New Phytol. (1995), **131**, 311–327

The influence of CO_2 concentration on stomatal density

BY F. I. WOODWARD[1] AND C. K. KELLY[2]

[1] *Department of Animal & Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK*
[2] *Department of Biological Sciences, University of North Texas, Denton, Texas 76203-5218, USA*

(*Received 27 February 1995; accepted 21 July 1995*)

SUMMARY

A survey of 100 species and 122 observations has shown an average reduction in stomatal density of 14·3 % (SE ±2·2 %) with CO_2 enrichment, with 74 % of the cases exhibiting a reduction in stomatal density. A sign test demonstrated that stomatal density decreases, in response to CO_2, significantly more often than expected by chance. Repeated observations on the same species indicated a significant repeatability in the direction of the stomatal response. Analyses which removed the potential effect of taxonomy on this data set showed no significant patterns in the dependency of the degree of stomatal change on growth form (woodiness vs. non-woodiness; trees vs. shrubs), habitat (cool vs. warm) or stomatal distribution on the leaf (amphi- vs. hypostomatous).

Forty-three of the observations had been made in controlled environments and under a typical range in CO_2 enrichment of 350–700 μmol mol^{-1}. For these cases the average stomatal density declined by 9 % (SE ±3·3 %) and 60 % of the cases showed reductions in stomatal density. When analyses were restricted to these 43 observations, amphistomatous samples more frequently had greater changes in stomatal density than did hypostomatous samples.

The degree of reduction in stomatal density with increasing CO_2 increases with initial stomatal density, after the influence of taxonomy is removed using analyses of independent contrasts. When the data were examined for patterns that might be due explicitly to the effects of relatedness, the subclasses of the Hamamelidae and the Rosidae showed highly significant reductions in stomatal density with CO_2 (87 % of the species studied in the Hamamelidae and 80 % of the species in the Rosidae showed reduction with CO_2 enrichment) and correlations between initial stomatal density and degree of reduction in stomatal density. The species sampled in the Hamamelidae were dominantly trees, whereas herbs dominated the species in the Rosidae. There were insufficient species studied at lower taxonomic levels to warrant further statistical analyses. This problem results from experimental and observational data being most often restricted to one species per taxonomic level, typically up to the level of order, a feature which can severely limit the extraction of taxonomically-related and ecologically-related plant responses.

Key words: Stomatal density, CO_2, climatic change, evolutionary comparative method, allometry.

INTRODUCTION

Stomatal density strongly influences water use efficiency in plants (Woodward, 1987; Woodward & Bazzaz, 1988; Mansfield, Hetherington & Atkinson, 1990), and it is therefore of interest that changes in CO_2 concentrations might induce changes in leaf stomatal densities which in turn control maximum values of stomatal conductances (Eamus, Berryman & Duff, 1993; Berryman, Eamus & Duff, 1994). Careful experimental studies (Ferris & Taylor, 1994) and a review of observations (Beerling, Putland & Woodward, 1995) have demonstrated that stomatal density responds to CO_2 concentration over the range from *c.* 200–700 μmol mol^{-1}, supporting earlier observations on individual species (Madsen, 1973; Oberbauer, Strain & Fetcher, 1985; Woodward, 1987; Woodward & Bazzaz, 1988).

Within species, stomatal density shows a regular linear or log-linear response to changes in CO_2 concentrations. Between species there are differences in degree as well as direction of response. In response to CO_2 enrichment, some species show decreases, others increases and others no change in stomatal density (Appendix). Especially because of this variability, understanding of interspecific patterns in stomatal density responses to CO_2 changes would be of substantial value, both in providing a better framework for interpreting the fossil evidence of stomatal densities (Beerling & Chaloner, 1991; Van

der Burgh *et al.*, 1993; Van der Water, Leavitt & Betancourt, 1994) and in projecting potential changes in plant responses to future increases in the CO_2 concentration of the atmosphere. Over the last 20000 yr, the CO_2 concentration of the atmosphere has varied approximately twofold – from 180–355 μmol mol^{-1} (Barnola *et al.*, 1987; Houghton, Callender & Varney, 1992); the current CO_2 level is predicted to increase by 1·8 μmol mol^{-1} per year, through the accumulation in the atmosphere of CO_2 released from the burning of fossil fuels and forest trees. A central goal of this paper is to identify a predictor variable that would allow knowledge about the response of stomatal density to elevated CO_2 in one species to be applied to another species and therefore predict future responses to changing CO_2 concentrations.

In this paper, we apply the tenets of the evolutionary comparative method (Harvey & Pagel, 1991) to analyse a large data set of observations on stomatal density responses to CO_2 enrichment. The aim is to extract predictor variables of stomatal density responses by either factoring out, or explicitly examining the effects of taxonomic differences between species. The evolutionary comparative method (ECM) rests upon the observation that closely related species are likely to be similar because they are related. A consequence of this pattern is that a statistical analysis might produce an apparent positive association between traits because it includes a preponderance of species from a group that has the traits in question, not because of any ecological relationship, but rather because the common ancestor of that group just happened to have those traits. In other words, treating individual species as independent data points can confound phylogeny and ecology. In order to avoid this problem, and to elucidate functional relationships, any potential effects due to relatedness must be factored out of the analysis. Therefore we have used methods that eliminate the effect of relatedness in examining interspecific associations between maximum stomatal change in response to elevated CO_2 and the variables stomatal position (amphi- vs. hypostomaty), life-form (woody vs. non-woody; tree vs. shrub), habitat (cool vs. warm) and initial stomatal density. We have performed these analyses, when possible, for experimentally and non-experimentally-derived data in a common data set, and for experimentally-derived data alone.

In addition to removing the effect of relatedness statistically, we have also explicitly examined the possibility that particular patterns in stomatal density response might be specific to one taxonomic group (Felsenstein, 1985; Harvey & Pagel, 1991; Peat & Fitter, 1994; Kelly & Beerling, 1995). We have applied nested ANOVA to determine the possible explanatory value of any taxonomic level, and then used these results as a basis for performing individual analyses within taxonomic groups shown by the ANOVA to differ in responses (cf. Harvey & Pagel, 1991; Harvey & Mace, 1992).

METHODS AND MATERIALS

Stomatal density measurements

This analysis is based primarily on published observations of stomatal densities in experiments and from field observations (herbarium and/or macrofossil leaves). The full data set is shown in the Appendix and the sources of the data are listed in the reference list. Stomatal density is sensitive to a wide range of environmental factors and also to position on the leaf (Salisbury, 1927; Tichá, 1982; Woodward, 1987; Beerling *et al.*, 1995; Kelly & Beerling, 1995). Therefore it is important that observations of stomatal density are made on leaves which developed under very similar environmental conditions, apart from CO_2 concentration, and that the observations are made on similar areas of the leaf lamina. Analyses outside these restrictions might well explain observations of extreme values of responses (Körner, 1988). Even time sequences of fossil leaves can be selected from very similar temperatures during periods of changing temperature, by adjusting the altitude and latitude of collection (Van der Water *et al.*, 1994), and there is little problem in identifying the same area of leaf for observations. The fact that some publications do not indicate the precise method of sampling suggests the possibility that differences in stomatal density might be recorded as responses to CO_2 concentration, when the actual responses might be due to systematic sampling errors on the leaf surface or to microclimatic differences, such as collections of sun and shade leaves.

In order to minimize the inclusion of such potentially inappropriate data, all of the observed data of stomatal density responses were entered into a frequency histogram. The percentage responses of stomatal density to CO_2 concentration formed a normal distribution including some outliers from field observations with uncertain microclimates and methods of recording. An analysis of the data collected only in controlled, experimental conditions indicated that 99·7 % (± 3 standard deviations) of the observations of stomatal density responses to CO_2 enrichment fell within the range of a ± 64 % change in density. No outliers were observed and so these data, from a wide taxonomic range, are taken to show the breadth of the stomatal density responses to CO_2. As a consequence, outliers with responses greater than 3 standard deviations of the mean in the full data set have been excluded from the analyses, on the grounds that they indicate the effects of other factors which are independent of CO_2. In practice this approach assumes that the stomatal density response to CO_2 is restricted within relatively small limits, a

feature which is supported by all careful experimental studies. We also note that this approach will allow the inclusion of responses due to environmental factors other than CO_2 if they are within this defined range.

Taxonomic analysis

Although the phylogenetic history of most plant species is imperfectly known, a taxonomic classification can provide a working representation of phylogeny (Harvey & Pagel, 1991; Gittleman & Luh, 1992). Therefore we have constructed the phylogenetic 'tree' necessary for our analyses by applying the taxonomic classification of Cronquist (1981), one of the most widely used plant classification systems. Our tree incorporates all species in our data set; by convention, we refer to the category 'species' as being 'low' in the hierarchical structure, with categories such as subdivision and division defining nodes 'high' in the overall structure.

The hypothesis that the patterns of response might differ depending upon the taxonomic level, or the specific taxonomic group to which a species belongs, is initially tested by applying a nested analysis of variance model to the target variable, if it is continuous (Harvey & Pagel, 1991; Harvey & Mace, 1992; Peat & Fitter, 1994). The nesting within the ANOVA model represents the nesting of taxonomic classification, such that the factors examined are species within genera (error), genera within families, families within orders, etc. A significant F-value, and/or having a large proportion of the variance accounted for by a particular taxonomic level might indicate that the target trait behaves differently within the groups that comprise that taxonomic level. For example, a significant F-value at the level of subclass for stomatal density response suggests that the pattern of stomatal response might depend on whether a species is in the Magnoliidae or the Hamamelidae. Absolute values of percent change in stomatal density under low and high CO_2 levels were entered into an eight-level, nested ANOVA model with unequal sample sizes (Sokal & Rohlf, 1995). However, because percent change is a derived value [(final stomatal density − initial stomatal density)/(initial stomatal density)], initial stomatal density and the absolute value of the change in stomatal density were also entered into similar nested ANOVA models.

Explanations of methods that account for relatedness among species are most easily divided into those dealing with categorical variables, and those dealing with continuous variables. We examined as categorical variables life form (woody vs. non-woody; shrub vs. tree) habitat type (cool vs. warm), stomatal distribution (hypo- vs. amphistomatous) and increased vs. decreased densities of stomata in relation to absolute value of percent change in stomatal densities with increased CO_2. Presence or absence of response was also treated as a categorical variable. To analyse this last factor, we assumed that response was demonstrated conclusively only if the percent change in stomatal density [(initial stomatal density − final stomatal density)/initial stomatal density] was greater than 10%. A frequency histogram of percent change over the complete data set formed a largely normal distribution, but with a notably higher frequency in categories $\leqslant 10\%$. We assumed that this was a function of placing in the lower echelons apparent change that was actually due to errors of measurement. Initial stomatal density was treated as a continuous variable, by comparing interspecific patterns of initial stomatal density (density of stomata at the lowest CO_2 level) with changes in stomatal density (initial density of stomata − final density) and CO_2 concentration. The common logarithms of the absolute values of initial stomatal density, percent change in stomatal density, and increase in stomatal density were the variables analysed.

Two or more observations were available for 17 species with responses within three standard deviations of the mean and were used in the larger data set, multiple intraspecific responses were available for five additional species which met this criterion but otherwise lacked information for one or more of the variables to be analysed. For categorical variables, intra-specific information was included explicitly where possible (e.g. *Vaccinium myrtillus* had both amphi- and hypostomatous examples, and these were used in a comparison of response versus stomatal distribution). However, differences between populations within a species might be less than those between two species, even if the latter are derived from an immediate common ancestor and approximately equal evolutionary distances between samples is an assumption of most ECM analysis. In consequence, we have analysed our data set both with and without the explicit use of subspecific examples. When not included explicitly, subspecific values were averaged into a composite value for the species (see Harvey & Pagel, 1991 and Garland, Harvey & Ives, 1992 for the reasoning behind this step).

Analyses of categorical variables are based on the idea that the important data points in demonstrating a functional relationship in a categorical variable are the 'changes' in the branches of the phylogenetic 'tree' that has been constructed with the available data (e.g. Ridley, 1983; Harvey & Pagel, 1991; Kelly & Purvis, 1993; Peat & Fitter, 1994; Kelly & Beerling, 1995; Kelly & Woodward, 1995). The particular analysis possible for a data set of this size focuses on points within the data set where one may infer that the categorical variable 'changed,' and a concomitant change in stomatal density response should occur if there is a functional relationship

between the two. 'Points of change' are identified by taxonomic groups that include more than one type of the categorical variable. The assumption is made that the common ancestor of this group existed in one of the two forms of the variable, and that the presence of more than one form of the variable indicates one such a point of change. We look for these points starting with the lowest taxonomic level available in the data set. Within a taxon, percent change in stomatal density is averaged over each form of the categorical target variable, for two reasons: (1) an average of percent change in stomatal density best recreates the response pattern of the presumed common ancestor (the one that originally exhibited that form of the categorical variable); (2) such averaging discounts differences between groups in speciation or sampling intensity. After completing comparisons at one level, we proceeded up to the next taxonomic level at which there was a point of change. A group that had been used for a comparison at a lower level was excluded from any higher-level comparisons, as such a group could not definitively be categorized as possessing one form or the other of the categorical target variable. For the next comparison, lower-level groups were averaged over each form of the target variable, in successive steps based on taxonomic level. Species were averaged within a genus to obtain an indication of the stomatal density response level of the ancestral species that gave rise to that genus; continuing, a family-level mean was the average of genus-level means; family level means were used to construct an average for an order, and so on.

For each categorical variable, a sign test was used to test the total number of comparisons against the number of disagreements with the hypothesis (Siegel, 1956). Frequencies of the signs of comparisons to categorical variables were also entered into a contingency table and segregated by taxonomic level. A random distribution of agreements and disagreements was assumed to be 50:50, as expected from a binomial distribution; and significant deviation from random would suggest that the relationship between the categorical variable and stomatal density response was in some way dependent upon the taxonomic level examined.

In addition, we investigated the possibility of interrelationships among stomatal distribution, the presence or absence of response, and the direction of response (increase vs. decrease in stomatal density) using a method introduced by Ridley (1983). Once again using the taxonomic tree constructed from Cronquist's classification, each species in the data set was categorized according to the state of the three categorical variables. Working up the tree step-by-step from species to genus to family, etc., the ancestral states of each of the variables at each step was then assigned as that state which would produce the minimum number of 'changes', as defined above, when considering the entire tree. After construction of this trait-tree of each of the three variables, changes in state of any one of the three traits was noted when moving from any higher to the next lower taxonomic level. At any point where any one of the three traits changed state, the state of all three of the traits was recorded in a $2 \times 2 \times 2$ contingency table, with each 'side' of the table possessing the two forms of the represented traits. For example, when moving from the level of subclass Hamamelidae to the level of order, in two of the orders, Fagales and Urticales, all three traits are in the same states as in the assumed ancestor from which the subclass arose, but the Capparales and Hamamelidales each have differences in one of the traits. The latter two orders thus contribute one data entry each, in the category represented by the traits that each of the groups possesses. Further entries in the three-way table were determined by proceeding down the trait-tree, and recording the state of all three traits each time one or more 'changes' were observed when moving from one taxonomic level to the next lowest. The table produced in this manner was analysed with log-linear model (SPSSPC), to determine the occurrence of significant associations between any two, or all three, of the traits.

The evolutionary comparative methods used to investigate continuous variables were derived from Felsenstein (1985), and applied the algorithms developed by Pagel (1992). In order to look at relationships between or among the continuous variables, subtaxa within each taxon were split into two groups based on similarity in the assumed causal variable (here, initial stomatal density). Again beginning at the species level, those species having an initial stomatal density greater than the average for the genus were placed in one group; those with an initial stomatal density less than the average were placed in a second group. The difference in the means between the two groups provided the contrast for initial stomatal density for the genus in question. Within-group averages in the dependent variable (here, changes in stomatal density) were also produced for the same two groups of species. and used to calculate a contrast for the dependent variable. The contrasts for independent and dependent variables are in agreement when the difference for each is in the same direction; if the contrasts for independent and dependent variables are in opposite directions, then, by convention, the independent variable is assumed to be positive, and the dependent variable negative (Garland *et al.*, 1992). In the next step, the overall average value of the dependent variable in a genus was taken to represent the ancestral species of that genus, and the same sort of contrasts were then performed between generic groups within a family, using the same criterion for dividing the family into two groups. This method of creating dichotomous contrasts within a taxon was

continued to the highest level of taxonomic organization possible.

The contrasts for the common logarithms of the two variables within each taxon were entered into a regression forced through the origin (Garland *et al.* 1992; SPSSPC), with contrasts for log (initial stomatal density) on the x-axis, and contrasts for log (difference in stomatal density) on the y-axis. A significant regression indicated a functional relationship between the two variables. When the regression proved significant, and the coefficient of determination sufficiently large, the data were then entered into a general structural relations model for the purpose of developing an allometric-type relationship between the untransformed variables of the form $y = x^a$.

The frequencies of the signs of the contrasts (i.e. + & + vs. + & −) between initial stomatal density and increase in stomatal density were tested for differences between subclasses, and between taxonomic levels, using heterogeneity χ^2 (Siegel, 1956). A group where any of the observed values was zero was excluded from the analysis. A significant result for any of these tests would indicate that the relationship between the variables differed among the groups tested.

Cross-species analyses

Evolutionary comparative analyses represent a new means of looking at potential predictors of response to changes in CO_2 levels. Until recently, cross-species analyses, in which species are treated as independent data points, have predominated. In order to relate previous studies to the analyses presented here, we have also applied simple cross-species regression models to the same data sets that were used in the analyses accounting for relatedness.

RESULTS

Data structure

The data set used for the comparative analysis has been arranged by taxonomy (Appendix). A total of 100 species has been investigated in 122 sets of observations. Cross-species analyses showed several strong patterns. Without accounting for any effects of relatedness, CO_2 enrichment induced a mean percentage reduction in stomatal density of 14·3 % (SE ±2·2 %). When only cases with CO_2 enrichment above current ambient CO_2 concentrations were considered ($n = 43$), the stomatal density was reduced by 9 % (SE ±3·3 %).

The percentage change in stomatal density with CO_2 concentration (Fig. 1) followed a close to normal distribution, with 74 % of all of the observations showing reductions in stomatal density with CO_2 enrichment. In controlled-environment experiments with large CO_2 enrichments (Fig. 2) a smaller percentage of cases (60 %) showed reductions in stomatal density. Further analysis of the data in the Appendix showed that species were thinly spread among taxa (Fig. 3*a–c*). Even at the level of the order (Fig. 3*a*) more than 40 % of the cases were for one species per order, which increased to more than 70 % of the cases with one species per genus (Fig. 3*c*).

In some cases one species may have been investigated more than once, by different investigators. Such cases have been selected (Table 1) to determine the fidelity of the stomatal density response by a species. Of the 21 cases of repeated observations on the same species, 16 retain the same sign of response and five differ between observations. This is a significant fidelity (sign test; $P = 0{\cdot}013$) in the stomatal density response to CO_2 by individual species. Additionally, 15 of the 21 consistently decrease in stomatal densities in response to elevated CO_2, allowing the conclusion that stomatal density generally declines with increasing CO_2 concentration (sign test; $P = 0{\cdot}039$).

Taxonomic analyses

Categorical variables. The primary goal of the study described in this paper was to identify factors that could predict reliably the degree of change in stomatal density with changes in CO_2 concentration. However, no categorical variables did successfully (Table 2) for the full data set. In addition, analyses of the frequencies of the signs of the contrasts showed no significant differences between taxonomic levels in the association between stomatal density response in any of the categorical variables (Table 3), a possible explanation for the lack of significance of the overall test. However, the small absolute number of comparisons for any one of the categorical variables would make detection of any such pattern difficult.

Interestingly, one factor became significant when the data set was restricted to experimentally-derived samples. When analyses included only the 43 experimentally-derived data points, amphistomatous samples had a greater change in stomatal density than hypostomatous samples significantly more often than expected by chance (Table 2). This did not correspond merely to amphistomatal samples having greater densities of stomata initially (see below), as there was no significant relationship between stomatal distribution on the leaf and initial stomatal density (eight comparisons/four disagreements; $P = 0{\cdot}637$).

No relationship was found (Table 4) between any two, or among all three of the variables in the log-linear analysis of presence or absence of response, direction of response, or stomatal distribution on the leaf (amphi- vs. hypostomaty).

Continuous variables. The continuous variables examined showed much stronger and more easily

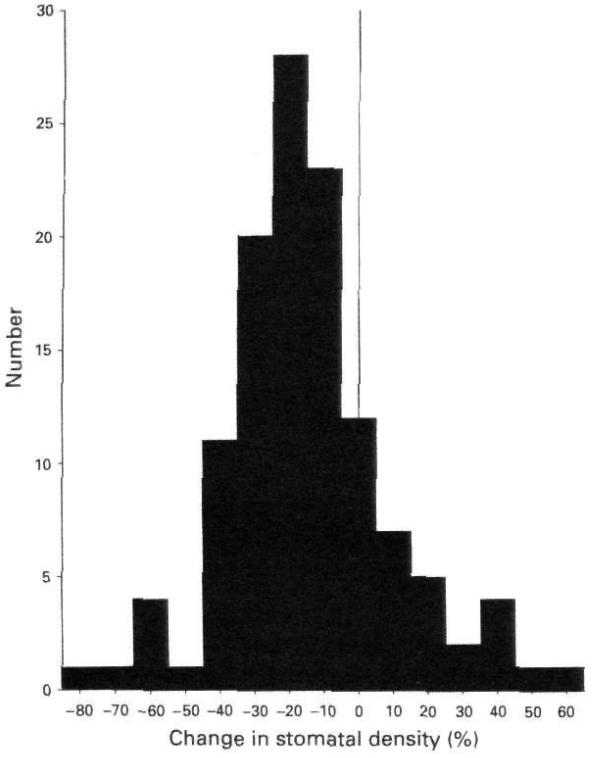


Figure 1. Stomatal density responses to CO_2 enrichment, measured as percentage changes relative to the lowest CO_2 concentration studied.

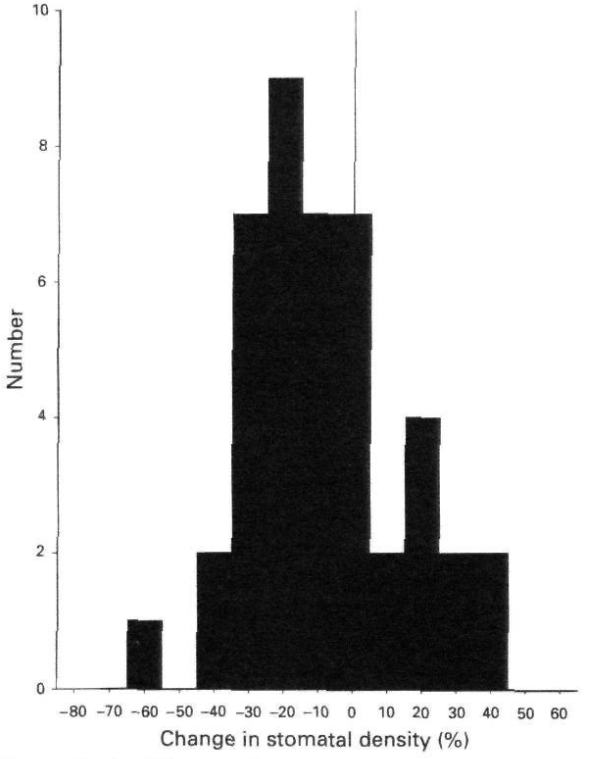


Figure 2. As Figure 1 but only for species studied in experimental CO_2 enrichment experiments.

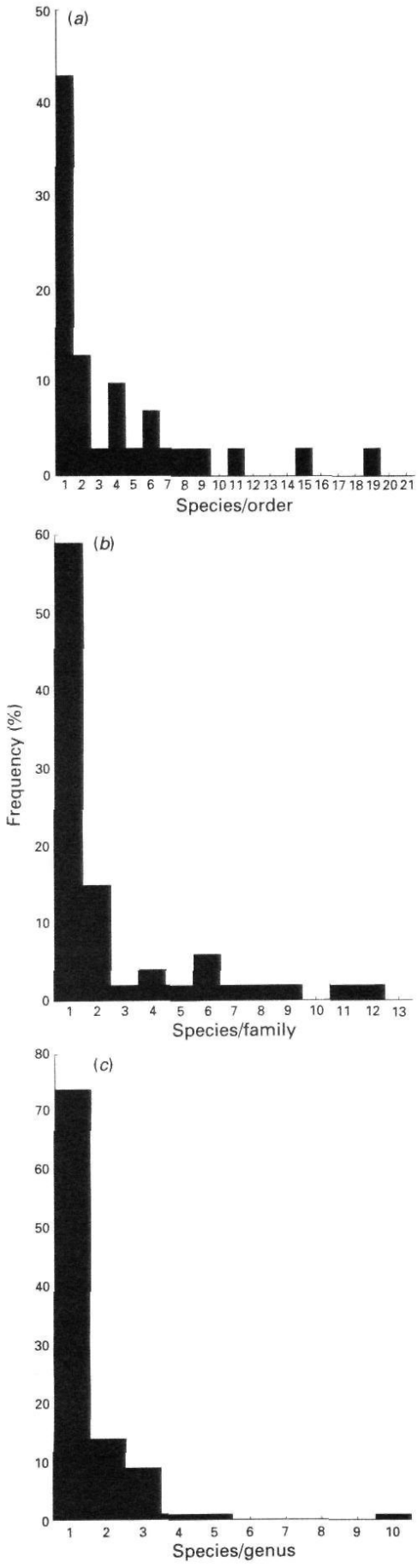
interpreted patterns than the categorical variables. Table 5 gives the results of the nested analysis of variances of the $\log_{10}$ transformed values of percent change, initial stomatal density and the difference in stomatal density. All three ANOVAs show that a large amount of the variance is accounted for by the error term, suggesting that species might respond as relatively independent entities. Unfortunately, the lack of replicates within cells at lower taxonomic levels (15 of the 44 families in the data set are monospecific) leaves the meaning of this pattern equivocal at best. Similarly, the significance of the effect of subdivisions within divisions in the nested ANOVA for $\log_{10}$ (difference in stomatal density) rests upon a comparison of only four species in one of the two subdivisions, abrogating the confidence that may be placed in the generality of the conclusion. However, the P-value for subclasses within classes is consistently one of the smallest across all three ANOVAs, and is significant ($P < 0{\cdot}005$) for this taxonomic level in the ANOVA of $\log_{10}$ (initial stomatal density), indicating that the simple interpretation of stomatal density response varying freely among species is not warranted at this time.

The degree of response to elevated CO_2 increased with increasing initial stomatal density (Table 6). A significant relationship was found between $\log_{10}$ (initial stomatal density) and $\log_{10}$ (change in stomatal density) for regressions of the full data set when forced through the origin (cf. Garland *et al.*, 1992). Further support is lent to the relationship by the additional analysis of the independent contrasts which showed that in 36 cases out of 40, the increase in stomatal density is accompanied by an increase in the strength of the response (response is change in stomatal density; heterogeneity χ^2; $P < 0{\cdot}001$). Interestingly, the regression relationship was stronger (had a larger coefficient of determination; R^2) when only species that decrease their stomatal density after exposure to elevated CO_2 were included in the analysis.

The relationship between $\log_{10}$ (initial stomatal density) and $\log_{10}$ (change in stomatal density) had larger R^2 when the data set was restricted to experimentally derived data. Further restriction of the data set to species that decreased stomatal density in response to elevated CO_2 produced the largest coefficient of determination of all regressions (Table 6).

It is not possible to infer that relationships between initial stomatal density and stomatal density response change among taxonomic levels. None of the regressions reported above showed significant heterogeneity among taxonomic level (Table 7).

Owing to the large and significant coefficients of determinations in the least squares regression models ($R^2 = 0{\cdot}127$–$0{\cdot}707$), a structural-relations model was used to determine the value of the power function $y = x^a$ (Harvey & Pagel, 1991). In this case, because λ, the ratio of the error variances of x and y, was not calculable from the data at hand, λ was assumed = 1·0, thus giving an equation equal to that for major axis regression (Sokal & Rohlf, 1995).



Excluding the individual analyses of subclasses, significant slopes produced by these analyses varied from 0·302 to 0·695 (the higher figure was from the data set that also yielded the highest R^2 value for the least squares regression; Table 6).

Differences among subclasses. The relationship between $\log_{10}$ (initial stomatal density) and $\log_{10}$ (change in stomatal density) also differed among subclasses, consistent with the patterns evidenced in the nested ANOVAs. Regressions (forced through the origin) for the five subclasses with sufficient independent contrasts to warrant individual analyses (Table 6) showed two with significant positive slopes, the Asteridae and the Hamamelidae. The differences among subclasses may be tested statistically with an ANOVA of the residuals from the general regression, designating subclass as a factor. This is a rather crude test of an hypothesis of differing relationships between the variables based on subclass, made less definitive by the relatively small number of contrasts within any one subclass. Hence, it is not too surprising that the ANOVA shows no significant difference among subclasses ($F_{4,39} = 1{\cdot}625$; $P = 0{\cdot}1878$).

However, a test to determine if the slope of any one subclass differed significantly from the overall slope did produce differences between two particular slopes and the general slope, and thus between subclasses (cf. Letcher & Harvey, 1994). Table 8 shows that for a regression of the residuals from the general relationship against the independent variable, the Caryophyllidae and Asteridae showed marginally significant results. The slope of a subclass could be a product of the composition of the data set within any one comparison. It may therefore be relevant that the two subclasses that showed slopes different from the general slope also had the largest percentages of species which increase, rather than decrease, the density of stomata in response to elevated CO_2 (Table 8).

Comparisons of taxonomic and non-taxonomic analyses

In comparing results of the least squares regressions for the cross-species analyses with those of the independent contrasts, we found that the signs of the slopes were in agreement significantly more often than expected by chance (17 comparisons, one disagreement; sign test; $P < 0{\cdot}001$). Similarly, those regressions that were significant for the regressions of the independent contrasts were also significant

Figure 3. The frequency of species observed per taxonomic order for stomatal density responses to CO_2, (*a*), species per order; (*b*), species per family; (*c*), species per genus.

Table 1. *Changes in stomatal density (%) with CO_2 enrichment, for species with more than one set of observations*

Species	Observation number			
	1	2	3	4
Acer pseudoplatanus	−52·6	−10·4		
Achillea moschata	−24·4	33·6		
Alnus glutinosa	−10	−24·7		
Anthyllis vulneraria[1]	−27·5	−11		
Arabidopsis thaliana[2]	−21	−41·5	−8·2	
Betula pendula	−6·2	−36		
Boehmeria cylindrica[3]	−10·6	−15·2		
Erigeron uniflorus	−16·2	−22·3		
Fagus sylvatica	+6·3	−26·7		
Linaria alpina	−9·5	−10·5		
Oxyria digyna	−15·7	+63·4	−22·5	
Phaseolus vulgaris	−6·7	−7·8		
Populus × *Beau*	−24·3	−5·3		
Populus × *Col R*	−33·8	−5·7		
Populus × *Robusta*	−31·5	6·3		
Quercus ilex[4]	−16·8	−24·3		
Quercus robur	−9·7	−44·2	−22·5	
Ranunculus glacialis	+9·1	+7·6		
Trifolium repens[5]	−56·6	+2·8	−4·6	−3·4
Triticum aestivum	−9·8	−12·9		
Vaccinium myrtillus	−44·5	−21·9	−27	

Notes on sources of data not presented in the Appendix (species lacking one or more of the variables for analysis). [1] Ferris & Taylor (1994); [2] V. Putland (pers. comm.); [3] G. B. Thompson (pers. comm.); [4] F. Miglietta (pers. comm.); [5] D. J. Beerling (pers. comm.).

for the cross-species regressions, with only one exception (17 comparisons/one disagreement; sign test; $P < 0{\cdot}001$).

Least squares regression of the slopes of the relationships shown in Table 6 (with cross-species slopes as the x variable and the independent contrasts slopes as the y variable), gives a significant result ($R^2 = 0{\cdot}72$, $P < 0{\cdot}0001$), as might be expected from the large amount of variance accounted for by the error terms in the nested ANOVAs (Table 4; N.B.: one explanation of a large variance component for error is that species might be operating as independent units, with regard to the largest variable). Also consistent with the possibility that species might to some extent operate independently, a reduced major axis analysis of the two groups of slopes (i.e. assuming variation in both the x and y axes; Harvey & Pagel, 1991) shows a slope of 1·056, not different from a 1:1 relationship.

However, 28% of the variation in the values for slopes of the independent contrast regressions remains unexplained; this figure might be a function of error, or in whole or part due to the effects of accounting for taxonomy. Although the data available in this study do not allow discrimination between the two possibilities, the differences among subclasses in the relationship between initial stomatal density and stomatal density response support an argument for a significant effect of taxonomy within this data set.

Table 2. *Results of analyses of discrete variables. Where appropriate, hypotheses are based on expectations of differences in stomatal density derived from Salisbury (1927)*

Hypothesis	Data set	*n*	C/D*	*P*-value
Woody species have greater responses than herbaceous species	Full	122	10/6	0·828
	Only those species with > 10% change	90	13/7	0·709
	Experimentally-derived data only	43	5/4	0·500
Tree species have greater responses than shrub species	Full	57	8/4	0·637
	Only those species with > 10% change	41	8/5	0·855
Species from cool habitats have greater responses than those from warm habitats	Full	122	12/6	0·613
Amphistomaty is associated with greater response than hypostomaty	Full	122	16/6	0·227
	Shade species only	18	4/2	n.c.†
	Experimentally-derived data only	43	8/1	0·035

* C/D, number of contrasts/number of disagreements.
† n.c., not calculable.

Table 3. *Contingency tables for the distribution among taxonomic level of the relative frequency of agreement and disagreement of comparisons with the hypothesis*

Taxonomic level	Woody vs. herbs				Trees vs. shrubs				Amphi- vs. hypostomaty		Cool vs. warm habitat	
	All species		Response > 10%		All species		Response > 10%					
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
Subdivision	1	1	0	1	0	0	0	0	0	0	0	0
Class	0	0	1	1	0	0	0	0	1	1	0	0
Subclass	3	2	3	2	1	2	3	2	1	1	1	2
Order	0	1	1	2	1	2	1	2	3	2	4	2
Family	1	2	1	2	2	1	1	1	3	4	1	3
Genus	1	1	1	1	0	0	0	1	0	1	0	0
G^2	0·541		0·814		0·908		0·541		0·345		1·989	
P-value	0·9098		0·9365		0·6351		0·7629		0·9514		0·3699	

The contingency tables presented here are for categorical variables only. Levels with either observed or expected values equal to 1 were excluded from the analysis.

Table 4. *Classification of the data used in the loglinear analyses, based on stomatal distribution and percentage change in stomatal density with increasing CO_2 concentration*

% change	Amphistomatous		Hypostomatous	
	Decrease	Increase	Decrease	Increase
> 10	4	9	4	8
⩽ 10	4	6	8	14

DISCUSSION

A major aim of this study has been to search for predictors of the direction and degree of stomatal density responses to CO_2 enrichment. The approach which has been taken is based on the evolutionary comparative method. This method has only rarely (e.g. Kelly & Purvis, 1993; Peat & Fitter, 1994; Kelly & Beerling, 1995; Kelly & Woodward, 1995) been applied to plant ecophysiological research, yet it is a powerful tool for understanding the interplay of genetic and ecological controls of plant responses. At one extreme the work investigated here could have been entirely constrained to studies on very closely related species, perhaps with the same common ancestor. A similarity of stomatal responses would be very likely, as already shown (Table 1). If the ecologies of the species were different, then it would be possible to relate the plant responses to ecological conditions. At the other extreme the work could have addressed the responses of only distantly related species from different taxa and perhaps different ecologies. In this case it would not be possible unequivocally to ascribe the stomatal responses to either genetic differences or to ecological differences. If the ecologies of the species were all the same, but the species were taxonomically unrelated, then there would be an inevitable spread of responses (e.g. Fig.

Table 5. *Nested ANOVA on species values of stomatal density and stomatal response*

Source of variation	df	Percent change		Initial stomatal density		Difference in stomatal density	
		Variance explained (%)	P-value	Variance explained (%)	P-value	Variance explained (%)	P-value
Subdivisions within divisions	1	0·1	< 0·90	0	< 0·75	8·4	< 0·005
Classes within subdivisions	1	0	< 0·90	5·1	< 0·50	0	< 0·90
Subclasses within classes	5	4·0	< 0·10	13·7	< 0·005	4·9	< 0·10
Orders within subclasses	22	0	< 0·75	0	< 0·50	9·1	< 0·50
Families within orders	15	26·5	< 0·10	0	< 0·50	6·6	< 0·50
Genera within families	36	0	< 0·75	41·0	< 0·10	1·1	< 0·50
Error	19	69·6		40·2		67	
Total	99						

The full data set ($n = 100$) was used for all three analyses; subspecific values were averaged to produce one value for the species before analysis. The proportion of the variance in the data set accounted for by each taxonomic level is presented. Negative variance components were set to zero before percentages were calculated. All values used in the analyses were $\log_{10}$ transformed absolute values of the base data. Significance is based on the F-value of the MS for that factor.

Table 6. *Results of least squares regression and major axis analyses*

Data set/ no. of samples/ no. of contrasts	Taxonomy taken into account				Species = independent units		
	R^2	*P*-value	Slope	Major axis slope	R^2	*P*-value	Slope
Non-experimental plus experimental data							
EDS, IDA/100/40	0·257	< 0·0001	0·926	0·348	0·201	< 0·0001	0·841
EDS/122/58	0·124	0·024	0·585	0·302	0·194	< 0·0001	0·854
Among subclasses							
Dilleniidae/20/8	0·178	0·259	0·934	0·286	0·003	0·818	0·081
Caryophyllidae/10/7	0·141	0·360	−0·780	−0·223	0·150	0·268	−0·874
Asteridae/22/9	0·070	0·461	0·426	1·122	0·280	0·011	1·075
Hamamelidae/23/11	0·731	0·0004	1·685	0·460	0·623	< 0·0001	1·134
Rosidae/30/9	0·430	0·040	2·019	0·226	0·215	0·010	1·339
OPDSD, IDA/98/50	0·490	< 0·0001	1·317	0·425	0·282	< 0·0001	0·908
OPDSD/80/37	0·418	< 0·0001	1·056	0·485	0·252	< 0·0001	0·87
OPISD, IDA/25/18	0·00004	0·979	−0·013	−0·004	0·003	0·805	−0·19
OPISD/21/17	0·00004	0·980	−0·013	−0·004	0·003	0·829	0·133
Experimental data only							
EDS, IDA/43/20	0·400	0·002	1·41	0·417	0·402	< 0·0001	1·331
EDS/34/14	0·402	0·011	0·851	0·635	0·411	< 0·0001	1·147
OPDSD, IDA/12/7	0·535	0·0392	1·420	0·418	0·391	0·030	1·586
OPDSD/24/13	0·707	0·0002	1·144	0·695	0·516	< 0·0001	1·121
OPISD/10/6	0·504	0·074	1·398	0·406	0·337	0·079	1·450

For least squares models, log(initial stomatal density) was the independent variable; log(difference in stomatal density) was the dependent variable.

* Abbreviations. EDS, entire data set; IDA, intraspecific data averaged; OPDSD, only plants that decrease stomatal density; OPISD, only plants that increase stomatal density.

1), with a probable interpretation of no dominant response. However, this interpretation is flawed in that it fails to account for the fact that the species have different ancestors, and therefore genotypes which respond in different directions and degrees. Such a state of affairs becomes increasingly likely if the genetic control of the plant response occurs only at one or a small number of genetic loci. Then the frequency with which species in a particular habitat show a particular stomatal density response is a complex function of the overall genotypic fit of a particular species to its environment and the selective advantage of possessing the stomatal response.

Plant ecophysiological studies are generally concerned with a number of species, often only distantly related and from different habitats. Such an approach makes it difficult to differentiate between the ecological and phylogenetic controls of the processes under study. However, if there are sufficient species at different taxonomic levels, then it is possible to factor out the phylogenetic controls on the stomatal responses, in order to paint a better picture of the processes in question. Unfortunately, as with this analysis (Fig. 3) one species per taxonomic level is more the norm than the exception. Extracting taxonomically-related responses of stomatal density can only, therefore, be achieved at higher taxonomic levels. In spite of this limitation, it has been possible to remove the effect of relatedness and show that the degree of stomatal density response to CO_2 enrichment increases as the initial stomatal density increases (Table 6). In addition, a greater amount of the variation in response is accounted for when only species which show a reduction in stomatal density are considered (Table 6). A further increase in accountable variance is seen when only results from controlled environment experiments are included (Table 6).

When taxonomy is taken into account, the subclasses Hamamelidae (dominated by trees in the data set) and Rosidae (dominated by herbs in the data set) show highly significant reductions in stomatal density with CO_2 enrichment and correlations with initial stomatal density. A nested ANOVA of species stomatal responses to CO_2 enrichment (Table 5) indicates that the error variance comprised the largest 'single' part of the total variance. This implies that species might respond independently, although consistent responses at the subclass level (Table 5, 6) indicate that this might not be the case. It is certainly the case that the Hamamelidae and Rosidae show consistent reductions in stomatal density. However, the Asteridae and Caryophyllidae in particular (Tables 6, 8) show a wider range of stomatal density responses, a feature which reduces the explanatory power at the subclass level in the ANOVA (Table 5).

Of the life forms which have been studied, 53 % of the cases were herbaceous and crop plants, 32 % trees and 15 % were shrubs. All of the observations on mature trees were from either herbarium or fossil leaf material, for which there is, as yet, no ex-

Table 7. *Contingency tables for the distribution among taxonomic levels of the relative frequency of contrasts which disagree in sign (i.e. log (difference in stomatal density decreases) when log (initial stomatal density) increases)*

Taxonomic level	Full data set		Decreased stomatal density only		Increased stomatal density only		Experimentally derived data		Decreased stomatal density only		Increased stomatal density only	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp
Subdivision	1	1	0	1	0	0	0	0	0	1	0	0
Class	1	1	0	1	1	1	0	1	0	1	0	1
Subclass	1	1	1	3	2	3	0	1	0	2	0	1
Order	0	5	1	5	0	1	1	2	1	2	1	1
Family	2	6	1	6	2	3	1	2	0	2	0	1
Genus	2	6	1	4	1	1	4	2	0	1	0	1
G^2	1·209		0·211		0·116		1·359		—		—	
P-value	0·8766		0·9758		0·9898		0·5068		—		—	

The contingency tables presented here are for continuous variables only. Levels with either observed or expected values equal to 1 were not used in the analysis. The G test is a likelihood ratio test, yielding a statistic which is closer to that of the theoretical chi-square than the more traditionally-used chi-squared test and is preferred for smaller sample sizes (Sokal & Rolf, 1995).

Table 8. *Differences among subclasses in slopes of the relationship between log(initial stomatal density) and log(difference in stomatal density)*

Subclass	R^2	P-value	% of species that increase stomatal density
Dilleniidae	0·002	0·906	20
Caryophyllidae	0·497	0·051	30
Asteridae	0·377	0·059	27
Hamamelidae	0·203	0·141	13
Rosidae	0·126	0·313	20

Values above are for comparisons of the residuals from the overall regression with the independent variable, on a subclass by subclass basis; a significant regression indicates that the slope of the relationship for that subclass is different from the overall slope. The final column in the table gives the percent of species in the comparison which increase, rather than decrease, stomatal density in response to elevated CO_2.

perimental comparison as no mature trees have been grown under CO_2 enrichment. When taxonomy is factored out for the complete data set, then no significant relationships were observed (Tables 2, 3) between stomatal density responses to CO_2 and growth form, habitat or stomatal distribution (amphi- or hypostomatous). An exception (Table 2) is the greater response of amphistomatous leaves in controlled environment experiments. This did not correspond to the presence of initially higher stomatal densities, as there was no significant relationship between stomatal distribution on the leaf and initial stomatal density.

The measure of stomatal density used here did not incorporate any potential responses of the leaf epidermal cells to CO_2. Therefore if stomatal density decreases and the epidermal cell density also decreases, then the proportion of epidermal cells which are stomata (the stomatal index, SI) might not change. In such a case, the CO_2 response might be due to the responses of epidermal cell expansion. There are many fewer cases where measurements of stomatal index are made alongside those of stomatal density. In a number of these cases (e.g. Woodward, 1987; Woodward & Bazzaz, 1988; Beerling *et al.*, 1992; Ferris & Taylor, 1994) it has been observed that stomatal index changes as well as density, indicating that stomatal initiation itself responds to CO_2 concentration.

In the studies which have been reviewed, it has been assumed for both fossil and herbarium leaves and for leaves from CO_2 experiments that the only environmental factor influencing stomatal density and stomatal index is a variation in CO_2 concentration. It is likely that for some cases other environmental factors, in particular the effects of varying solar radiation and drought, might have exerted some influence (Tichá, 1982). Such variations might mask relationships between taxonomy,

life form or habitat and the stomatal density response. An important requirement for further study is to define the mechanism by which stomatal density responds to CO_2, and for species which respond by increases and decreases to CO_2 enrichment. Details of this mechanism will increase the potential for predicting species responses and might improve the potential for differentiating between the environmental responses of stomatal density. Our results suggest that this mechanism might be tied somehow into the controls of initial stomatal density.

ACKNOWLEDGEMENTS

We are grateful for comments on the manuscript by Professor W. G. Chaloner and Dr D. J. Beerling.

REFERENCES

(*Some of the references are only sources of data for Appendix 1 and are not referenced within the main text*)

Anderson VJ, Briske DD. 1990. Stomatal distribution and conductance of three perennial grasses native to the southern true prairie of Texas. *American Midland Naturalist* **123**: 152–159.

Au S. 1969. Internal leaf surface and stomatal abundance in arctic and alpine populations of *Oxyria digyna*. *Ecology* **50**: 131–135.

Barnola JM, Raynaud D, Korotkevich YS, Lorius C. 1987. Vostok ice core provides 160,000-years record of atmospheric CO_2. *Nature* **329**: 408–414.

Beerling DJ. 1993. Changes in the stomatal density of *Betula nana* L. leaves in response to increases in the atmospheric CO_2 concentration since the late-glacial. *Special Papers in Palaeobotany* **49**: 181–187.

Beerling DJ, Chaloner WG. 1991. Stomatal density as an indicator of atmospheric CO_2 concentration. *The Holocene* **2**: 71–78.

Beerling DJ, Chaloner WG. 1993*a*. The impact of atmospheric CO_2 concentration and temperature change in stomatal density: observations from *Quercus robus* lammas leaves. *Annals of Botany* **71**: 231–235.

Beerling DJ, Chaloner WG. 1993*b*. Stomatal density responses of Egyptian *Olea europaea* L. leaves to CO_2 change since 1327 BC. *Annals of Botany* **71**: 431–435.

Beerling DJ, Chaloner WG. 1993*c*. Evolutionary responses of stomatal density to global CO_2 change. *Biological Journal of the Linnean Society* **48**: 343–353.

Beerling DJ, Chaloner WG, Huntley B, Pearson JA, Tooley MJ. 1993. Stomatal density responds to the glacial cycle of environmental change. *Proceedings of the Royal Society* **B251**: 133–138.

Beerling DJ, Putland V, Woodward FI. 1995. Stomatal density responses to global environmental change. *Advances in Bioclimatology* (in press).

Beerling DJ, Woodward FI. 1993. Ecophysiological responses of plants to global environmental change since the Last Glacial Maximum. *New Phytologist* **125**: 641–648.

Berryman CA, Eamus D, Duff GA. 1994. Stomatal responses to a range of variables in two tropical tree species grown with CO_2 enrichment. *Journal of Experimental Botany* **45**: 539–546.

Broadmeadow MSJ, Griffiths H. 1993. Genotypic variation in *Plantago major* in response to elevated CO_2. *Journal of Experimental Botany* (Supplement) **44**: 15.

Cronquist A. 1981. *An integrated system of classification of flowering plants*. New York: Columbia University Press.

Drake BG. 1992. A field study of the effects of elevated CO_2 on ecosystem processes in a Chesapeake Bay Wetland. *Australian Journal of Botany* **40**: 579–595.

Eamus D. 1991. The interaction of rising CO_2 and temperatures with water use efficiency. *Plant Cell and Environment* **14**: 843–852.

Eamus D, Berryman CA, Duff GA. 1993. Assimilation, stomatal conductance, specific leaf area and chlorophyll responses to elevated CO_2 of *Maranthes corybosa*, in a tropical monsoon rain forest species. *Australian Journal of Plant Physiology* **20**: 741–755.

Eamus D, Jarvis PG. 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Advances in Ecological Research* **19**: 1–55.

Felsenstein J. 1985. Phylogenies and the comparative method. *American Naturalist* **125**: 1–15.

Ferris R, Taylor G. 1993. Contrasting effects of elevated CO_2 on the root and shoot growth of four native herbs commonly found in chalk grassland. *New Phytologist* **125**: 855–866.

Ferris R, Taylor G. 1994. Stomatal characteristics of four native herbs following exposure to elevated CO_2. *Annals of Botany* **73**: 447–453.

Friend AD, Woodward FI. 1990. Evolutionary and ecophysiological responses of mountain plants to the growing season environment. *Advances in Ecological Research* **20**: 59–124.

Garland Jr T, Harvey PH, Ives AR. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology* **41**: 18–32.

Gaudillère JP, Mousseau M. 1989. Short term effect of CO_2 on leaf development and gas exchange of young poplars (*Populus euramericana* cv. I 214). *Oecologia Plantarum* **10**: 95–105.

Gittleman JL, Luh HK. 1992. On comparing comparative methods. *Annual Review of Ecology and Systematics* **23**: 383–404.

Harvey PH, Mace GM. 1992. Comparisons between taxa and adaptive trends: problems of methodology. In: King's College Sociobiology Group, ed. *Current Problems in Sociobiology*. Cambridge: Cambridge University Press, 343–361.

Harvey PH, Pagel MD. 1991. *The comparative method in evolutionary biology*. Oxford: Oxford University Press.

Houghton JT, Callender BA, Varney SK. 1992. Intergovernmental panel for climate change. *The Supplementary Report to the IPCC Scientific Assessment*. Cambridge: Cambridge University Press.

Hunt R, Hand DW, Hannah MA, Neal AM. 1991. Response to CO_2 enrichment in 27 herbaceous species. *Functional Ecology* **5**: 729–739.

Kelly CK, Beerling. 1995. Plant life form, stomatal density and taxonomic relatedness: a reanalysis of Salisbury (1927). *Functional Ecology* **9**: 422–431.

Kelly CK, Purvis A. 1993. Seed size and establishment conditions in tropical trees: on the use of taxonomic relatedness in determining ecological patterns. *Oecologia* **94**: 356–360.

Kelly CK, Woodward FI. 1995. Ecological correlates of carbon isotope composition of leaves: a comparative analysis testing for the effects of temperature, CO_2 and O_2 partial pressures and taxonomic relatedness on $\delta^{13}C$. *Journal of Ecology* **83**: 509–515.

Kelly DW, Hicklenton PR, Reekie EG. 1990. Photosynthetic response of geranium to elevated CO_2 as affected by leaf age and time of CO_2 exposure. *Canadian Journal of Botany* **69**: 2482–2488.

Körner C. 1988. Does global increase in CO_2 alter stomatal density? *Flora* **181**: 253–257.

Lee HSJ. 1993. *Biomass growth*: Betula pendula. *An investigation into the impact of elevated CO_2 upon the response of European Forests*. EC EPOC-0013 final report.

Letcher AJ, Harvey PH. 1994. Variation in geographical range size among mammals of the palaeoarctic. *American Naturalist* **144**: 30–39.

Madsen E. 1973. Effect of CO_2-concentration on the morphological, histological and cytological changes in tomato plants. *Acta Agriculturae Scandinavica* **23**: 241–246.

Malone SR, Mayeux HS, Johnson HB, Polley HW. 1993. Stomatal density and aperture length in four plant species grown across a subambient CO_2 gradient. *American Journal of Botany* **80**: 1413–1418.

Mansfield TA, Hetherington AM, Atkinson CJ. 1990. Some current aspects of stomatal physiology. *Annual Review of Plant Physiology and Molecular Biology* **41**: 55–75.

Miglietta F, Raschi A. 1993. Studying the effect of elevated CO_2 in the open in a naturally enriched environment in Central Italy. *Vegetatio* **104/105**: 391–400.

Miglietta F, Raschi A, Bettarini I, Resti R, Selvi F. 1993. Natural CO_2 springs in Italy: a resource for examining long-term response of vegetation to rising atmospheric CO_2 concentrations. *Plant Cell and Environment* **16**: 873–878.

Mousseau M, Enoch HZ. 1989. Carbon dioxide enrichment reduces shoot growth in sweet chestnut seedlings (*Castanea sativa* Mill.) *Plant Cell and Environment* **12**: 927–934.

Oberbauer SF, Strain BR. 1986. Effects of canopy position and irradiance on the leaf physiology and morphology of *Pentaclethra macroloba* (Mimosaceae). *American Journal of Botany* **73**: 409–416.

Oberbauer SF, Strain BR, Fetcher N. 1985. Effect of CO_2-enrichment on seedling physiology and growth of tropical tree species. *Physiologia Plantarum* **65**: 352–356.

O'Leary JW, Knecht GN. 1981. Elevated CO_2 concentration increases stomate numbers in *Phaseolus vulgaris* leaves. *Botanical Gazette* **142**: 438–441.

Pagel MD. 1992. A method for the analysis of comparative data. *Journal of Theoretical Biology* **156**: 431–442.

Pearcy RW, Björkman O. 1983. Physiological effects. In: Lemon ER, ed. *CO_2 and Plants. The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide*. Boulder: Westview Press, 65–105.

Peat HJ, Fitter AH. 1994. A comparative study of the distribution and density of stomata in the British flora. *Biological Journal of the Linnean Society* **52**: 377–393.

Peñuelas J, Matamala R. 1990. Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. *Journal of Experimental Botany* **41**: 1119–1124.

Peñuelas J, Azcón-Bieto J. 1992. Changes in leaf $\Delta^{13}C$ of herbarium plant species during the last 3 centuries of CO_2 increase. *Plant Cell and Environment* **15**: 485–489.

Polley HW, Johnson HB, Marino BD, Mayeux HS. 1993. Increase in C_3 plant water use efficiency and biomass over glacial to present CO_2 concentrations. *Nature* **361**: 61–64.

Ragdolou KM, Jarvis PG. 1990. Effects of CO_2 enrichment on four Poplar clones. II. Leaf surface properties. *Annals of Botany* **65**: 627–632.

Ragdolou KM, Jarvis PG. 1992. The effects of CO_2 enrichment and nutrient supply on growth, morphology and anatomy of *Phaseolus vulgaris* L. seedlings. *Annals of Botany* **70**: 245–256.

Ragdolou KM, Jarvis PG. 1993. Effects of atmospheric CO_2 enrichment on early growth of *Vicia faba*, a plant with large cotyledons. *Plant Cell and Environment* **16**: 93–98.

Retuerto R, Woodward FI. 1993. The influences of increased CO_2 and water supply on growth, biomass allocation and water use efficiency of *Sinapis alba* L. grown under different wind speeds. *Oecologia* **94**: 415–427.

Ridley M. 1983. *The explanation of organic diversity: the comparative method and adaptations for mating*. Oxford: Oxford University Press.

Robinson JM. 1994. Speculations on carbon dioxide starvation, Late Tertiary evolution of stomatal regulation and floristic modernization. *Plant, Cell and Environment* **17**: 1–10.

Rowland-Bamford AJ, Nordenbrock C, Baker JT, Bowes G, Hartwell Allen L. 1990. Changes in stomatal density in rice growth under various CO_2 regimes with natural solar irradiance. *Environmental and Experimental Botany* **30**: 175–180.

Ryle GJA, Stanley J. 1992. Effect of elevated CO_2 on stomatal size and distribution in perennial ryegrass. *Annals of Botany* **69**: 563–565.

Salisbury EJ. 1927. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Philosophical Transactions of the Royal Society of London*. **B216**: 1–65.

Siegel S. 1956. *Non-parametric statistics*. Tokyo, Japan: McGraw-Hill Kogakusha.

Sokal RR, Rohlf FJ. 1995. *Biometry*, 3rd edition. New York: W. H. Freeman.

Thomas JF, Harvey CN. 1983. Leaf anatomy of four species grown under continuous CO_2 enrichment. *Botanical Gazette* **144**: 303–309.

Tichá I. 1982. Photosynthetic characteristics during ontogenesis of leaves: stomatal densities and sizes. *Photosynthetica* **11**: 375–471.

Van der Burgh J, Visscher H, Dilcher DL, Kürschner WM. 1993. Paleoatmospheric signatures in Neogene fossil leaves. *Science* **260**: 1788–1790.

Van der Water PK, Leavitt SW, Betancourt JL. 1994. Trends in stomatal density and $^{13}C/^{12}C$ ratios of *Pinus flexilis* needles during last glacial–interglacial cycle. *Science* **264**: 239–243.

Van Gardingen PR, Jeffree CE, Grace J. 1989. Variation in stomatal aperture in leaves of *Avena fatua* L. observed by low-temperature electron microscopy. *Plant Cell and Environment* **12**: 887–898.

Van Gardingen PR, Grace J, Jeffree CE. 1993. Long-term effects of CO_2 increase on a Mediterranean ecosystem. *Journal of Experimental Botany* (Supplement) **44**: 13.

Woodward FI. 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia* **70**: 580–586.

Woodward FI. 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. *Nature* **327**: 617–618.

Woodward FI. 1988. The responses of stomata to changes in atmospheric levels of CO_2. *Plant Today* **1**: 132–135.

Woodward FI. 1993. Plant responses to past concentrations of CO_2. *Vegetatio* **104/105**: 145–155.

Woodward FI, Bazzaz FA. 1988. The responses of stomatal density to CO_2 partial pressure. *Journal of Experimental Botany* **39**: 1771–1781.

Woodward FI, Beerling DJ. 1995. Plant-CO_2 responses in the long term: plants from CO_2 springs in Florida and tombs in Egypt. In: Miglietta F, Raschi A, eds. *High CO_2 Springs and their Use in Biological Research*. Cambridge: Cambridge University Press, in press.

Woodward FI, Thompson GB, McKee IF. 1991. The effects of elevated concentrations of carbon dioxide on individual plants, populations, communities and ecosystems. *Annals of Botany* (Suppl. 1) **67**: 23–38.

Appendix. *Full data set*

Subdivision Class Subclass Order Family Genus & species	$[CO_2]$ range (ppm)	Life form	Habitat	Initial stomatal density (mm^{-2})	Final stomatal density (mm^{-2})
Gymnospermae					
Coniferophyta					
Pinales					
Cupressaceae					
Juniperus communis	280–345	Shrub	Cool	329	229
Pinaceae					
Pinus pinea	280–345	Tree	Warm	425	399
Pinus uncinata	280–345	Tree	Warm	438	339
Pinus flexilis	190–350	Tree	Cool	116	85
Magnoliopsida					
Magnoliidae					
Ranunculales					
Ranunculaceae					
Helleborus foetidus	280–345	Shrub	Cool, shade	106	79
Ranunculus glacialis	219–249	Herb	Alpine	220	240
Ranunculus glacialis	227–249	Herb	Alpine	223	240
Ranunculus lappaceus	287–301	Herb	Cool	68	67
Papaverales					
Papaveraceae					
Papaver alpinum	280–345	Herb	Alpine	172	139
Hamamelidae					
Fagales					
Fagaceae					
Castanea sativa	350–700	Tree	Cool	649	597
Fagus sylvatica	285–316	Tree	Cool	111	118
Fagus sylvatica	285–338	Tree	Cool	479	351
Nothofagus menziesii	297–336	Tree	Cool	180	149
Quercus pubescens	550–750	Tree	Cool	515	536
Quercus robur	280–350	Tree	Cool	600	542
Quercus robur	225–340	Tree	Cool	720	402
Quercus robur	287–329	Tree	Cool	591	458
Quercus petraea	283–338	Tree	Cool	575	450
Betulaceae					
Alnus glutinosa	280–345	Tree	Cool	218	196
Alnus glutinosa	300–331	Tree	Cool	434	327
Betula nana	290–350	Shrub	Cool	216	150
Betula pendula	350–700	Tree	Cool	45	42
Betula pendula	280–345	Tree	Cool	200	128
Carpinus betulus	285–338	Tree	Cool	361	287
Urticales					
Urticaceae					
Boehmeria cylindrica	350–550	Herb	Warm, shade	216	193
Moraceae					
Ficus pumila	350–550	Shrub	Tropical, shade	163	115
Capparales					
Brassicaceae					
Cardamine resedifolia	227–249	Herb	Alpine, shade	463	432
Hutchinsia alpina	219–249	Herb	Alpine	471	362
Biscutella laevigata	273–307	Herb	Warm	236	150
Sinapsis alba	350–700	Herb	Warm, crop	73	58
Arabidopsis thaliana	350–550	Herb	Cool	655	520
Hamamelidales					
Hamamelidaceae					
Liquidambar styraciflua	340–910	Tree	Warm	275	327
Caryophyllidae					
Carophyllales					
Carophyllaceae					
Cerastium uniflorum	227–249	Herb	Alpine	142	186
Silene acaulis	255–316	Herb	Alpine	150	107
Amaranthaceae					
Amaranthus caudatus	280–345	Herb	Warm	232	194
Amaranthus retroflexus	350–700	Herb	Warm	358	224

Appendix (*cont.*)

Subdivision Class Subclass Order Family Genus & species	$[CO_2]$ range (ppm)	Life form	Habitat	Initial stomatal density (mm^{-2})	Final stomatal density (mm^{-2})
Polygonales					
Polygonaceae					
Oxyria digyna	251–317	Herb	Alpine	134	113
Oxyria digyna	240–340	Herb	Alpine	172	281
Oxyria digyna	227–249	Herb	Alpine	173	134
Rumex acetosella	287–350	Herb	Cool	61	62
Rumex crispus	225–340	Herb	Cool	247	145
Rumex scutatus	260–308	Herb	Alpine	186	79
Dilleniidae					
Salicales					
Salicaceae					
Populus alba	350–700	Tree	Cool	429	463
Populus euramericana	330–660	Tree	Cool	292	340
Populus nigra	297–338	Tree	Cool	279	209
Populus × Beau	350–700	Tree	Cool	267	202
Populus × Beau	350–700	Tree	Cool	151	143
Populus × Col R	350–700	Tree	Cool	263	174
Populus × Col R	350–700	Tree	Cool	123	116
Populus × Ras	350–700	Tree	Cool	132	128
Populus × Robusta	350–700	Tree	Cool	292	200
Populus × Robusta	350–700	Tree	Cool	142	151
Salix herbacea	200–340	Shrub	Cool	182	108
Malvales					
Tiliaceae					
Tilia cordata	300–338	Tree	Cool	366	296
Ericales					
Ericaceae					
Arctostaphylos uva-ursi	264–316	Shrub	Cool	87	62
Gaultheria mundula	205–222	Shrub	Warm	356	330
Rhododendron hirsutum	272–316	Shrub	Cool, shade	107	73
Vaccinium myrtillus	265–305	Shrub	Cool, shade	164	91
Vaccinium myrtillus	250–450	Shrub	Cool, shade	512	400
Vaccinium myrtillus	285–316	Shrub	Cool, shade	89	65
Epacridaceae					
Styphelia suaveolenus	191–222	Shrub	Warm, Shade	316	305
Primulales					
Primulaceae					
Primula auricula	264–316	Herb	Alpine	118	52
Rosidae					
Rosales					
Pittosporaceae					
Pittosporum pullifolium	222–259	Tree	Warm	290	441
Saxifragaceae					
Saxifraga moschata	219–249	Herb	Alpine	190	123
Rosacae					
Geum montanum	258–285	Herb	Alpine	220	186
Geum reptans	251–317	Herb	Alpine	358	303
Geum urbanum	225–340	Herb	Cool, shade	221	158
Sorbus aucuparia	285–316	Tree	Cool	89	60
Chrysobalanaceae					
Maranthes corymbosa	350–700	Tree	Tropical	86	74
Fabaceae					
Anthyllis vulneraria	264–315	Herb	Cool	154	137
Glycine max	340–910	Herb	Warm, Crop	381	521
Phaseolus vulgaris	400–1200	Herb	Warm, crop	300	280
Phaseolus vulgaris	350–700	Herb	Warm, crop	167	154
Trifolium repens	350–600	Herb	Cool	316	137
Trifolium repens	350–600	Herb	Cool	180	185
Trifolium repens	350–600	Herb	Cool	173	165
Trifolium repens	350–600	Herb	Cool	176	170
Vicia faba	350–700	Herb	Warm, crop	111	131
Ceratonia siliqua	280–345	Tree	Warm	299	230
Pentaclethra macroloba	350–675	Tree	Tropical	332	308

Appendix (*cont.*)

Subdivision Class Subclass Order Family Genus & species	$[CO_2]$ range (ppm)	Life form	Habitat	Initial stomatal density (mm^{-2})	Final stomatal density (mm^{-2})
Myrtales					
Myrtaceae					
Eucalyptus pauciflora	270–306	Tree	Warm	177	141
Cornales					
Cornaceae					
Griselinia littoralis	318–335	Shrub	Cool	94	110
Euphorbiales					
Buxaceae					
Buxus sempervirens	280–345	Shrub	Warm, shade	128	117
Rhamnales					
Rhamnaceae					
Rhamnus catharticus	225–340	Shrub	Cool, shade	325	140
Sapindales					
Aceraceae					
Acer pseudoplatanus	225–340	Tree	Cool	485	230
Acer pseudoplatanus	288–318	Tree	Cool	442	396
Anacardiaceae					
Pistacia lentiscus	280–345	Shrub	Warm, shade	311	287
Geraniales					
Geraniaceae					
Pelargonium × hortorum	350–1000	Herb	Warm	158	154
Trapaeolaceae					
Tropaeolum majus	350–550	Herb	Warm	306	245
Malpighiales					
Polygalaceae					
Polygala amara	272–316	Herb	Cool	130	80
Umbellales					
Araliaceae					
Hedera helix	350–550	Shrub	Cool, shade	255	256
Asteridae					
Gentiales					
Gentianaceae					
Gentiana alpina	280–345	Herb	Alpine	147	150
Gentiana verna	219–249	Herb	Alpine	119	166
Polemoniales					
Solanaceae					
Lycopersicum esculentum	350–3200	Herb	Warm, crop	390	278
Plantaginales					
Plantaginaceae					
Plantago major	263–355	Herb	Cool	381	281
Scrophulariales					
Oeaceae					
Olea europaea	270–350	Tree	Warm	524	226
Scrophulariaceae					
Linaria alpina	251–317	Herb	Alpine	214	193
Linaria alpina	227–249	Herb	Alpine	239	214
Globulariaceae					
Globularia cordifolia	292–316	Shrub	Alpine	120	101
Globularia nudicaulis	272–299	Herb	Cool	150	123
Acanthaceae					
Hypoestes sanguinolenta	350–550	Herb	Tropical, shade	110	61
Campanulales					
Campanulaceae					
Campanula barbata	227–249	Herb	Alpine	348	480
Lobelia telekii	180–213	Shrub	Warm	217	305
Asterales					
Asteraceae					
Achillea moschata	227–249	Herb	Cool	205	155
Achillea moschata	219–249	Herb	Cool	116	155
Ambrosia artemisifolia	350–700	Herb	Warm	519	419
Erigeron uniflorus	219–249	Herb	Alpine, shade	278	233

Appendix (*cont.*)

Subdivision Class Subclass Order Family Genus & species	$[CO_2]$ range (ppm)	Life form	Habitat	Initial stomatal density (mm^{-2})	Final stomatal density (mm^{-2})
Erigeron uniflorus	227–249	Herb	Alpine, shade	300	233
Hypochaeris radicata	307–350	Herb	Cool	47	43
Leontodon hispidus	251–317	Herb	Cool	319	321
Tussilago farfara	260–285	Herb	Cool	85	54
Bellidiastrum michelii	260–316	Herb	Alpine	155	32
Lamiales					
Labiatae					
Solenostemon scutellarioides	350–550	Herb	Tropical, shade	175	130
Liliopsida					
Commelinidae					
Cyperales					
Cyperaceae					
Scirpus olneyi	343–681	Sedge	Warm	166	177
Poaceae					
Cynodon dactylon	280–345	Grass	Warm	321	249
Lolium perenne	340–680	Grass	Cool, crop	176	203
Oryza sativa	160–900	Grass	Warm, crop	644	931
Schizachyrium scoparium	200–350	Grass	Warm	209	235
Setaria faberii	350–700	Grass	Warm	140	104
Triticum aestivum 'yaqui'	200–350	Grass	Warm, crop	82	74
Triticum aestivum 'seri'	200–350	Grass	Warm, crop	93	81
Zea mays	340–910	Grass	Warm, crop	192	142