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Variations in the stomatal density of *Salix herbacea* L. under the changing atmospheric CO_2 concentrations of late- and post-glacial time

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SUMMARY

The rapidly rising CO_2 concentration of the past 200 years has been shown to be accompanied by a fall in stomatal density in the leaves of temperate trees. The present study attempts to investigate the relationship of atmospheric CO_2 change and stomatal density in the arctic–alpine shrub, *Salix herbacea*, over the longer time span of 11 500 years offered by fossil leaves from post-glacial deposits. Comparisons of fossil material from Scotland and Norway are made with leaves from living populations growing in Austria, Greenland and Scotland. The Austrian material, from an altitudinal gradient between 2000 and 2670 m above sea level, gives added comparison of contemporary differences of CO_2 partial pressure with altitude. The results of our investigation indicate, rather surprisingly, that the rising CO_2 concentration of the past 11 500 years has been accompanied by an increase in the stomatal density of *S. herbacea* in contrast to the shorter-term observations on the herbarium material of temperate trees. The most likely explanation appears to centre on the temperatures and water availability of the early post-glacial environment overriding the effect of the lower CO_2 régime. However, the scale of the time interval involved may also be significant. Natural selection over the 11 500 year period concerned may have favoured a different response to what is, in effect, an acclimatory response observed in trees within the period of rapid CO_2 rise of the past 200 years.

1. INTRODUCTION

Woodward (1987) and Beerling & Chaloner (1992) have recently emphasized the importance of stomatal density as a major ecophysiological parameter controlling the productivity of the world's vegetation, and the importance of understanding its response to changing atmospheric CO_2 concentrations. Variations in atmospheric CO_2 concentrations have occurred throughout the Earth's history (Gammon *et al.* 1985), particularly during successive glaciations of the Quaternary period. During the glacial–interglacial phases of the past 160 000 years the CO_2 concentration varied between 180 and 300 p.p.m. (by volume) (Barnola *et al.* 1987) and, over the past 200 years, the CO_2 concentration has risen far more rapidly to the current ambient level of 340 p.p.m.v., due mainly to the combustion of fossil fuels during the industrial era (Friedli *et al.* 1986). Current projections by general circulation models (GCMs), assuming atmospheric CO_2 emissions are kept at present day rates, suggest that a rise in the mean global atmospheric CO_2 level to 520 p.p.m.v. by the year 2100 will lead to an increase in the global surface air temperature of 1.5–4.5°C (Mitchell *et al.* 1990). Although the rate of climate change forecast for the next century is 10–100 times faster than the rate of deglacial warming, the magnitude of both events is comparable (Huntley 1991). Utilizing the Quaternary fossil record therefore provides evidence of long-term plant responses to atmospheric and climatic change of a similar magnitude to that forecast by GCMs for the future.

Woodward (1987) has shown that the stomatal density of eight temperate tree species decreased in response to the increasing atmospheric CO_2 levels of the past 200 years with an ensuing improvement in water use efficiency. These stomatal density measurements were made on herbarium leaves collected over that time span. These results were confirmed in separate experiments by growing plants under elevated CO_2 concentrations in controlled environments (Morison & Gifford 1984; Morison 1985; Woodward 1987; Woodward & Bazzaz 1988; Eamus & Jarvis 1989). Clearly, the duration of exposure to such elevated CO_2 régimes is central to understanding and interpreting the observed responses. Most of these

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experiments were short-term, lasting for days, weeks or months and represent only an immediate response of the plants, in terms of leaf development and physiological behaviour. The trees that formed the basis of Woodward's (1987) observations over a 200 year period may, however, fairly be said to have become acclimated to the ever-rising CO_2 levels, over many seasons. But even over this time span it is most unlikely to represent any genetic change, much less natural selection acting on any genetic heterogeneity of response (cf. the response of grass populations to heavy metal toxicity (Bradshaw & McNeilly 1991)). In nature, plants have been exposed to gradual fluctuations in the atmospheric CO_2 concentrations rather than single-step perturbations, and as Jarvis (1989) recommended 'there is considerable need for long-term experiments in which plant growth is not artificially restricted by unsuitable experimental conditions'.

Current collaborative work between the Universities of London, Durham and Cambridge aims to follow changes in stomatal density and index (the percentage of epidermal cells which differentiate to form stomata) as seen in fossil cuticles of identified extant species. This offers the potential of following these changes over the past 200 000 years (through a glacial–interglacial cycle) under the changing atmospheric and climatic conditions documented by Barnola *et al.* (1987). To assess the significance of changes in stomatal density seen in the fossil record it is essential to measure the inherent variability of densities in a single species growing in a range of habitats and at different geographical locations (Beerling *et al.* 1991). This paper describes the present day stomatal density of *Salix herbacea* L. (dwarf willow) from a variety of habitats in Scotland, Greenland and Austria, and from four fossil samples of *S. herbacea*, giving densities from the Devensian late-glacial. This species was abundant in many full-glacial and late-glacial habitats in N.W. Europe, where conditions suited the plant, and is well represented in deposits of this age (Godwin 1975). The effects of reduced CO_2 partial pressures on stomatal density in *S. herbacea* from an altitudinal gradient in Austria were also examined. On this basis we consider the long-term response of the plant, interpreted from the fossil material, in relation to the documented changes of atmospheric CO_2 levels.

2. MATERIALS AND METHODS

(*a*) *Salix herbacea*

S. herbacea is typically less than 6 cm high and forms loose flattened mats with a large number of aerial shoots arising from an extensive rhizome system (Wijk 1986*a*). Shoots are distinguishable from rhizomes because the latter are segmented; each segment corresponds to an annual shoot increment making it possible to determine the age structure of populations (Wijk 1986*b*). *S. herbacea* is characteristic of alpine snow-bed communities on acidic soils (Caseldine 1989), and often forms an alpine turf with *Carex bigelowii* Torr. ex. Schwein., *C. brunnescens* L., *Anthoxanthum odoratum* L. and *Deschampsia flexuosa* L. (Wijk 1986*b*). The restriction of *S. herbacea* to harsh environments such as exposed rock ledges and summits may be the result of its low competitive ability: it is classified as a 'stress-tolerator' under the C–S–R model of primary strategies (*sensu* Grime 1979). *S. herbacea* has a wide circumpolar distribution in Europe, Asia and North America, and within Britain is confined to upland sites in Scotland, Wales and the Lake District (Meikle 1984). The current distribution of *S. herbacea* in the U.K. is limited by the 23°C maximum summit temperature isotherm in Ireland, Highland Scotland and the Isle of Man, the 24°C isotherm in southern Scotland and the 25°C isotherm in Wales and England (Conolly 1970). This correlation, and the abundance of *S. herbacea* in Quaternary deposits, has been used to reconstruct the temperature during the Devensian glacial, when the distribution of *S. herbacea* extended much further south than the present day (Conolly 1970).

(*b*) *Sources of* Salix herbacea *leaves for stomatal counts*

Stomatal counts were made on fresh, herbarium and fossil leaves. The geographical location of sites from which fossil, herbarium and fresh material was collected are shown in figure 1. Fresh leaves of *S. herbacea* were collected from the Ötztaler Alpen in Austria during July 1989 at 2000 m, 2200 m (male and female plants), 2300 m and 2670 m (north and south facing slopes) and stored in formalin-aceto-alcohol (FAA) solution (ratios of ethanol, ethanoic acid and formalin were, by volume, 18:1:1) before making stomatal counts. Leaves were also obtained from the herbaria at Royal Holloway and Bedford New College (RHBNC) and the Natural History Museum, London (NHM). Leaves from the RHBNC herbarium came from three sites in Perthshire, Scotland (Ben Lawers at 1200 m, Meall Tairneachan at 780 m and Meall Greigh at 1000 m) all collected in the early 1940s. Leaves used from the NHM herbarium were from five sites in Greenland, three on the eastern side at Kronprins Frederiks Bjerge, Kangerdlugssuaq and Christian Bay, and two on the western side at Disko and Melville Bugt, all from sites below 10 m altitude; plants were deposited in the collection during the late 1970s. All herbarium and fresh leaves sampled were of a similar size and where possible were removed from the top 3 cm of a shoot to minimize ontogenetic drift (Tichá 1982).

Fossil leaves were collected from three sites, one in Scotland and two in southern Norway. Scottish leaves were collected from Morrone Birkwoods National Nature Reserve, Braemar, Scotland and sorted from core segments taken at 260 and 325 cm depth from a radiocarbon-dated sequence of late-glacial and post-glacial deposits. Radiocarbon dating gave interpolated ages for the material of 11 087 and 11 500 years before present (BP) respectively. Site details and methods of coring, sorting and dating are given by Huntley (1976). In Norway fossil leaves were obtained

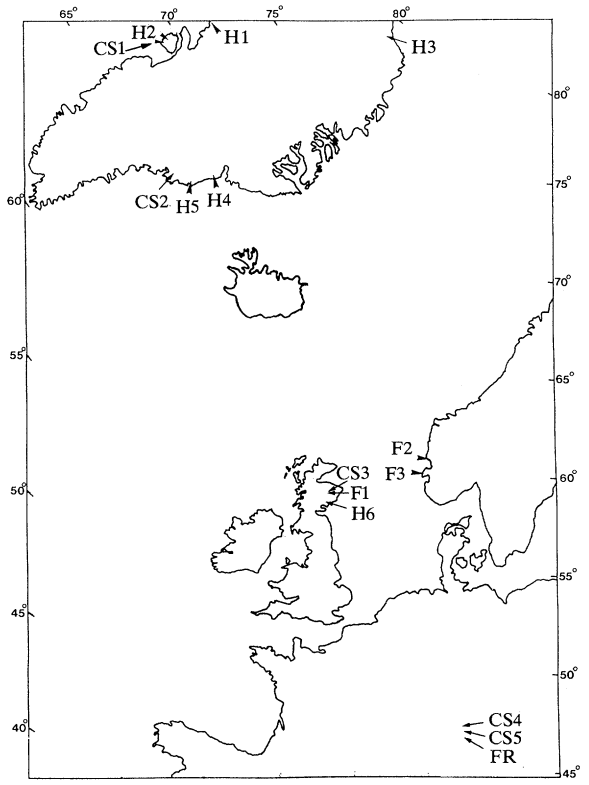

Figure 1. Geographical location of climate stations (CS) and sites from which fossil (F), fresh (FR) and herbarium (H) material were collected. Key to stations and sites: CS1, Godhavn, Greenland; CS2, Angmagssalik, Greenland; CS3, Braemar, Scotland; CS4 & CS5, Innsbruck (at 582 m & 1817 m), Austria. F1, Braemar, Scotland; F2, Blomøy, Norway; F3, Utsira, Norway. FR, fresh material from the Ötztaler Alpen, Austria. H1, Melville Bugt, Greenland; H2, Disko, Greenland; H3, Christian Bay, Greenland; H4, Kangerdlugssuaq, Greenland; H5, Kronprins Frederiks Bjergeis; H6, Perthshire, Scotland.

from Øvre Kvilhaugmyra, Utsira, west of Stavanger (figure 1) at a depth of 168–170 cm and from Blomøy, west of Bergen (figure 1) at a depth of 222–224 cm (material supplied by Dr H. H. Birks, Bergen). Both sites had a radiocarbon date of 10 500 years BP and have been described in full by Paus (1990).

Climatic data from the nearest meteorological stations for Austria, Scotland and Greenland are given in figure 2. Data for Austria are given for two sites, one geographically close to the Ötztaler Alpen and one at a higher altitude (figure 2*a*, *b*). Both diagrams illustrate the wetter climate of these regions with high rainfall during the summer months and consistently cooler temperatures during the year at the higher altitude. Figure 2*d*, *e* illustrates the typical temperature climate of eastern Greenland and Scotland with high rainfall during the winter months and peak temperatures during the summer. Western Greenland has a markedly reduced rainfall compared with the eastern Greenland site (figure 2*c*).

(*c*) *Methods of obtaining stomatal counts*

Stomatal density counts involve the preparation of either a replica of the leaf epidermal features (from both fresh and herbarium material) or in the case of a fossil, the leaf cuticle. These preparations were then photographed at standard magnifications and counts made from projected images. Stomatal densities were estimated from the abaxial and adaxial leaf surfaces of both fresh and herbarium material. The leaf surface was flooded with acetone and a film of cellulose acetate applied; the leaf-plus-acetate was then compressed between flat surfaces, and the acetone allowed to evaporate (10–15 min). The resulting replica is a negative version of the leaf epidermal topography, and was mounted dry (i.e. without mounting medium) and photographed by transmitted light at $\times 40$ magnification on a Zeiss Photomikroskop (Beerling *et al.* 1991). Replicas were made from three fresh leaves per site and photographed, on both sides of the mid-rib, mid-way between the leaf apex and base. Stomatal counts were made on five fossil leaves from each sample by oxidation of the coalified mesophyll tissue with sodium hypochlorite (8% by volume) for 2 min and mounting the resulting cuticles in glycerol jelly with safranin; two to five counts were made for each leaf. In the fossil material it proved too difficult to retain the identity of the adaxial and abaxial cuticles after oxidation of the mesophyll tissue. Specimens were examined and photographed as before.

The photographic negatives were mounted as slides and magnified by projection on to a screen where all stomata within a random equivalent 200 μm × 200 μm quadrat of interveinal area were counted. Stomatal numbers were expressed per square millimetre, after back calculation for the projection factor. The quadrat was calibrated against a photographic slide of a graticule taken at the same magnification as for the specimen. The quality of both the acetate peels from the fresh material and of the mounted fossil cuticles allowed epidermal cells to be distinguished. Stomatal index was calculated as: number of stomata per unit area/(number of stomata + number of epidermal cells per unit area) × 100. This to some extent standardizes for the conditions under which the plants grew (Tichá 1982), and is independent of the extent of leaf expansion during growth.

(*d*) *Stomatal nearest-neighbour measurements*

In addition to the stomatal density and index measurements, the distance of the nearest-neighbouring stoma for each of the stomata within the leaf surface quadrats was noted. Nearest-neighbour measurements were made in millimetres on the projected image and back calculated to micrometres by the projection factor. If the pattern of stomatal distribution is approximately random then the nearest-neighbour value should be inversely proportional to an exponential function of density. However, if as is likely, the stomatal distribution is clumped (underdispersed) this parameter may prove to be a more sensitive measure of response to CO_2 partial pressure. We have recorded it principally to test whether, as the

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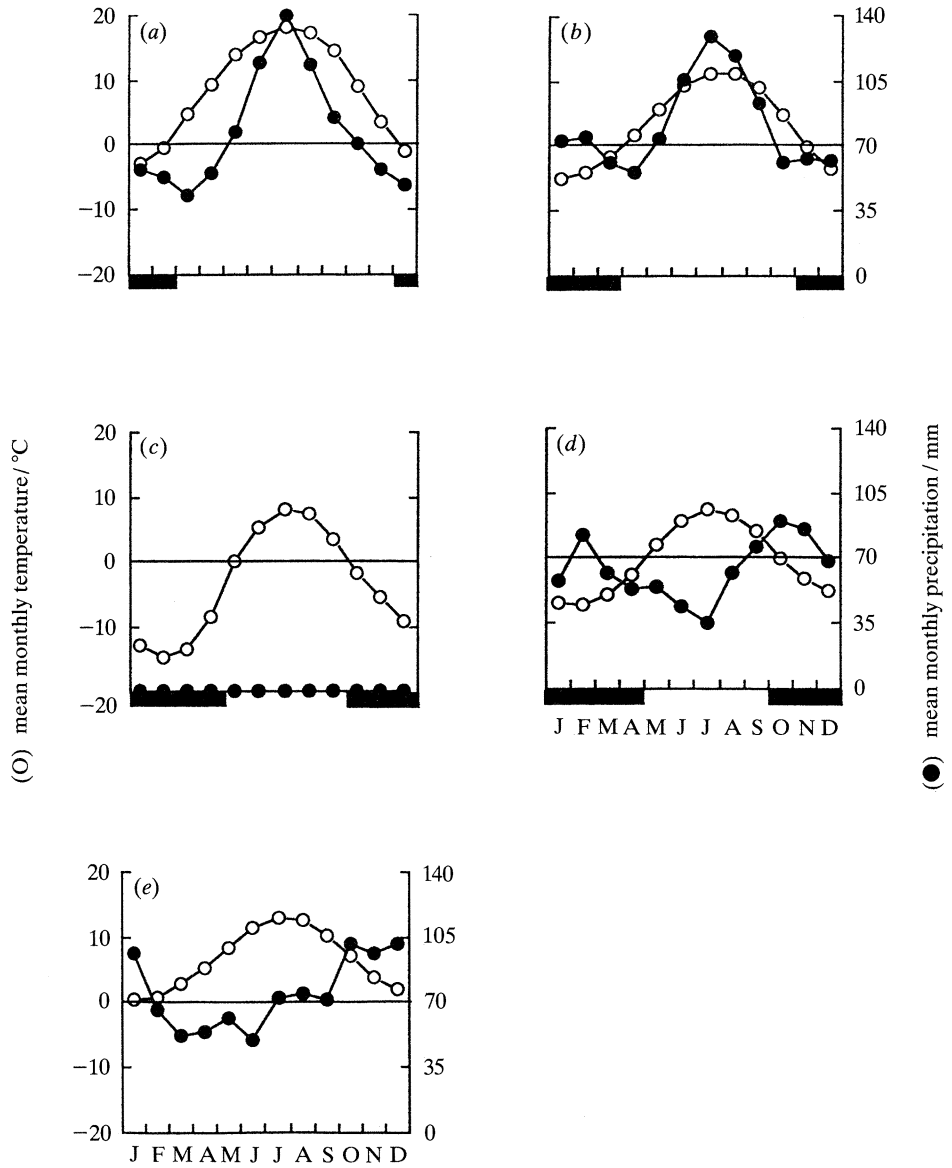

Figure 2. Climate diagrams for the principal sites from which *S. herbacea* material was collected (see figure 1 for geographical locations). Diagrams indicate mean monthly temperatures (open circles), mean monthly precipitation (solid circles). Solid bars indicate months when the mean monthly temperatures fell below 0°C. (*a*) Innsbruck (582 m), Austria, 47°27′ N 11°40′ E, $n = 30$ years; (*b*) Hochserfaus (1817 m), Austria, 47°3′ N 10°60′ E, $n = 30$ years; (*c*) Godhavn (8 m), west Greenland, 69°25′ N 53°52′ E, $n = 19$ years; (*d*) Angmagssalik (29 m), east Greenland, 65°62′ N 37°65′ E, $n = 16$ years; (*e*) Braemar (339 m), Scotland, 57°0′ N 3°40′ E, $n = 30$ years.

work progresses further, this possibility is borne out. It may also prove a simpler measurement to make when dealing with fragmentary material.

(*e*) *Statistical analysis*

The stomatal density data were analysed in two blocks for each locality; adaxial and abaxial values, using one-way analysis of variance performed on SPSSX (1984). *A posteriori* comparisons were subsequently made using Tukeys' procedure (Sokal & Rohlf 1981). Each data set was checked for normality and homogeneity of variance and $\log_{10}$ transformation used when necessary (Sokal & Rohlf 1981). The stomatal index data from the fresh Austrian material was analysed in two blocks; abaxial and adaxial values, using the same methods.

3. RESULTS

(*a*) *Variations in stomatal density between localities and habitats in herbarium and living material*

Statistical comparisons of stomatal density counts obtained from populations of *S. herbacea* growing in Austria, Scotland and Greenland were not valid because plants were not sampled from comparable habitats and so were not attempted. However, table 1 summarizes the coefficient of variation values derived from figures 3–5 and shows that it was greatest in the Scottish material. In the Greenland material, where the effects of altitude are virtually eliminated because all sites were below 10 m, the coefficient of variation was lowest (table 1).

One-way analysis of variance identified significant ($p < 0.05$) differences between the abaxial stomatal

Table 1. *Coefficients of variation calculated from stomatal density counts of* Salix herbacea *plants growing in a range of habitats in three countries and from four fossil samples representing the Devensian late-glacial*

(Values in parentheses indicate mean stomatal densities (per square millimetre). Values for fossil material are means for both surfaces because cuticles were too difficult to categorize after oxidation of the leaf.)

	coefficient of variation (leaf surface)		
country/material	abaxial	adaxial	mean for both surfaces
Austria	22.1 (134)	12.8 (96)	17.5 (115)
Greenland (west)	8.1 (203)	15.1 (203)	11.6 (203)
Greenland (east)	9.0 (171)	6.0 (175)	7.5 (173)
Greenland (all)	12.2 (84)	10.5 (186)	11.4 (135)
Scotland	22.0 (84)	36.1 (78)	29.0 (81)
Scotland (fossil) 11 500 years BP	not applicable		8.9 (64)
Scotland (fossil) 11 087 years BP	not applicable		14.3 (54)
Norway (fossil) Utsira 10 500 years BP	not applicable		18.8 (115)
Norway (fossil) Blomøy 10 500 years BP	not applicable		5.8 (130)

densities from plants growing in different habitats in Austria but not between the adaxial values (figure 3*a*). *A posteriori* comparisons showed that female plants from 2200 m had significantly ($p < 0.05$) lower abaxial stomatal densities than plants sampled from all other localities except those from the south facing slope at an altitude of 2670 m (figure 3*a*). No significant differences ($p > 0.05$) were detected for either leaf surface in stomatal index (figure 3*b*). The Scottish material had lower stomatal densities on both leaf surfaces relative to those from Austria and Greenland. One-way analysis of variance identified significant ($p < 0.05$) differences between adaxial stomatal densities from different habitats, but not on the abaxial densities with higher stomatal densities on plants from Ben Lawers relative to the other two sites (figure 4). In Greenland abaxial and adaxial densities were very similar, and both generally higher than in the Austrian or Scottish material. Stomatal densities varied significantly ($p < 0.05$) only on the adaxial surface where they were lowest at Kangerlugssuaq, Greenland (site 2, figure 5).

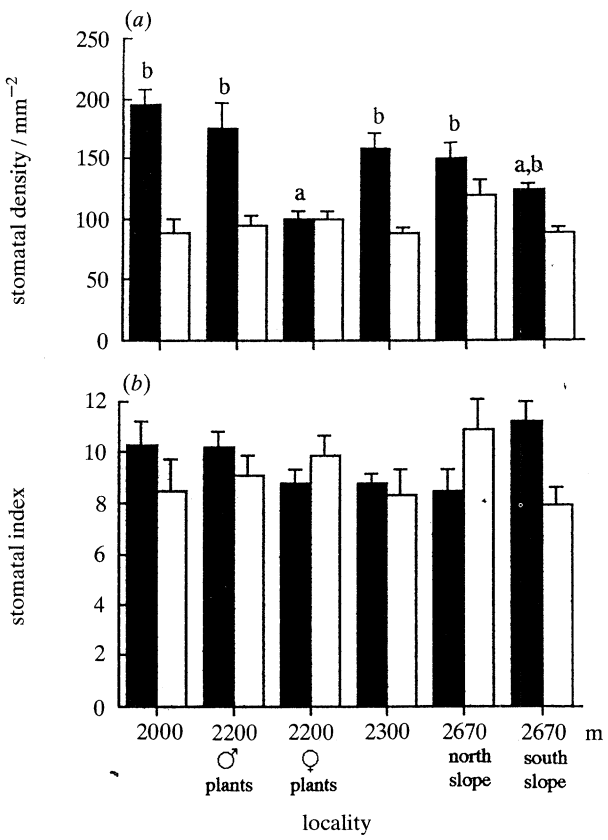

Figure 3. (*a*) Stomatal density and (*b*) stomatal index on the abaxial (solid bars) and adaxial (open bars) leaf surfaces of *Salix herbacea* plants (FR) collected along an altitudinal gradient from the Ötztaler Alpen, Austria. All values are means ± 1 s.e. Bars with the same letter did not differ significantly ($p < 0.05$) by Tukeys' *a posteriori* multiple comparison procedure. Bars without letters did not differ significantly ($p < 0.05$) after one-way analysis of variance; those with two letters did not differ significantly ($p < 0.05$) from any other value.

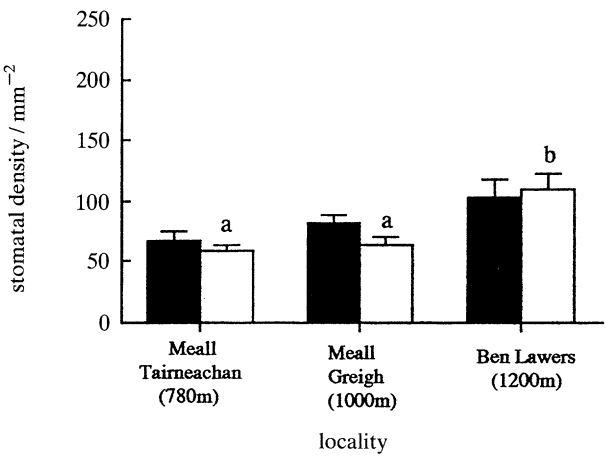

Figure 4. Stomatal density on the abaxial (solid bars) and adaxial (open bars) leaf surfaces of *Salix herbacea* plants (H) from three sites in Perthshire, Scotland. All values are means ± 1 s.e. Bars with the same letter did not differ significantly ($p < 0.05$) by Tukeys' *a posteriori* multiple comparison procedure. Bars without letters did not differ significantly ($p < 0.05$) after one-way analysis of variance.

(*b*) *Relationship between stomatal density and nearest-neighbour measurements*

Figure 6*a*, *b* shows the linear relation of decreasing

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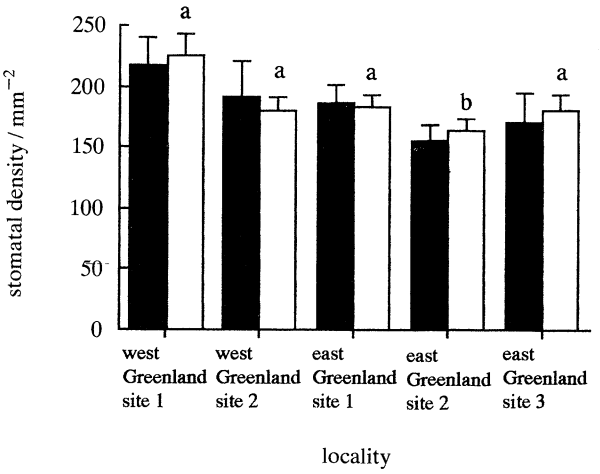

Figure 5. Stomatal density on the abaxial (solid bars) and the adaxial (open bars) leaf surfaces of *Salix herbacea* plants from five sites in Greenland. West Greenland site 1 (H1) = Melville Bugt; west Greenland site 2 (H2) = Disko; east Greenland site 1 (H5) = Kronprins Frederiks; east Greenland site 2 (H4) = Kangerdlugssuaq; east Greenland site 3 (H3) = Christian bay. All values are means ± 1 s.e. Bars with the same letter did not differ significantly ($p < 0.05$) by Tukeys' *a posteriori* multiple comparison procedure. Bars without letters did not differ significantly ($p < 0.05$) after one-way analysis of variance.

nearest-neighbour distance with increasing stomatal density for pooled data sets from all three countries for the abaxial and adaxial leaf surfaces respectively. Significant ($p < 0.05$) exponential equations were fitted to describe the relation (see figure 6) so that stomatal density could be predicted from nearest-neighbour measurements. Nearest-neighbour measurements and stomatal indices calculated for Austrian *S. herbacea* leaves were not significantly ($p > 0.05$) correlated.

(*c*) *Stomatal densities in relation to changing atmospheric CO_2 concentrations*

Figure 7a illustrates the stomatal densities derived from fossil, herbarium and living *S. herbacea* plants in relation to the changing atmospheric CO_2 concentrations (figure 7*b*) determined from the Antarctic ice cores at Vostok and Siple stations by Barnola *et al.* (1987) and Friedli *et al.* (1986). No change is shown in CO_2 concentration between 11 500 years BP and 10 500 years BP (figure 7*b*), but this may merely reflect the coarse resolution of the ice core analyses rather than an absence of a steady gradual rise. The changing stomatal density and index over this period, may therefore, have occurred under the slowly rising

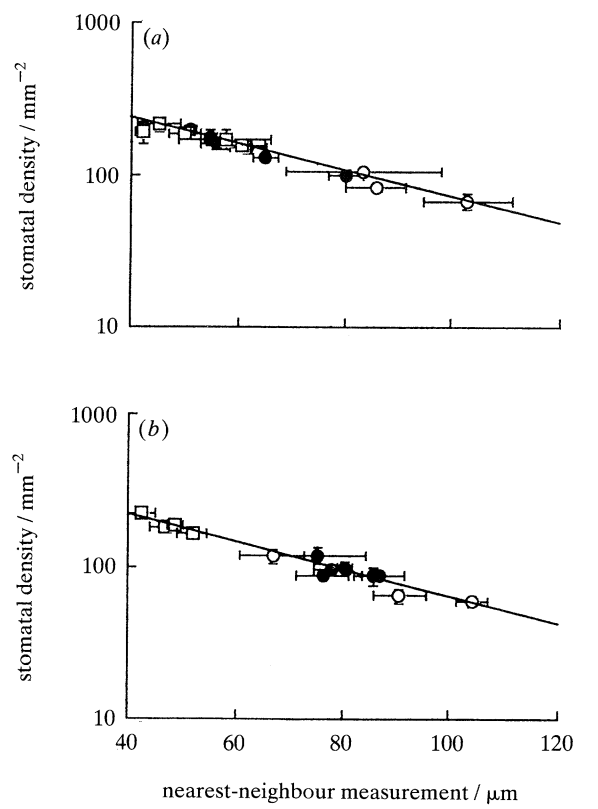

Figure 6. The relationship between (*a*) abaxial and (*b*) the adaxial stomatal density (SD, per square millimetre) and mean nearest-neighbour measurements (NNM, microlitres) for the pooled data set from Austria (solid circle), Scotland (open circle) and Greenland (open square) plotted on a log scale. The relationship for abaxial and adaxial leaf surfaces were described by the following exponential equations respectively: SD, $mm^{-2} = 497.7 \times 10^{(0.0085\ NNM,\ \mu m)}$ ($r^2 = 0.968$) and SD, $mm^{-2} = 504.0 \times 10^{(0.0091\ NNM,\ \mu m)}$ ($r^2 = 0.954$).

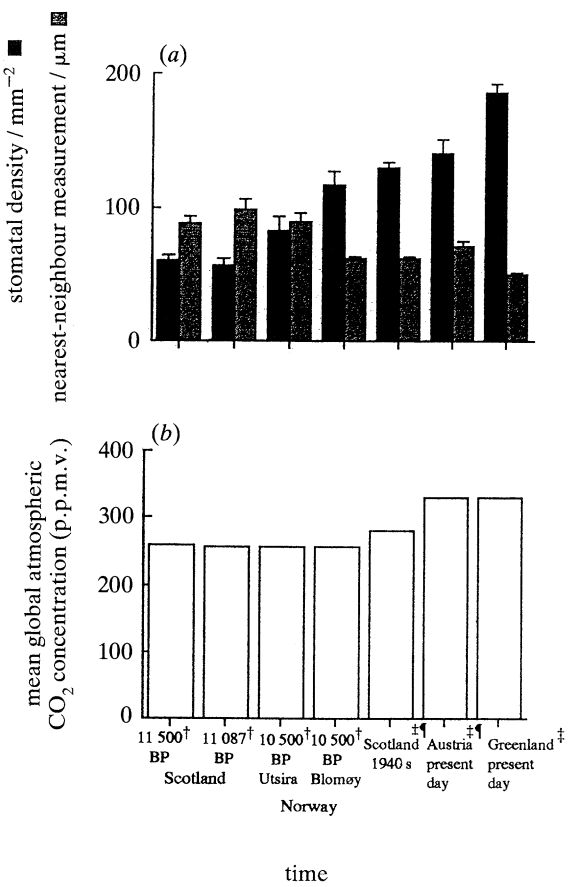

Figure 7. Comparisons of (*a*) the mean stomatal density and nearest-neighbour measurements of *Salix herbacea* for both leaf surfaces combined, from fossil and present day material in relation to (*b*) the mean global atmospheric CO_2 concentrations as determined from ice-core studies. †, Data from Barnola *et al.* (1987); ‡, data from Friedli *et al.* (1986); ¶, figures corrected for mean altitude after Jones (1983).

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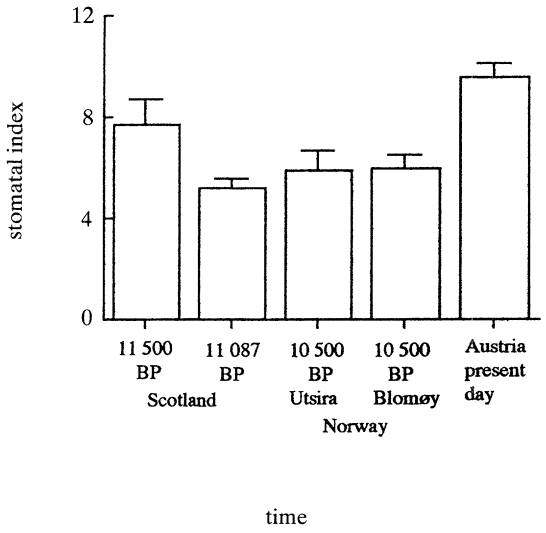

Figure 8. The stomatal index of fossil and modern *Salix herbacea* leaves.

CO_2 concentration of that period which is, as yet, undetermined. The densities presented for the fossil leaves represent a mean for the abaxial and adaxial leaf surfaces (because it was too difficult to keep the cuticles separate after oxidation of the leaf); for comparison, the means of the two leaf surfaces was also calculated for the fresh and herbarium material examined in the study. Fossil cuticles showed markedly lower densities (figure 7*a*) than the present day plants sampled from all three countries, and lower stomatal indices (figure 8) than the Austrian material. Nearest-neighbour measurements (figure 7*a*, *b*) paralleled the stomatal density counts, decreasing with increasing density.

4. DISCUSSION

(*a*) *Variations in stomatal density*

The variation in the stomatal density of *S. herbacea* leaves sampled in this study was low, given the wide range of habitats and geographical locations from which they were collected (table 1). Binns & Blunden (1980) reported the adaxial and abaxial stomatal density of *S. herbacea* as 35–140 mm^{-2} and more than 140 mm^{-2} respectively. Our results do not confirm these values with some plants possessing higher values on the adaxial surface rather than the abaxial (see figures 2 and 3); Binns & Blunden (1980), however, only sampled leaves from a single plant growing in Fermanagh, Ireland. The coefficient of variation in stomatal density determined from the fossil material ranged from 6–19%, which compares with 18% for populations growing in Austria along a wide altitudinal range (table 1). The response of stomatal density of other species to environmental gradients (light, CO_2 concentration, altitude) show a similar range of values from 10–36% (table 2). Given that these values represent an extremely broad range of abiotic factors it is considered that the between plant variability due to genotypic or local environmental effects of a species is not sufficiently great as to mask the response of stomatal density shown in the late- and post-glacial leaves.

S. herbacea leaves collected from plants growing along an altitudinal gradient in Austria were formed under progressively reduced CO_2 partial pressures at higher altitudes (Gale 1972). In this situation the leaves responded by reducing the abaxial stomatal density with increasing altitude and keeping the adaxial density constant (figure 3). Woodward (1986) demonstrated the same pattern of results on the abaxial leaf surface for the shrub *Vaccinium myrtillus* L. growing along an altitudinal gradient on Ben More in central Scotland, and independently confirmed these by growing the shrub at reduced CO_2 molar fractions in controlled environments. Most species with amphistomatous leaves have higher densities of stomata on the adaxial surface (Mott *et al.* 1982). This trend was observed in *S. herbacea* at some sites in Greenland, Scotland and Austria, but was particularly absent in Austrian female plants which had low numbers of stomata on the abaxial leaf surface (figure 3*a*). This was surprising because it suggested that female plants had reduced demands for CO_2 fixation compared with male plants. This is contrary to what might be expected because female plants would require greater amounts of assimilate for the production of fruit and seeds. Leaf conductance measurements made on *S. polaris* L. showed that females had a higher leaf resistance than males where they occurred in Spitsber-

Table 2. *Coefficients of variation calculated from reported stomatal density counts for different plant species growing along environmental gradients calculated from the authors' original data*

(Values in parentheses indicate mean stomatal density (per square millimetre.)

Species (environmental gradient)	coefficient of variation (leaf surface) abaxial	adaxial	author
Vaccinium myrtillus (200–1100 m)	12.3 (251.6)	91 (30.6)	Woodward (1986)
Nothofagus menziesii (30–1200 m)	9.4 (165.6)	—	Körner *et al.* (1986)
Griselinia littoralis (50–550 m)	8.1 (101.0)	—	Körner *et al.* (1986)
Eucalyptus pauciflora (940–2040 m)	10.2 (159.9)	10.2 (160.0)	Körner & Cochrane (1985)
Pentaclethra macroloba (photon flux density 10–500 $\mu mol\ m^{-2}\ s^{-1}$)	29.9 (237.5)	—	Oberbauer & Strain (1986)
Zea mays (340–910 p.p.m. CO_2)	18.7 (86.2)	15.0 (71.6)	Thomas & Harvey (1983)
Glycine max (340–910 p.p.m. CO_2)	35.8 (283.8)	22.7 (117.6)	Thomas & Harvey (1983)
Liquidambar styraciflua (340–910 p.p.m. CO_2)	9.9 (319.2)	—	Thomas & Harvey (1983)

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gen, and it has been suggested that this attribute may have reduced female mortality (Crawford & Balfour 1983). Both *S. herbacea* and *S. polaris* exhibit female-biased sex ratios of 59:41, close to the theoretical optimum 60:40, and it may be that by reducing CO_2 fixation and water loss, female plants increase their ability to persist in harsh habitats under severe climatic conditions. Clearly, further work is required to elucidate the ecophysiological differences between the male and female plants of *S. herbacea*.

(*b*) *Response of stomatal density to changing atmospheric CO_2 concentrations*

Previous work on the effects of changing CO_2 concentrations on stomatal density using controlled environments, altitudinal gradients, leaf macrofossils and herbarium leaves has been reviewed in detail elsewhere (Beerling & Chaloner 1992). Woodward (1987) provides the longest timescale of response, using herbarium leaves of temperate British trees up to 200 years old. His results showed a *reduction* in stomatal density over this period corresponding to the rising atmospheric CO_2 concentrations documented by the ice-core studies of Friedli *et al.* (1986). In the present study, evidence from fossil *S. herbacea* leaves shows that stomatal density has *increased* (figure 7) since the Devensian late-glacial, based on the rather limited fossil material analysed, and that a similar trend is shown by stomatal index (figure 8).

The short-term response of trees examined by Woodward (1987) seems to have represented a direct response to a rising atmospheric CO_2 concentration. However, the leaves of *S. herbacea* examined in this study were responding to changing régimes of CO_2 concentration, temperature and water availability. It seems therefore, that over the short time interval studied by Woodward (1987), plants effected improved water use efficiency at the expense of increasing their productivity. Over the longer time period represented by this study, the response (at least for *S. herbacea*) is different; with rising mean temperature *S. herbacea* may have been able to increase its carbohydrate production by increasing stomatal density (figure 7), at the expense of water use efficiency. Such a response may have been because climatic amelioration brought increased availability of moisture, or that increased water use efficiency was compensated for in other ways (e.g. increased root production). This suggests that for *S. herbacea* the changing temperature and water availability régime over this long time span, overrode the CO_2 change. It also indicates the possibility of a hierarchical set of environmental factors controlling stomatal density and index. One such factor, environmental temperature, could explain the differences between the stomatal density response of temperate tree species and the arctic–alpine *S. herbacea*. Warren Wilson (1966) proposed that in an arctic–alpine environment low temperatures depress respiration and growth, resulting in slow utilization and subsequent accumulation of assimilates, especially sugars. It has also been shown that slow growth, a trait exhibited by many arctic–alpine species as a consequence of reduced day length and temperature (e.g. *Salix arctica* L. Warren Wilson 1964; *Salix glauca* L.; Sampson & Jones 1977; *Cassiope tetragona* L., Callaghan *et al.* 1989), leads to photosynthate accumulation (Bagnall *et al.* 1988). This accumulation then acts as a negative feedback process causing depression of assimilation by end-point inhibition (Bagnall *et al.* 1988; Wulff & Strain 1982). Although it has recently been shown that in the temperate species *Arabidopsis thaliana*, storage of photosynthate allows rapid growth where excess nitrogen is available and carbon availability is limited (Schulze *et al.* 1991), this situation is unlikely to prevail in arctic–alpine regions. Analyses of arctic plants have shown that their sugar content can be 2–3 times higher than temperate species. *S. herbacea* had a sugar content of 28.1% (expressed as a percentage of alcohol insoluble material) where it grew on Jan Mayen Island (71° N) in the Greenland Sea, and this level was maintained irrespective of habitat type and sampling time (Warren Wilson 1966). For comparison, the heathland plant species *Calluna vulgaris* L. and *Erica tetralix* L. had mean sugar concentrations of less than 10% when sampled from different geographical locations during March, December and August (Bannister 1981). It would seem, therefore, that there is no net benefit for plants in fixing more CO_2 in a cold environment, if they cannot already utilize all the assimilates currently being produced.

(*c*) *Limitations of the evidence*

The results presented in this paper must be considered with three important caveats. Firstly, fossil leaves have been obtained from relatively few geographical locations and have also been examined in small numbers. As more material of the appropriate age becomes available it will be possible to further test the current results. Secondly, as Conolly (1970) has pointed out, ecotype evolution may have occurred since the late-glacial which, while not affecting the morphological characters of a species, may have affected its environmental range, tolerance and ability to compete. Thirdly, comparisons of stomatal density counts from fossils with living material were made from different locations. However, in the absence of living material from southern Norway and Scotland the limited variation in stomatal density observed (table 1) suggests that this factor is unlikely to affect the observed trend of lower late-glacial densities compared with the present day, as measured from two different countries.

Further investigations of our results using controlled environment cabinets with temperature regulation facilities would clearly be worthwhile, especially because it has recently been demonstrated that there are genetically determined intraspecific differences in species response to the combined effects of elevated temperature and CO_2 concentration (Wulff & Alexander 1985). Such an approach was adopted by Tissue & Oechel (1987) who exposed, *in situ* for ten weeks, a tussock tundra dominated by *Eriophorum vaginatum* to elevated CO_2 (680 p.p.m.v.) and tem-

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perature (+4°C above ambient). The results, in comparison with plant growth under an elevated atmospheric CO_2 concentration of 680 p.p.m.v. but ambient temperature, showed that this ecosystem did not have significantly higher photosynthetic rates. Furthermore, these plants exhibited reduced tillering rates and had similar non-structural carbohydrate concentrations. Our current limited understanding of the interactive effects of elevated temperature and CO_2 concentration on plant growth is also one of the major factors constraining the ability of dynamic-growth crop-simulation models to accurately predict water use requirements and crop yields in a 'greenhouse' world (Adams *et al.* 1990).

There is of course the further consideration that extant populations of *S. herbacea* have had some 11 500 years to respond to the rising CO_2 concentrations. It may be that over this time interval, long-term natural selection favours a different response to elevated CO_2 than that observed by Woodward (1987) in forest trees under the much more rapid change seen over the past 200 years. The time available for the action of natural selection, as well as differences in water availability and temperature, may have contributed to this seemingly contradictory response of *S. herbacea* compared with forest trees. Many of the tree species examined by Woodward (1987) have generation times in excess of 100 years (Bennett 1986) and the response shown over the 200 year period in question is probably due to phenotypic response. With a maximum recorded shoot age of 10 years (Wijk 1986*b*) the arctic–alpine shrub *S. herbacea* is much more likely to have undergone genetic change over a period of 11 500 years – this representing some 1150 possible generations – and so have been subject to natural selection. Our results suggest that the long-term response of plants cannot be predicted by direct extrapolation of Woodward's (1987) observations, especially where they are likely to be accompanied by changing water availability and temperature. Great interest must now be attached to the changing stomatal densities of Quaternary leaves from lower latitudes. These will have experienced the same changing CO_2 régime, but under less drastic temperature changes than those of northwestern Europe (Frakes 1979). Work on these lines is currently in progress.

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