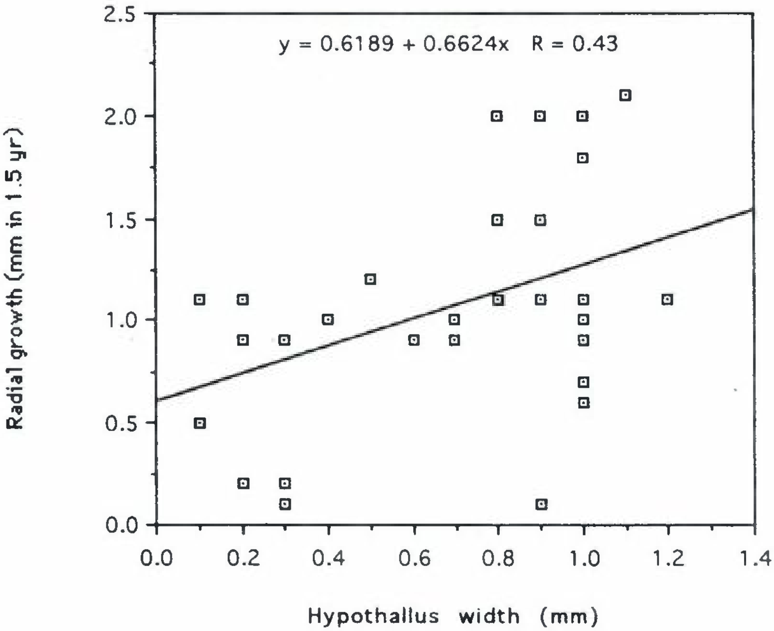

Figure 2. Relationship between the radial growth of the hypothallus, averaged over five locations, (mm in 18 months) and the initial width of the hypothallus (mm) ($r=0.43$, $P<0.05$) in thalli of *Rhizocarpon geographicum* (L.) DC.

Variation in hypothallus width and in radial growth in successive three-month growth periods in five thalli is shown in Table 1. Hypothallus width varied significantly within thalli in two individuals and between three-month growth periods in four individuals. With the exception of one thallus, in which the variations were especially small, the largest component of variance in hypothallus width was associated with differences between the growth periods. There was no significant variation in radial growth at different locations within any of the thalli studied but significant variation in radial growth between growth periods was evident in two individuals.

The relationship between total radial growth over 18 months and initial hypothallus width is shown in Fig. 2. Radial growth over this period was positively correlated with hypothallus width ($r=0.43$, $P<0.05$). The stepwise multiple regression analysis (Table 2) suggested that differences in thallus diameter could not account for the correlation between hypothallus width and radial growth.

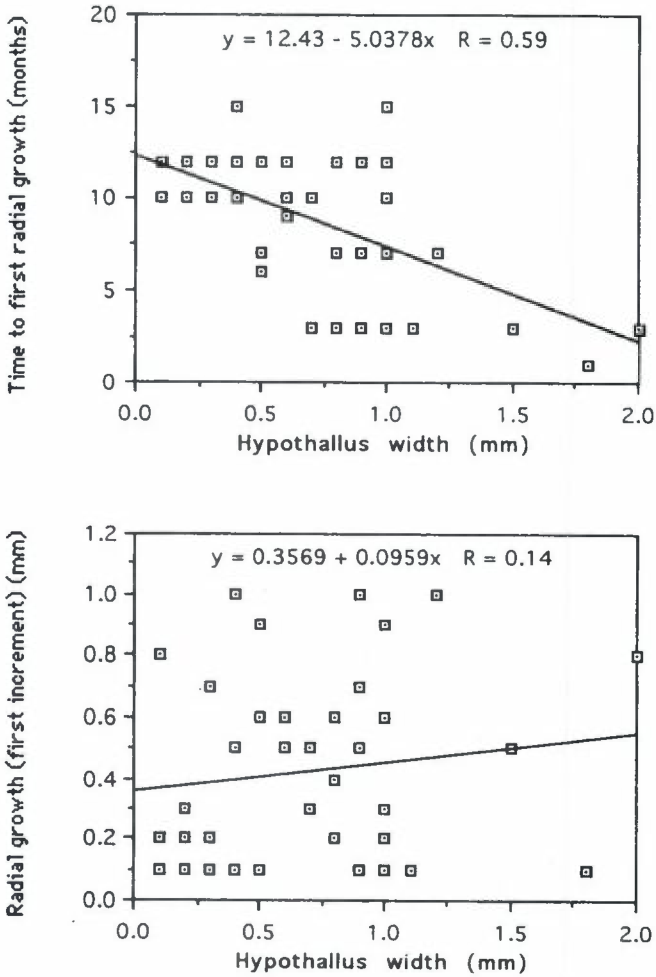

Figure 3. Correlation between initial hypothallus width (mm), the time interval until measurable radial growth (months) (r=-0.62, P<0.001) and the first increment of radial growth (mm) averaged over five locations, (r=0.11, P>0.05) in thalli of *Rhizocarpon geographicum* (L.) DC.

Correlations between hypothallus width, the time elapsed before there was measurable growth of the hypothallus, and radial growth during this first growth increment are shown in Fig. 3. Whereas the length of time before measurable radial growth of the hypothallus was recorded was negatively correlated with hypothallus width (r=-0.59,P<0.001) (Fig. 3), there was no

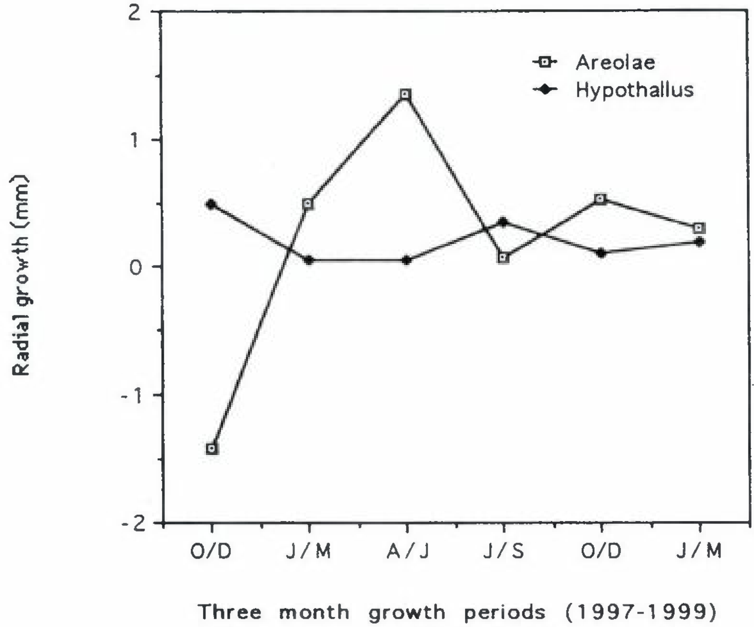

Figure 4. Radial growth of the hypothallus and changes at the margin of the areolae, averaged over four locations, in six successive three-month growth periods in a single thallus of *Rhizocarpon geographicum* (L.) DC.

significant correlation between radial growth during the first measurable growth period and initial hypothallus width ($r=0.14$, $P>0.05$) (Fig. 3).

The radial growth of the hypothallus and changes at the margin of the areolae in successive three-month growth periods is shown for a single thallus in Fig. 4. Changes in hypothallus width resulting from changes at the margin of the areolae were much greater than those attributable to hypothallus growth. The stepwise multiple regression analysis, including data from all thalli (Table 2), suggested that 91% of the variation in the change in hypothallus width in a three-month period was attributable to changes at the margin of the areolae and that only 2% was attributable to radial growth of the hypothallus.

4. Discussion

Although variations in hypothallus width were observed at different points around individual thalli of *R. geographicum*, there was little evidence for significant variation in the radial growth of the hypothallus. More uniform radial growth at different points around thalli of *R. geographicum* contrasts with the considerable lobe to lobe variation in radial growth of foliose thalli (Philips, 1969; Lawrey and Hale, 1977; Benedict and Nash, 1990; Armstrong and Smith, 1992). Carbohydrate may move more readily within the hypothallus of *R. geographicum* than between the lobes of a foliose lichen (Armstrong and Smith, 1996b) and would tend to equalise growth at different locations around the thallus (Aplin and Hill, 1979).

The largest component of variance in hypothallus width was associated with thalli of similar size. At any time, hypothallus width reflects the summation of at least two processes, viz., the growth of the hypothallus and changes at the margin of the areolae which can be either positive or negative (Armstrong and Smith, 1987). Analysis of successive three-month growth periods in individual thalli suggested that changes at the margin of the areolae in some periods were much greater than hypothallus growth. Retreat of areolae could be attributable to seasonal changes in climate or to seasonal variations in the effects of possible predators such as gastropods which are known to graze thalli of *R. geographicum* (1991). Hence, much of the variation in hypothallus width observed between individual thalli may be attributable to fluctuations in the position of the margin of the areolae.

Nevertheless, total radial growth over 18 months was positively correlated with initial hypothallus width; a wider hypothallus indicating a faster growing thallus as reported previously (Proctor, 1983). This correlation may be a consequence of hypothallus width being negatively correlated with the time elapsed before measurable hypothallus growth was recorded, i.e., individuals with narrow hypothalli take considerably longer before growth of the hypothallus can be measured and therefore, show less growth over an 18 month period. Growth may be delayed in thalli with narrow hypothalli because there may be alternating phases of areolae and hypothallus growth (Armstrong and Smith, 1987) and narrow hypothalli indicate a phase of areolae growth.

The present data have two implications for studies of the growth curve of the yellow-green *Rhizocarpon* species by direct measurement. First, if there is a declining phase of RGR in larger thalli as suggested by many lichenometric studies (Beschel, 1973; Mottershead and White, 1972; Miller, 1973) and the growth study of Armstrong (1983), then this phase is unlikely to be attributable to a change in hypothallus width with thallus size. Second, studies which have attempted to determine the growth curve of *Rhizocarpon* species have generally been inconclusive because of the high degree of variation in RGR between thalli (Proctor, 1983; Mathews, 1994). Between thallus variation may be attributable to several factors including differences in microclimate under which the thalli were grown (Armstrong, 1983) or the inclusion of different species of *Rhizocarpon* within an aggregated *R. geographicum* species (Innes, 1985). In addition, the present data suggest that variation in hypothallus width may also contribute significantly to growth variability. Hence, to determine the growth curve of a yellow-green *Rhizocarpon* by direct measurement in the field, studies should standardise, as far as possible, the species studied, the environmental conditions under which the thalli are grown and the initial hypothallus width of the thalli.

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