

A comparative study of the distribution and density of stomata in the British flora

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The distribution of stomata over both leaf surfaces may affect both the photosynthetic rate and water use efficiency of species, implying that species with different photosynthetic and water requirements may also have different stomatal distributions. A database containing data on the distribution of stomata on the leaves of 469 British plant species was used to look for relationships between stomatal distribution (including both location on the leaf and density) and both habitat and morphological variables. Statistical models were applied to the data that minimized any effects that phylogenetic constraints may have had on the data.

Hypostomaty is common in woody species, species which typically occur in shaded habitats and species with large or glabrous leaves. Amphistomaty, however, predominates in species which occur in non-shaded habitats, species with small, dissected or hairy leaves, and in annual species. Amphistomaty, therefore, tends to occur in species where CO₂ may be limiting photosynthesis (unshaded environments), or where there are structures to prevent water loss from the leaf (e.g. hairs). Hypostomaty, however, occurs in slow-growing species (e.g. trees), species with leaves which have large boundary layers (large or entire leaves) and in species where CO₂ is unlikely to limit photosynthesis (shaded habitats).

ADDITIONAL KEY WORDS:—hypostomaty – amphistomaty – comparative analyses – habitat – morphology – stomatal density – shade.

CONTENTS

Introduction	377
Material and methods	379
Results	382
Discussion	388
Acknowledgements	391
References	391
Appendix	392

INTRODUCTION

Stomata occur in all terrestrial flowering plants, but there is great variation between species in the distribution and density of stomata on the leaf. Leaves may be completely or predominantly hypostomatous (i.e. have all the stomata on the lower epidermis) or hyperstomatous (all the stomata on the upper epidermis) or amphistomatous (stomata distributed on both leaf epidermes). The distribution of stomata on the leaves of 469 British plant species (Fig. 1)

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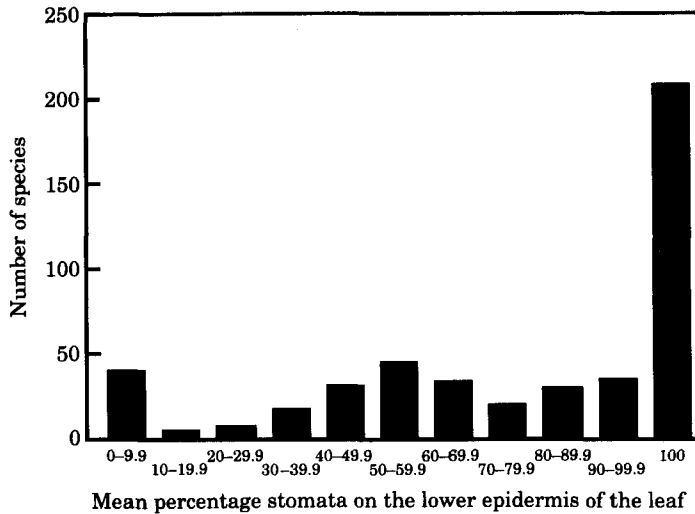


Figure 1. The stomatal distribution of 469 British species. Stomatal distribution is measured as the percentage number of stomata found on the lower epidermis.

demonstrates wide inter-specific variation, but 44% of species in the sample are hypostomatous. Differences in stomatal distribution between species might be expected to be related to differences in the morphology or ecology of these species, as the stomatal distribution affects both the photosynthetic rate and water use efficiency of a species (Woodward & Bazzaz, 1988).

The diffusion of CO_2 into the leaf or water vapour out of the leaf involves a pathway comprising several different resistances. The distribution of stomata will directly affect the boundary layer resistance for water vapour and CO_2 , since amphistomatous leaves have two boundary layers in parallel whereas hypostomatous and hyperstomatous leaves only have one, and also the intercellular mesophyll resistance for CO_2 , as the mean distance between a stomatal pore and a mesophyll cell will be less. Mesophyll and stomatal resistances will also be decreased by a higher density of stomata.

Explanations for the differences in distribution and density of stomata have been derived from comparative analyses (Salisbury, 1927; Parkhurst, 1978; Mott, Gibson & O'Leary, 1982), models (Parkhurst, 1978; Jones, 1985; Foster & Smith, 1986), and experimental approaches (Jones & Slatyer, 1972). Comparative methods have concentrated on one or two factors. Parkhurst (1978) found stomatal distribution to be associated with leaf thickness. Thicker leaves, which have greater intercellular mesophyll resistance, tended to be amphistomatous and thinner leaves hypostomatous. Mott *et al.* (1982) also found a weak trend towards amphistomaty in species with thick leaves but concluded that the relationship was a secondary one, and provided anecdotal evidence that light levels are an important determining factor. Amphistomaty may have evolved in species living in unshaded environments where CO_2 is limiting the rate of photosynthesis since it would increase maximum leaf conductance to CO_2 . Salisbury (1927) discovered a predominance of hypostomaty in temperate woody species.

Modelling approaches have been used to search for an optimal stomatal distribution that gives maximum photosynthetic CO₂ uptake without allowing excessive water loss. Parkhurst (1978) included many variables such as mesophyll thickness, transpiration rate, air temperature, relative humidity, boundary layer resistance and stomatal spacing in his models and found that amphistomatous leaves appeared to be better adapted than hypostomatous leaves under most conditions. Leaf thickness, however, was the most important determining factor in the models, with hypostomaty being optimal for very thin leaves. Jones (1985) found that amphistomaty may be advantageous as it allows greater CO₂ transfer through the mesophyll, especially in thicker leaves, and greater leaf conductance in high light conditions when CO₂ is limiting photosynthetic rate. However, his models showed that hypostomaty would be advantageous when there are high boundary layer resistances (for example in large leaves or deep within a plant canopy), high humidity or a temperature gradient across the leaf.

Comparative analyses using species data have recently come under scrutiny and criticism (e.g. Harvey & Pagel, 1991) because often no attempt is made to take account of phylogeny. Phylogeny is important because two species may have similar values for one or more characteristics either because they have evolved similarly as a result of the same evolutionary pressures, or because they share a common ancestor and evolution of the characteristic(s) has not occurred since the species diverged. If this second explanation is the case then the species values are not independent of one another and many statistical techniques of analysis will consequently be invalid. It is therefore vital to account for relatedness between species in any comparative analyses. There are, however, problems in accounting for the phylogeny of flowering plants due both to the poor fossil record and the possibility of genetic material being exchanged between plant species by hybridization. Taxonomic relationships between species, however, which are the best available representation of phylogeny, can be accounted for by using a hierarchical analysis of variance to determine at which taxonomic level(s) the majority of variance in a characteristic occurs (Clutton-Brock & Harvey, 1977; Harvey & Mace, 1982). Comparative analyses are then conducted using mean values for the characteristic at the taxonomic level at which the majority of the variance occurs.

Comparative analyses on the distribution and density of stomata were therefore conducted, investigating both habitat variables (shade and water availability) and morphological variables (woodiness, leaf shape, leaf area and leaf hairiness) and accounting for taxonomic relationships between species. The aim was to see if previously found relationships still held (i.e. that species of high light habitats were more amphistomatous than species of more shaded habitats and that woody species tend to be hypostomatous) and whether other relationships could be found. It was not possible either to relook at leaf thickness or to include intraspecific variation in the analyses due to a lack of data in the literature.

MATERIAL AND METHODS

The Ecological Flora Database compiled at the University of York (Fitter & Peat, in press) contains data on a wide variety of ecological characteristics of

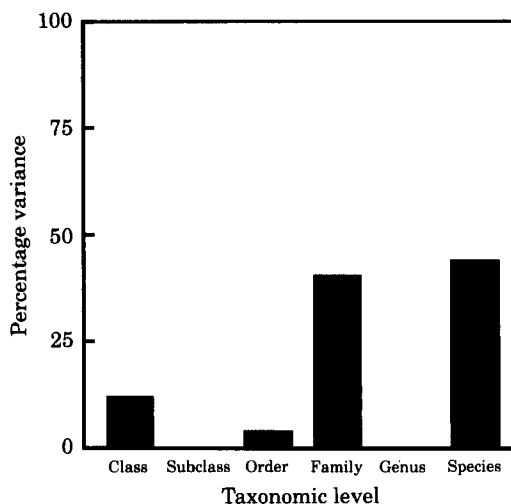


Figure 2. The percentage variance in stomatal distribution at different taxonomic levels of 469 British species. Zero values are true values and not omissions.

British plant species extracted predominantly from the scientific literature. It includes data on stomatal location (whether on top, bottom or both leaf surfaces) for 469 British species and the density on each surface for 353 species. The former represents 26% of the British flora representing over 70% of all families. The distribution of these data over families is shown in the appendix.

For each species the proportion of stomata on the lower epidermis of the leaf was calculated and arcsin transformed to normalize the data. A hierarchical analysis of variance shows that the majority of the variance in stomatal distribution occurs at two taxonomic levels: between-species-within-genera and between-families-within-orders (Fig. 2). This means that an analysis using species data would use spurious degrees of freedom because of the lack of independence of species values in some families (Harvey & Pagel, 1991), while conducting analyses using family means alone would lose information from the amount of variance at the species level. Families were therefore grouped into four groups, depending on the amount of intra-familial variance and the mean stomatal distribution of the family (Table 1). The appendix lists each family, the

TABLE 1. Characterization of the four groups of families according to their stomatal distributions. The mean distribution of a family represents the criterion for group membership. Distributions given are the proportion of stomata on the lower epidermis of the leaf

Group	Mean distribution of family	Mean distribution of group	Standard deviation of distribution	Number of species in the group
1. Hypostomatous families	> 0.92	0.98	0.0663	170
2. Mainly hypostomatous families	0.81–0.92	0.88	0.1555	52
3. Weakly hypostomatous families	0.50–0.80	0.72	0.2703	159
4. Weak-strongly hyperstomatous families	< 0.50	0.31	0.2834	81

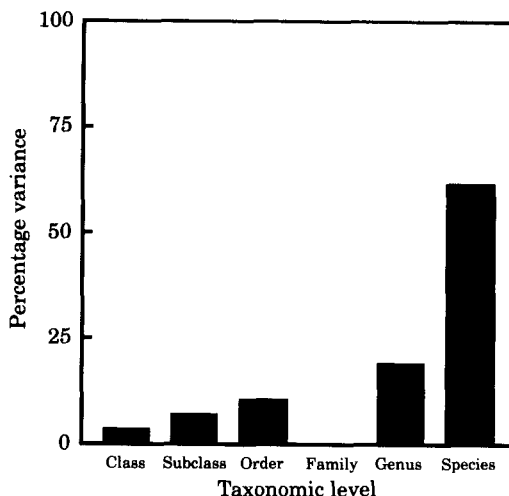


Figure 3. The percentage variance in stomatal distribution at different taxonomic levels of species in families which have a mean of 50–80% of stomata on the lower epidermis (group 3 species). Zero values are true values and not omissions.

group it belongs to and also the minimum and maximum proportion of stomata on the lower epidermis for species in each family as these do not necessarily lie within the same bounds as the family mean.

The nested analysis of variance of stomatal distribution at different taxonomic levels was repeated using only species in families in group 3, i.e. families with a mean stomatal distribution of 0.5–0.8 (Fig. 3). The majority of the variance in the species of this group occurs at the between-species-within-genus level showing that analyses for this group can be done using the data values for species. Factors such as habitat variables, e.g. shade and water availability, vary from species to species meaning it would not be relevant to calculate mean family values for such factors. Analyses involving these factors must be done at the species level. Therefore many of the analyses conducted concentrate on the species in families within group 3, the weakly hypostomatous families, as the group is reasonably large (159 species) and the majority of the variance in stomatal distribution occurs at the between-species-within-genus level.

One-way analyses of variance were conducted to test for significant ($P < 0.05$) relationships between stomatal distribution/density and the following variables: shade, water availability, leaf area, leaf type, leaf outline and leaf hairiness. Data on leaf thicknesses were not available. Details of the tests are given in Table 2. In all cases the arcsin square-root of the proportion of stomata on the lower epidermis and the natural logarithm of the density data have been used to normalize the data.

Habitat preference in terms of shade was classified into zero, light, mid and deep using data from a variety of sources but predominantly Fitter (1978), and in terms of water availability into dry, moist, damp, wet and flooded/submerged using Ellenberg (1988). Leaf area data, classified into four categories (0.1–1, 1–10, 10–100 and $> 100 \text{ cm}^2$), were extracted from the Ecological Flora Database. Data on leaf morphology and hairiness were retrieved from standard

TABLE 2. A list showing the data used in each of the one-way analyses of variance that were conducted. Distribution = proportion of stomata on the lower epidermis, density = number of stomata mm^{-2} (total density = sum of densities on upper and lower leaf surfaces). Group 1 = species in families with on average 92–100% of stomata on the lower epidermis. Group 3 = species in families with on average 50–80% of stomata on the lower epidermis. Groups 2 and 4 were not used in any analyses

Stomatal data used	Species used (cf. Table 1)	Second variable
distribution	group 3	shade
total density	group 1	shade
total density	group 3	shade
density on lower epidermis	group 1	shade
density on lower epidermis	group 3	shade
density on upper epidermis	group 3	shade
distribution	group 3	water availability
total density	group 3	water availability
density on lower epidermis	group 3	water availability
distribution	group 3	leaf area
distribution	group 3 species which occur in environments which are lightly shaded or with no shade	leaf area
distribution	group 3	leaf type
distribution	group 3 species with leaf area < 10 cm^2	leaf outline
distribution	group 3 species with leaf area > 10 cm^2	leaf outline
distribution	group 3	leaf hairiness

floras, e.g. Clapman, Tutin and Moore (1987) and Stace (1991). Leaf type was categorized as simple or compound, leaf outline as entire, toothed or lobed margins and hairiness as hairy, hairy/glabrous and glabrous.

Although some families contain woody and non-woody species (e.g. Rosaceae), in general they are either entirely woody or non-woody, largely because primitive angiosperms were woody, and while herbaceous species have evolved from woody species on a number of occasions, the reverse has rarely occurred (Stebbins, 1974). It was therefore appropriate to analyse woodiness and stomatal distribution using mean family values for both stomatal distribution and woodiness. These family means are independent of one another as there is very little variance at taxonomic levels higher than families for either stomatal distribution or woodiness (Peat, 1992). Families were split into woody and non-woody, with non-woody families being those with < 10% woody species and woody families being those with > 90% woody species. A chi-squared analysis was conducted to test whether woody families tended to have a different stomatal distribution from non-woody families.

In addition to these tests, the mean stomatal distribution of species in different habitats and in different life form categories was calculated. Habitats were classified using the CORINE biotopes codes (Moss, 1991).

RESULTS

Stomatal distributions differ markedly between species living in habitats receiving different amounts of shade ($F_{3,154} = 14.82$, $P < 0.001$). Hypostomaty is more prevalent in species which live in deep shade and amphistomaty in species living in non-shaded environments (Fig. 4).

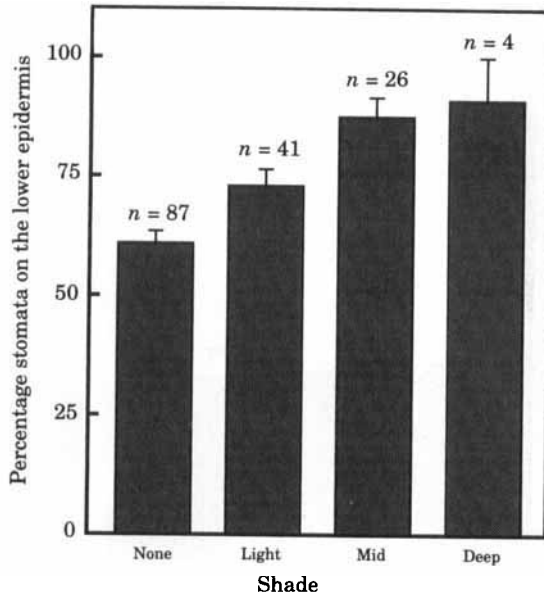


Figure 4. The mean stomatal distribution of species in weakly hypostomatous families typical of different levels of habitat shade. Error bars represent one standard error.

There is also a significant difference between total stomatal density (sum of stomata mm^{-2} on the lower and upper epidermes) and shade level both in species of families in group 3, the weakly hypostomatous families ($F_{3,140} = 11.67$, $P < 0.001$) and species of families in group 1, the hypostomatous families ($F_{3,99} = 8.54$, $P < 0.001$). The total number of stomata/ mm^2 is greater in species of non-shaded environments than in species of shaded environments (Figs 5 & 6). Species of strongly hypostomatous families, however, have a much higher total density of stomata in lightly shaded habitat than those of weakly hypostomatous families.

When the analyses were repeated using the density solely on the lower epidermis, the result for species in group 1 was still significant, as expected, since the majority of these species are hypostomatous ($F_{3,99} = 7.08$, $P < 0.001$). The result for the weakly hypostomatous species in group 3 was also significant ($F_{3,140} = 3.55$, $P = 0.016$), but the majority of the decrease in total density in increasing shade is due to a reduction in stomata on the upper epidermis ($F_{3,140} = 29.33$, $P < 0.001$, Figs 5 & 6). No significant differences were found between the typical water availability of a species' habitat and either the stomatal distribution ($F_{4,131} = 1.79$, $P = 0.135$) or the stomatal density (total density— $F_{4,108} = 1.83$, $P = 0.129$; density on the lower epidermis— $F_{4,108} = 1.16$, $P = 0.335$).

Species with leaves belonging to different leaf area categories have significantly different stomatal distributions ($F_{3,127} = 7.24$, $P = 0.001$). This is also the case when species living in shaded habitats are omitted ($F_{2,101} = 3.53$, $P < 0.05$). Larger-leaved species tend to have a higher proportion of stomata on the lower epidermis of the leaf than do species with smaller leaves (Fig. 7).

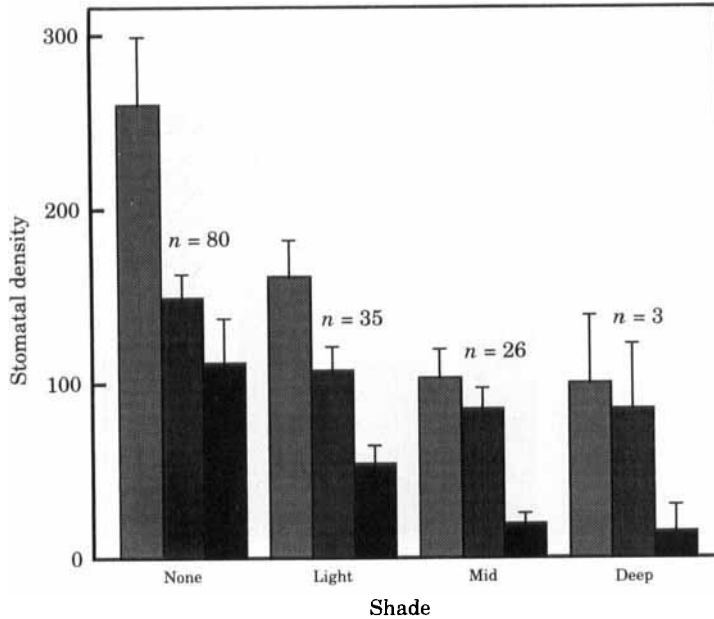


Figure 5. The density of stomata (number/mm²) of species from weakly hypostomatous families which are typical of different levels of shade. ▨ total density, ▩ density on lower epidermis, ■ density on upper epidermis. Error bars represent one standard error.

The stomatal distribution of species with compound leaves and species with simple leaves does not differ ($F_{1,156} = 2.47$, $P = 0.12$). Neither are there any significant differences in the stomatal distribution of species with leaf areas

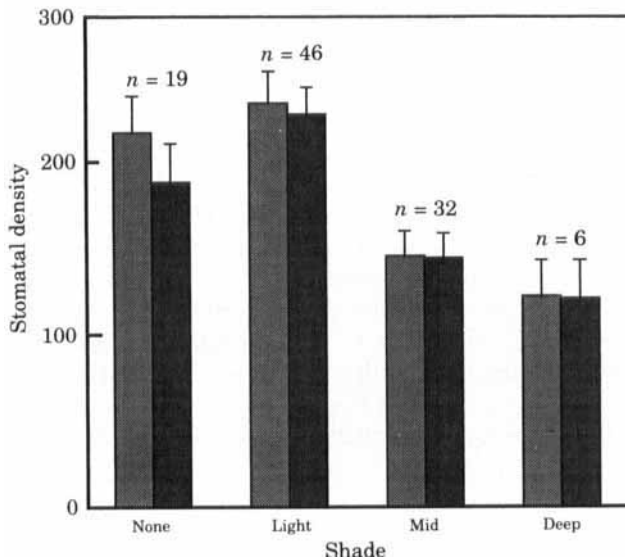


Figure 6. The stomatal density of species in hypostomatous families which are typical of different shade levels. ▨ = total density. ▩ = density on the lower epidermis. Error bars represent one standard error.

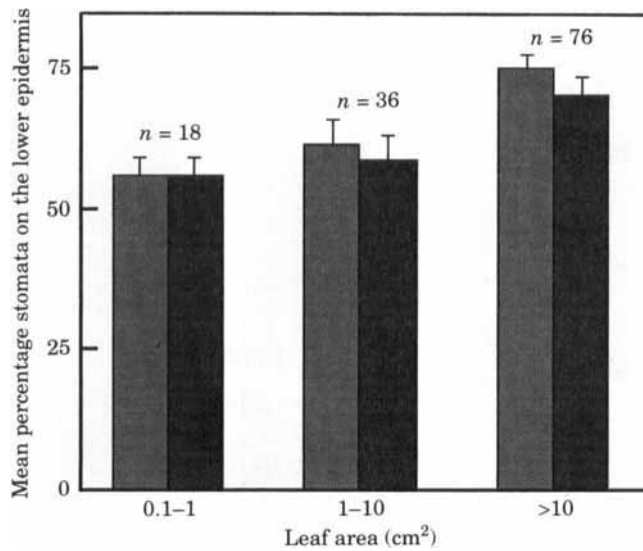


Figure 7. The mean percentage of stomata on the lower epidermis of species with different sized leaves. = all species in the weakly hypostomatous families, = species of none or lightly shaded habitats. Error bars represent one standard error.

> 10 cm² having different types of leaf margin ($F_{3,51} = 0.03$, $P = 0.991$). The analysis using only those species with leaf areas < 10 cm² was also not significant at the 5% level ($F_{2,50} = 2.25$, $P = 0.11$), although there is a trend for species with an entire margin to have a greater proportion of stomata on the lower epidermis than species with either toothed or lobed margins (Fig. 8).

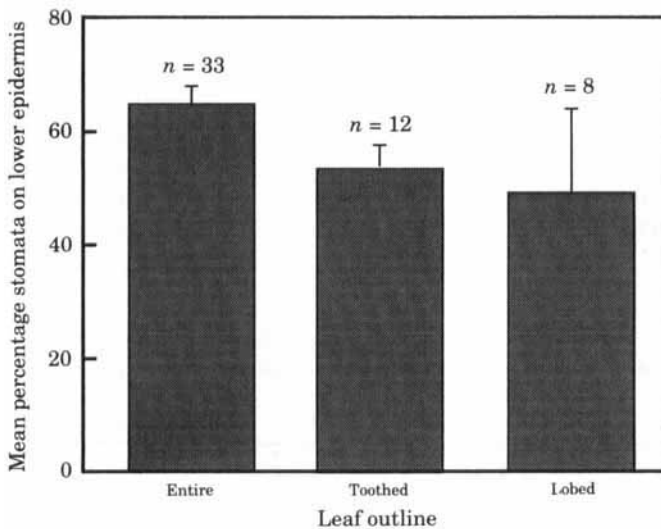


Figure 8. The mean percentage of stomata on the lower epidermis of species in weakly hypostomatous families which have different types of leaf outline. Error bars represent one standard error.

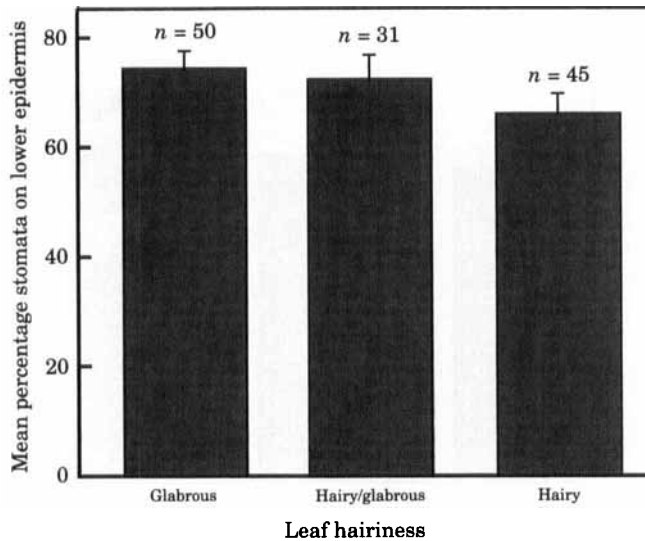


Figure 9. The mean percentage of stomata on the lower epidermis of species in weakly hypostomatous families which have different degrees of leaf hairiness. Error bars represent one standard error.

Species with glabrous leaves have a significantly higher proportion of stomata on the lower epidermis of the leaf than species with hairy leaves ($F_{1,93} = 4.39$, $P < 0.05$) although species with hairy or glabrous leaves do not differ significantly from either species with always hairy or always glabrous leaves ($F_{2,123} = 2.15$, $P = 0.12$, Fig. 9).

Woody families have a significantly different stomatal distribution from non-woody families ($\chi^2 = 17.5$, $P < 0.01$, Table 3). The number of families in each group indicate that this is due to woody families having a very strong tendency to be hypostomatous. Non-woody families however do not tend to any particular stomatal distribution.

Phanerophytes and geophytes tend to be hypostomatous whereas stomata on the upper epidermis are more common in hydrophytes, helophytes and therophytes (Table 4). Aquatic and dry grassland communities contain a large proportion of amphi-hyperstomatous species (i.e. species with 50–100% of stomata on the upper epidermis) and woodland communities a large proportion of hypostomatous species (Table 5). The predominance of hypostomy in

TABLE 3. The number of woody/non-woody families having different mean stomatal distributions, measured as the percentage of stomata on the lower epidermis. Woody families are those containing $> 90\%$ woody species and non-woody families those with $< 10\%$ woody species

Percent stomata on lower surface	No. woody families	No. non-woody families
92–100%	20	18
81–92%	0	6
50–80%	2	19
$< 50\%$	1	11

TABLE 4. The mean percentage of stomata on the lower epidermis of species in different life form classes

Life form	Mean percent stomata on the lower epidermis
therophyte	64
hemicytophyte	73
helophyte	66
hydrophyte	24
chamaephyte	74
geophyte	83
phanerophyte	96
all species	74

TABLE 5. The mean percentage of stomata on the lower epidermis of species occurring in different habitat types. Only habitats for which there was data for over 10 species and which have a particularly low or high stomatal distribution are included. The mean stomatal distribution of all species and of non-woody species is given

Corine code	Habitat description	Mean stomatal distribution of all species	Mean stomatal distribution of non-woody species
Habitats containing species with a low mean percentage of stomata on the lower epidermis			
C22.42	Rooted submerged vegetation	30.3	30.3
C22.43	Rooted floating vegetation	33.9	33.9
C16.13	Sand beach perennial communities	45.5	45.5
C17.32	Channel sea kale communities	52.7	50.1
C34.33	Sub-atlantic very dry calcareous grasslands	53.0	50.7
C81.1	Dry improved grasslands	53.6	53.6
C53.12	Common clubrush beds	53.8	51.6
C53.4	Small reed beds of fast-flowing waters	58.9	56.9
C15.34	Pearlwort-saltmarsh grass swards	60.0	60.0
C16.24	Heather brown dunes	60.0	48.0
C18.21	Atlantic cliff communities	62.3	59.2
C54.11	Soft water springs	62.3	61.1
C1A	Machair	62.3	61.1
C35.2	Medio-European open siliceous grasslands	62.5	57.0
C31.85	Gorse Thickets	62.8	60.3
Habitats containing species with a high mean percentage of stomata on the lower epidermis			
C54.5	Transition mires	83.1	82.2
C41.31	Ash-rowan-mercury forests	84.0	78.9
C37.71	Watercourse veils	85.0	85.0
C44.31	Ash-alder woods of rivulets and springs	85.4	79.8
C44.91	Alder swamp woods	85.7	80.2
C84	Tree lines, hedges, small woods, bocage, parkland dehesa	86.1	82.8
C44.A1	Sphagnum birch woods	86.5	80.0
C41.12	Atlantic acidophilous beech forests	86.5	74.1
C41.41	Ravine ash-sycamore forests	87.3	81.5
C41.16	Beech forest on limestone	87.4	80.8
C44.12	Lowland, collinar and mediterraneo-montane willow brush	87.5	81.9
C31.83	Atlantic poor soil thickets	88.3	84.5
C41.23	Sub-Atlantic oxlip ash-oak forests	89.0	85.3
C44.33	Ash-alder woods of slow rivers	89.5	85.1
C42.A7	Yew woods	90.8	73.6

woodland habitats is not solely due to woody species as the mean stomatal distribution of the non-woody species in these habitats is also high.

DISCUSSION

The results presented here show that shade, leaf area, and possibly leaf outline of small leaves and leaf hairiness are important factors determining the distribution of stomata over the leaf surface. Other ecological and morphological features may also be important but were not included in these analyses. Altitude, for example, can have an effect on stomatal distribution with species occurring at higher altitudes having a greater proportion of stomata on the upper epidermis (Korner *et al.*, 1989).

The results of the analyses considering habitat shade levels support the suggestions of Mott *et al.* (1982). Species living in unshaded environments have a significantly lower proportion of stomata on the lower epidermis than species living in shaded environments (Fig. 4). It does, therefore, appear that there is selection for amphistomaty in species living in unshaded habitats. The total density of stomata is also significantly greater in species living in unshaded habitats than in species living in shaded habitats (Figs 5 & 6). This is true both for species in group 1, i.e. those in families which are almost entirely hypostomatous, and species in group 3, weakly hypostomatous families which include species with a wide range of stomatal distributions. Our data show there has been selection for a morphology which will increase the uptake of CO₂ in situations where CO₂ availability is likely to limit the rate of photosynthesis. When, however, the density of stomata on each epidermis is considered, the species in weakly hypostomatous families (group 3) show that while the density on both epidermes increases as shade decreases, the increase on the upper epidermis is much greater than that on the lower epidermis. This indicates that the increase in density in high-light habitats is largely achieved by adding stomata to the upper epidermis rather than to both epidermes.

There are two possible explanations for this result, depending on whether or not the species in the hypostomatous families (group 1) have the capacity to develop stomata on the upper surface and are hypostomatous because they would be at a selective disadvantage if they were not, or whether these species are unable to develop stomata on the upper surface due to phylogenetic constraints. If their hypostomaty is solely due to selection then these results suggest that species in the weakly hypostomatous families (group 3) living in high light environments do not suffer the same selective disadvantage as do the species in entirely hypostomatous families (group 1) in having stomata on the upper epidermis. For example, the upper epidermis of species in group 3 families may be better adapted in some way to avoid water loss than those in group 1. It has been shown that the species in weakly hypostomatous families (group 3) with hairy leaves are more likely to be amphistomatous than species with glabrous leaves (Fig. 9), suggesting that the presence of leaf hairs is important in amphistomatous species. Again, however, there may be two explanations for this. Species with stomata on the upper surface could have evolved hairs because they reduce water loss; alternatively, species with hairy leaves may have been able to evolve stomata on the upper epidermis, whereas those with glabrous

leaves could not because of the competitive disadvantage of extra water loss. Some insight into this problem may be gained by studying the function of the hairs on the leaves: for example, reducing water loss may be an important function, as has been shown in more severe environments (Ehleringer, 1980). In addition, species which may or may not have hairs on the leaves can be used to determine whether leaves with hairs have a different stomatal distribution from those which do not.

It is not, however, the case that the species in hypostomatous families (group 1) are unable to evolve stomata on the upper epidermis because they lack hairs. There is no particular tendency in this group for species to have glabrous leaves: 44% of species are glabrous, 18% glabrous/hairy and 38% hairy. Unless there is some other feature preventing amphistomaty in the hypostomatous families this would suggest that species in these families have never evolved stomata on the upper epidermis—hypostomaty is thought to be the primitive condition (Mott *et al.*, 1982). If this is the case then the results of the shade analysis from the species in weakly hypostomatous families (group 3) indicate that there is a greater advantage in adding extra stomata onto a second surface rather than to the same surface. This may indeed be so as they will be benefiting from two parallel boundary layers. It is also possible that too high a density of stomata on a single surface may lead to the stomata interfering with one another. Parlange & Waggoner (1970) used models to infer that stomatal interference is negligible if interstomatal spacing is at least three times the length of the stomatal aperture. If an average stomatal opening is rectangular and 20 μm long by 10 μm wide (typical figures for the length and maximal stomatal opening: Meidner & Mansfield, 1968), and there are three stomatal lengths/widths between each pair of stomata, maximum density is 300 mm^{-2} . While this is an extreme simplification of a real leaf, 93% of the 353 British species for which data are available have densities below 300 mm^{-2} on the lower epidermis. On the upper epidermis, 94% of species have less than 200 stomata mm^{-2} , possibly because water loss from stomata on the upper epidermis is greater than from those on the lower epidermis, or because many species have less air space in the upper mesophyll than in the lower.

The models of both Parkhurst (1978) and Jones (1985) predict that hypostomaty will be advantageous over amphistomaty when there is a high boundary layer resistance. The results presented here provide some support for this. Species with large leaves or smaller leaves with an entire leaf margin, which will have the largest boundary layer resistance, tend to have a larger proportion of stomata on the lower epidermis than smaller/more dissected leaves (Fig. 8). Dissection of leaf margins has a much larger effect on the boundary layer of a small than of a large leaf. Species of shaded environments often have larger leaves than those in unshaded environments (Givnish, 1987) but the relationship between shade and stomatal distribution did not cause the relationship between leaf area and stomatal distribution, since the relationship survived when species of shaded habitats were omitted from the analysis.

Neither stomatal distribution nor density was related to habitat water availability, possibly because plants can control water loss by opening and closing stomata, so the morphological distribution of stomata over the leaf may not be the same as the functional distribution. Stomata on the upper and lower epidermes can respond differently (Turner, 1979; Pospisilova & Solarova, 1980);

upper stomata are more sensitive and may often be shut while the lower ones are open. Other factors than water availability may therefore be far more important in determining the distribution and density of stomata. In addition, some wetland species have xeromorphic adaptations which reduce both transpiration and the uptake of toxic ions such as Fe^{2+} (Etherington, 1983). Parkhurst's models predict that stomatal location will have very little effect on CO_2 uptake when water stress is high and that in general climatic variables are relatively unimportant compared with differences in leaf structure for determining whether stomatal location is important. Parkhurst (1978), however, did find that amphistomaty was more common in xeric and hydric habitats and hypostomaty in mesic habitats. The data from the British flora (Table 5) also show that dry grassland and aquatic and wetland habitats contain species with a high proportion of stomata on the lower epidermis. Hypostomaty, however, is prevalent in woodland habitats, in both woody and non-woody species, and also in some wetland habitats where woody species or rushes and sedges predominate.

The results also provide evidence that hypostomaty in woody species is an adaptive trait and not simply due to shared ancestry between woody and hypostomatous species. This may be because woody species often have a better developed canopy than herbaceous species and boundary layers of leaves protected by a plant canopy are likely to be greater than unprotected leaves. Furthermore, Parkhurst (1978) suggests that the air inside the canopy will be enriched with water vapour and reduced in CO_2 concentration, but will also have increased humidity. The proportional increase in humidity will be greater than the proportional decrease in CO_2 concentration, and pores on the lower surface on the leaf will tend to be more exposed to this favourable air than any on the upper surface. Amphistomaty is only likely to evolve if CO_2 is limiting the photosynthetic rate or if rapid water use is advantageous, in slow-growing woody species neither is likely to be the case. Amphistomaty is especially common in annual species (therophytes have, on average, 64% of their stomata on the lower surface of the leaf), whereas slower growing species tend to be more hypostomatous (geophytes with 83% and phanerophytes, with 96% of their stomata on the lower leaf surface; Table 4).

Many other factors may play a role in determining the stomatal distribution. Leaf angle may be important: horizontal leaves might be expected to be mainly hypostomatous to reduce water loss, and amphistomaty or even hyperstomaty to be more common in vertical leaves. Different types of leaves such as stem or rosette leaves may also have different stomatal distributions. Pathogens might also be important as the stomata are natural openings in the cuticle of a leaf and are used by fungi, bacteria or viruses to gain entry into a leaf (Martin & Juniper, 1970), so it is possible that the susceptibility of species to pathogens may vary according to the distribution and density of stomata. The analyses conducted here using data from the Ecological Flora Database, however, suggest that hypostomaty is prevalent in woody species, species of shaded environments, species with large leaves or with smaller leaves but an entire leaf margin and species with glabrous leaves. Hypostomaty is likely to have been the primitive condition (Mott *et al.*, 1982) and it appears that while hypostomaty may be an adaptive condition in some species, there are others which have never evolved the capacity to produce stomata on the upper epidermis. Amphistomaty is selected in conditions where CO_2 is likely to limit the photosynthetic rate, such as

unshaded habitats and is also commoner in conditions where water loss from the leaf will be limited by, for example, structures on the leaf surface such as trichomes.

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APPENDIX

A list of all the families in the British flora, the mean proportion of stomata on the lower epidermis of species within each family (stomatal distribution), the group to which the family belongs (see Table 1), minimum and maximum stomatal distribution of individual species within each family and both the number of species for which stomatal data was available and the total number in each family. ND means no data were available for that family

Family	Stomatal distribution	Group	Minimum	Maximum	No. species with data	No. species in family
Aceraceae	1.00	1	1.00	1.00	3	3
Adoxaceae	1.00	1	1.00	1.00	1	1
Aizoaceae	ND	ND	ND	ND	0	3
Alismataceae	0.36	4	0.30	0.42	2	7
Apiaceae	0.93	1	0.47	1.00	15	57
Apocynaceae	1.00	1	1.00	1.00	1	2
Aquifoliaceae	1.00	1	1.00	1.00	1	1
Araceae	0.63	3	0.63	0.63	1	4
Araliaceae	1.00	1	1.00	1.00	1	1
Aristolochiaceae	ND	ND	ND	ND	0	1
Asteraceae	0.67	3	0.00	1.00	37	176
Balsaminaceae	0.67	3	0.67	0.67	1	4
Berberidaceae	1.00	1	1.00	1.00	1	2
Betulaceae	1.00	1	1.00	1.00	6	6
Boraginaceae	0.89	2	0.63	1.00	6	28
Brassicaceae	0.64	3	0.32	1.00	11	85
Buddlejaceae	ND	ND	ND	ND	0	1
Butomaceae	ND	ND	ND	ND	0	1
Buxaceae	1.00	1	1.00	1.00	1	1
Callitrichaceae	ND	ND	ND	ND	0	7
Campanulaceae	0.86	2	0.63	1.00	6	15
Cannabaceae	ND	ND	ND	ND	0	1
Caprifoliaceae	1.00	1	1.00	1.00	6	11
Caryophyllaceae	0.65	3	0.32	1.00	16	78
Celastraceae	1.00	1	1.00	1.00	1	1
Ceratophyllaceae	ND	ND	ND	ND	0	2
Chenopodiaceae	0.61	3	0.57	0.64	4	33
Cistaceae	0.62	3	0.50	0.73	2	4
Clusiaceae	1.00	1	1.00	1.00	7	13
Convolvulaceae	0.79	3	0.61	0.97	2	5
Cornaceae	1.00	1	1.00	1.00	1	2
Crassulaceae	0.49	4	0.34	0.72	3	14
Cucurbitaceae	1.00	1	1.00	1.00	1	1
Cupressaceae	0.00	4	0.00	0.00	1	1
Cuscutaceae	ND	ND	ND	ND	0	2
Cyperaceae	0.82	2	0.00	1.00	22	100
Diapensiaceae	1.00	1	1.00	1.00	1	1
Dioscoreaceae	ND	ND	ND	ND	0	1
Dipsacaceae	0.84	2	0.74	0.91	3	5
Droseraceae	0.50	3	0.50	0.50	1	3
Elaeagnaceae	ND	ND	ND	ND	0	1
Elatinaceae	ND	ND	ND	ND	0	2
Empetraceae	ND	ND	ND	ND	0	1
Ericaceae	0.98	1	0.87	1.00	8	29
Eriocaulaceae	ND	ND	ND	ND	0	1
Euphorbiaceae	0.97	1	0.93	1.00	2	15
Fabaceae	0.48	4	0.00	1.00	20	84
Fagaceae	1.00	1	1.00	1.00	3	5
Frankeniaceae	1.00	1	1.00	1.00	1	1
Fumariaceae	1.00	1	1.00	1.00	1	12
Gentianaceae	0.71	3	0.58	0.80	3	16
Geraniaceae	0.96	1	0.74	1.00	6	19
Grossulariaceae	1.00	1	1.00	1.00	3	6
Gunneraceae	ND	ND	ND	ND	0	1
Haloragaceae	ND	ND	ND	ND	0	3
Hippuridaceae	0.41	4	0.41	0.41	1	1

APPENDIX—*cont.*

Family	Stomatal distribution	Group	Minimum	Maximum	No. species with data	No. species in family
Hydrocharitaceae	0.31	4	0.00	0.61	2	9
Iridaceae	ND	ND	ND	ND	0	10
Juncaceae	1.00	1	1.00	1.00	25	35
Juncaginaceae	0.50	3	0.50	0.50	1	2
Lamiaceae	0.94	1	0.81	1.00	14	51
Lemnaceae	0.00	4	0.00	0.00	3	6
Lentibulariaceae	1.00	1	1.00	1.00	1	9
Liliaceae	0.76	3	0.44	1.00	12	43
Linaceae	0.46	4	0.46	0.46	1	4
Lythraceae	0.87	2	0.87	0.87	1	3
Malvaceae	0.65	3	0.56	0.74	4	6
Menyanthaceae	0.00	4	0.00	0.00	1	2
Monotropaceae	ND	ND	ND	ND	0	1
Myricaceae	ND	ND	ND	ND	0	1
Najadaceae	ND	ND	ND	ND	0	2
Nymphaeaceae	0.00	4	0.00	0.00	2	3
Oleaceae	1.00	1	1.00	1.00	2	2
Onagraceae	0.77	3	0.39	1.00	10	22
Orchidaceae	1.00	1	1.00	1.00	4	49
Orobanchaceae	ND	ND	ND	ND	0	10
Oxalidaceae	1.00	1	1.00	1.00	1	5
Papaveraceae	0.65	3	0.43	0.87	2	7
Pinaceae	0.50	3	0.50	0.50	1	2
Pittosporaceae	ND	ND	ND	ND	0	1
Plantaginaceae	0.56	3	0.46	0.46	4	6
Plumbaginaceae	0.39	4	0.33	0.44	2	5
Poaceae	0.21	4	0.00	1.00	42	149
Polemoniaceae	1.00	1	1.00	1.00	1	1
Polygalaceae	0.57	3	0.57	0.57	1	4
Polygonaceae	0.73	3	0.00	1.00	14	38
Portulacaceae	0.50	3	0.50	0.50	1	4
Potamogetonaceae	0.02	4	0.02	0.02	1	22
Primulaceae	0.87	2	0.68	1.00	5	18
Pyrolaceae	0.96	1	0.92	1.00	2	5
Ranunculaceae	0.78	3	0.00	1.00	22	40
Resedaceae	0.60	3	0.60	0.60	1	2
Rhamnaceae	1.00	1	1.00	1.00	2	2
Rosaceae	0.97	1	0.37	1.00	21	107
Rubiaceae	0.98	1	0.95	1.00	3	17
Ruppiaceae	ND	ND	ND	ND	0	2
Salicaceae	0.94	1	0.52	1.00	11	24
Santalaceae	ND	ND	ND	ND	0	1
Sarraceniaceae	1.00	1	1.00	1.00	1	1
Saxifragaceae	0.60	3	0.24	1.00	3	20
Scheuchzeriaceae	ND	ND	ND	ND	0	1
Scrophulariaceae	0.87	2	0.53	1.00	16	85
Simaroubaceae	ND	ND	ND	ND	0	1
Solanaceae	0.78	3	0.62	1.00	4	8
Sparganiaceae	ND	ND	ND	ND	0	4
Taxaceae	1.00	1	1.00	1.00	1	1
Thymelaeaceae	1.00	1	1.00	1.00	2	2
Tiliaceae	1.00	1	1.00	1.00	2	2
Typhaceae	ND	ND	ND	ND	0	2
Ulmaceae	ND	ND	ND	ND	0	3
Urticaceae	1.00	1	1.00	1.00	1	4
Valerianaceae	1.00	1	1.00	1.00	1	9
Verbenaceae	0.80	3	0.80	0.80	1	1
Violaceae	0.93	1	0.78	1.00	4	14
Visaceae	ND	ND	ND	ND	0	1
Vitaceae	ND	ND	ND	ND	0	1
Zannichelliaceae	ND	ND	ND	ND	0	1
Zosteraceae	ND	ND	ND	ND	0	3