

Interspecific covariation between stomatal density and other functional leaf traits in a local flora

Jessy Loranger and Bill Shipley

Abstract: Despite the importance of stomata in leaf functioning, and despite the recent interest in interspecific leaf trait covariation in functional ecology, little is known about how stomatal density relates to other leaf traits in a broad interspecific context. This is especially important because stomatal density has been widely used to deduce temporal variation in atmospheric CO₂ concentrations [CO_{2atm}] from fossilized or herbarium leaves. We therefore measured stomatal density, specific leaf area (SLA) and its components, leaf thickness, and leaf chlorophyll content in both sun and shade leaves of 169 individuals from 52 angiosperm species in southwestern Quebec. Using mixed models, we show that stomatal density decreases allometrically with increasing SLA and chlorophyll content, and increases allometrically with increasing lamina thickness. The sun–shade contrast changes the intercepts, but not the slopes, of these relationships. It is important to take into consideration these relations when correlating stomatal density with [CO₂], to avoid spurious interpretations.

Key words: SLA, stomatal density, allometry, comparative ecology, leaf lamina thickness, variance components.

Résumé : Malgré l'importance des stomates dans le fonctionnement des plantes et malgré les récents intérêts pour la covariation interspécifique des traits foliaires en écologie fonctionnelle, peu est connu sur comment la densité stomatique est reliée aux autres traits foliaires. Ceci est particulièrement important parce que la densité stomatique a largement été utilisée pour déduire les variations dans le temps des concentrations de CO₂ atmosphérique [CO_{2atm}] à partir de feuilles fossilisées ou de feuilles d'herbiers. Cependant, la relation entre [CO_{2atm}] et densité stomatique n'est pas claire et est incertaine. Nous avons donc mesuré la densité stomatique, la surface spécifique foliaire (SSF) et ses composantes, l'épaisseur des feuilles et la concentration de chlorophylle des feuilles, pour les feuilles de soleil et d'ombre, de 169 individus provenant de 52 espèces d'angiospermes dans le sud-ouest du Québec. À l'aide de modèles mixtes, nous montrons que la densité stomatique diminue allométriquement avec la SSF et avec la concentration de chlorophylle et qu'elle augmente allométriquement avec l'épaisseur des feuilles. Le contraste entre feuilles de soleil et feuilles d'ombre change les interceptes, mais pas les pentes. Il est important de prendre en considération ces relations quand la densité stomatique est corrélée avec [CO_{2atm}], afin d'éviter des interprétations erronées.

Mots-clés : SSF, densité stomatal, allométrie, l'écologie comparée, épaisseur de la lame foliaire, composantes de la variance.

Introduction

Stomata are known to have an important role in the control of transpiration, and thus in the regulation of water loss, leaf temperature, as well as in gas exchange. Moreover they are responsible for the trade-off between CO₂ gain and water loss (water use efficiency) (Morison 1998; Gutschick 1999). Given this, it is surprising that so little is known about large scale interspecific covariation between this leaf attribute and other leaf traits. Recent work documenting and quantifying the major axes of variation in leaf functional traits, including some components of gas exchange (photosynthetic rate and stomatal conductance), have ignored variation in stomatal density (*D*) simply because this variable is rarely studied in an interspecific context (Reich et al. 1997;

Wright et al. 2004; Shipley et al. 2006; Marino et al. 2010). However, recent comparative work on covariation between *D*, genome size, and cell size suggests some tantalizing relationships between *D* and leaf functional traits. Beaulieu et al. (2008) reported a positive correlation between genome size and cell size (guard and epidermal cells) and a negative correlation with stomatal density in 101 species. Lomax et al. (2009) showed that these scaling relationships were preserved in *Arabidopsis thaliana* in different environments. Since the model of Shipley et al. (2006) hypothesizes that the major axis of leaf functional trait coordination is generated by the ratio between leaf cytoplasmic and cell wall volumes, and since this ratio would decrease in leaves having more, but smaller, cells, it is possible that variation in *D* would also covary with these other leaf traits, including specific leaf area. Thus, the objective of this paper is to quantify the degree of interspecific covariation between *D* and other leaf functional traits.

Besides functional plant ecology, the importance of understanding the interspecific covariation between *D* and other leaf traits, and how such interspecific covariation might vary with external environments is relevant in other

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J. Loranger and B. Shipley.¹ Département de biologie and Centre des études de la forêt, Université de Sherbrooke, Sherbrooke, QC J1K 2R9, Canada.

¹Corresponding author (e-mail: Bill.Shipley@USherbrooke.ca).

areas. For instance, based on an assumed link between D and atmospheric CO_2 concentration $[\text{CO}_{2\text{atm}}]$, many studies have tried to link variation in $[\text{CO}_{2\text{atm}}]$ over geological time with variation in D measured on fossil leaves (Paoletti and Gellini 1993; Beerling and Woodward 1997; Kouwenberg et al. 2003; Roth-Nebelsick et al. 2004; Carter 2007). However, if interspecific variation in D is strongly constrained by covariation with other leaf functional traits, then this becomes potentially problematic if such functional traits also respond to other environmental factors. For instance, Royer et al. (2007) have recently linked the leaf economics spectrum (Wright et al. 2004) of functional traits (but not D) to variation in paleoclimates. The bivariate link between D and $[\text{CO}_{2\text{atm}}]$ is not clear (Morison 1998), since different papers have reported negative relationships (Woodward and Bazzaz 1988; Woodward and Kelly 1995; Kürschner et al. 1998), positive relationships (Ferris and Taylor 1994; Marchi et al. 2004), or no relationship at all (Reddy et al. 1998). Moreover, Tricker et al. (2005) observed that an elevation of $[\text{CO}_{2\text{atm}}]$ leads to a decrease of D in three *Populus* species, but only for the first 2 years, after which there was no significant relationship. Other environmental factors can also affect variation in D , including temperature (Luomala et al. 2005) and light intensity (Casson and Gray 2008).

Part of the difficulty lies in the fact that such relationships are often measured from rather small numbers of species. Another complication is that D might covary with other leaf traits, requiring such covariation to be taken into account before comparing across environments. For instance, the same environmental factors that have been implicated in changing D also affect specific leaf area (SLA). Cao et al. (2008) have shown that an elevated $[\text{CO}_{2\text{atm}}]$ can cause a decrease in SLA. Pardos et al. (2006) point out that water and light stresses have a greater impact on all morphological leaf traits than does elevated $[\text{CO}_2]$. Yañez-Espinosa et al. (2003) clearly showed that for a wide range of environmental factors (including temperature, vapour pressure deficit, relative humidity), the variations of both SLA and stomatal density are the opposite of each other. It is well known that shade leaves have a smaller SLA than sun leaves (Pardos et al. 2006; Al Afas et al. 2007), and this also leads to a greater D (Al Afas et al. 2007). Despite such possible links, only a few studies (Tay and Furukawa 2008) exist concerning the relation between stomata and other leaf morphological characteristics. Although SLA and D are often present in the same study, the relationship between them is rarely made although it is usually, but not always (Luo et al. 2006), found that for the same factor, SLA and D respond in different directions (Al Afas et al. 2007; Avramov et al. 2007). These studies therefore suggest that a relationship between D and SLA should exist, and that this relationship might complicate interpretations of variation in D with environments. Unfortunately each study involves only a small number of species, and so we do not know what the general relationship might be.

Because of these uncertainties and contradictory studies, here we report a first attempt to establish a general relation between D and some leaf traits implicated in the “worldwide economics spectrum” (Reich et al. 1997; Wright et al. 2004; Shipley et al. 2006). Specifically, we measured D , SLA and its two components (projected leaf

area and leaf dry mass), leaf lamina thickness, and leaf chlorophyll concentration on a wide selection of plant species, and quantified the allometric relationships between them at four hierarchical levels: between families, between species, between individuals, and within leaves of the same individual. Few studies consider the hierarchical nature of such data, but it is important to do so for at least three reasons. The statistical reason is to properly take into account the partial dependence of the data (“pseudo-replication”) when conducting tests of significance. There are two biological reasons. First, the relationships might differ at different hierarchical levels. Second (and related to the first reason), differences in the quantitative relationships at different hierarchical levels provides insight into evolutionary and taxonomic constraints that might be forcing such relationships; if such relationships exist primarily at higher taxonomic levels then species-level comparisons would not be informative or necessary.

Material and methods

The data set includes 52 angiosperm species in 36 genera and 21 families, including 6 herbaceous species and 46 woody species (Appendix A, Table A1); nomenclature follows Marie-Victorin et al. (1995) unless otherwise indicated. All data came from plants growing in the field (including the non-indigenous species). Each species included an average of three individuals and, when possible, came from different sites. Each individual included an average of two leaves from the exterior of the canopy and exposed to direct sunlight (“sun leaves”) and two leaves from the interior of the canopy (“shade leaves”). Rijkers et al. (2000) showed that the canopy position of a leaf is a good approximation of the actual photon flux density experienced by it. Plants were sampled in 14 different sites in the south of Quebec, Canada (latitude, 45°15'N to 46°12'N; longitude, 71°38'W to 72°31'W), a region that contains mixed hardwood forests. This sampling was done over 3 months, from 22 May 2007 to 16 August 2007.

When sampling, a part of the plant (stem or branch) including at least six complete and healthy leaves was harvested on a randomly chosen individual in the population. The branch or stem tip was placed in a small receptacle containing water, stored in a cooler, and directly brought to the laboratory where the stems were re-cut under water and stored in water in darkness, for approximately 16 h to allow non-structural carbohydrates to be respired from the leaf (Garnier et al. 2001). The next day, we sampled three or four healthy well-developed leaves.

Trait analysis

First, after the leaf was cut from the stem, the fresh mass of the leaf blade and petiole were measured. Next, leaf thickness was measured with a digital micrometer while avoiding veins as much as possible. The leaf area was then measured with Winfolia software (Instruments Régent, Québec, Quebec, Canada).

D (abaxial and adaxial) was measured from imprints of leaf surfaces made with clear nail polish and examined with a compound microscope (100×) (Hodgson et al. 1993). The number of stomata per square millimetre was counted using

a grid ocular, again avoiding veins as much as possible. Only one count per film was made and the average of three films gave D of the surface of the leaf. Finally, the leaf blade and petiole dry mass were measured after 48 h at 70°C.

For chlorophyll content, a part of each leaf (~50 mg, depending on the species) was taken and put in dimethyl sulphoxide (DMSO) at 65 °C until no colour remained in the tissues of the leaf. Then, the chlorophyll content was analysed with a spectrophotometer, following Arnon (1949) and Hiscox and Isrealstam (1979).

Statistical analysis

Because of a lack of appropriate sample size for the herbaceous species, only the woody species were used for all the statistical analysis at the species, individuals, and leaf levels. Different authors have used different models (reduced major axis or least-squares) to estimate allometric slopes. Both standardized major axis and least-squares regression slopes are biased (Sprent 1969), but knowing which (if either) is more appropriate requires a knowledge of the causal structure linking the variables (Shiple 2000). When such knowledge is not available (as here) then it is impossible to know whether standardized major axis regression is less biased than least squares regression. Furthermore, standardized major axis regression cannot yet be extended to a hierarchical context. Given the nested structure of data (between families, between species in the same family, between individuals in the same species, and between leaves in a same individual), we therefore used mixed models (Pinheiro and Bates 2000) fitted using the R statistical software that gives least-squares (maximum likelihood) slopes. Given that intercepts were always part of the random part of the models (which means that intercepts are allowed to vary), we used centered values of dependant variables; in this way, variation in intercepts matched variation in means. All variables discussed in the next sections were always transformed in natural logarithms to better approximate a normal distribution of residuals.

Results

Relations with SLA

Table 1 lists the variance components of each measured trait; all statistical analyses were based on natural-log (ln)-transformed variables. It is clear that these leaf traits display non-negligible variation at every hierarchical level, thus justifying a mixed model analysis. We regressed abaxial D on specific leaf area (SLA) in a mixed model with the same slope for all leaves but allowing variation of the intercepts between levels (and thus means because variables are centered). The allometric slope was -0.344 (± 0.046 SD, $p < 0.0005$) showing that D decreases with increasing SLA ($D \propto 1/\text{SLA}^{0.344}$). This model explained 17.5% of the initial variance of D between the species of the same family and likewise between the individuals belonging to the same species. Only 7.0% of this variance between the leaves of the same individual was explained. However, the residual variance between families increased.

Next, we added the leaf's location (shade–sun) as a covariable. Sun leaves with the same SLA tend to have a

Table 1. Components of variance (percent total variance) for stomatal density (no. \cdot mm $^{-2}$), specific leaf area (SLA, cm 2 \cdot g $^{-1}$), leaf lamina thickness (mm), chlorophyll content (μ mol \cdot g $^{-1}$), leaf area (cm 2), and leaf mass (g).

Level	ln(stomatal density)	ln(SLA)	ln(leaf thickness)	ln(chlorophyll)	ln(leaf area)	ln(leaf mass)
Family	0.138 (33.4%)	0.012 (7.0%)	1×10^{-9} (0.0%)	0.026 (15.9%)	1.323 (75.3%)	1.174 (68.8%)
Species	0.110 (26.5%)	0.074 (43.9%)	0.061 (60.7%)	0.032 (19.7%)	0.280 (15.9%)	0.305 (17.9%)
Individual	0.133 (32.3%)	0.052 (31.0%)	0.021 (21.0%)	0.065 (39.9%)	0.081 (4.6%)	0.111 (6.5%)
Leaf	0.032 (7.8%)	0.030 (18.1%)	0.018 (18.3)	0.040 (24.5%)	0.073 (4.2%)	0.116 (6.8%)
Total	0.412	0.168	0.101	0.163	1.758	1.706

higher D , 0.107 ± 0.022 ln units ($p < 0.0005$) than do shade leaves, but the allometric slope decreased to -0.183 (± 0.055 , $p = 0.0010$). However, this result assumes that the allometric slope (whatever its value) is the same over all hierarchical levels. To relax this assumption, we allowed the slopes to vary between the hierarchical levels by introducing SLA in the random part of the model. In this new model, the average allometric slope was -0.230 (± 0.078 , $p = 0.0033$) and the difference between the intercept of the sun leaves and the shade leaves decreased to 0.090 (± 0.023 , $p < 0.0005$). Therefore, D decreases in species having a larger SLA even when comparing leaves at the same canopy position, and sun leaves have a slightly larger D on average, even after taking into account differences in SLA. Therefore, the allometry for sun leaves was $D \propto 1.09/\text{SLA}^{0.23}$ and for shade leaves was $D \propto 1.0/\text{SLA}^{0.23}$. This model showed that 58.8% of the variation between the allometric slopes came from the variation between the species belonging to the same family, and 41.2% came from the variation between individuals belonging to the same species. Therefore, almost half of the variation in allometric slopes was due to some combination of individual plastic phenotypic variation and genetic variation between genotypes of the same species.

The interaction between the slopes and the intercepts explained a part of the variance of each except at the family level. At the species level, there was a weak negative correlation between the slope values and the intercept values ($r = -0.172$), while the correlation was strong and positive at the individual level ($r = 0.751$). Thus, when comparing between species, those species whose average D was lower than average had a steeper negative allometric slope with respect to SLA. However, when comparing between individuals of the same species, those individuals having a lower D had a shallower negative allometric slope with respect to SLA. Finally, SLA again had a slightly higher mean for shade leaves than sun leaves. Figure 1 summarizes these results; as can be seen, the negative correlation between specific leaf area and D exists both within and between species, but not within a single individual or at the family level.

Relations with leaf area and leaf mass

Since SLA is leaf projected area divided by leaf dry mass, it follows that $\ln(\text{SLA}) = \ln(\text{leaf area}) + \ln(1/\text{leaf mass})$. To see whether area or mass was most responsible for the previous regression results, we regressed D on both leaf area and leaf mass in the same kind of mixed model that we had used earlier. This model gave partial allometric slopes of -0.365 (± 0.056 , $p < 0.0005$) for $\ln(\text{leaf area})$ and -0.347 (± 0.046 , $p < 0.0005$) for $\ln(1/\text{leaf mass})$ giving an allometry of $D \propto \text{mass}^{0.347}/\text{area}^{0.365}$. These two slopes were significantly different from zero but not significantly different from each other. Both components of SLA therefore affect variation in D ; increasing dry mass increases stomatal density and increasing surface area decreases stomatal density. This model explained 17.8% of the initial variance of D between the species belonging to the same family, 17.2% of this variance between the individuals belonging to the same species and 6.9% of this variance between the leaves belonging to the same individual.

Relations with leaf thickness and chlorophyll concentration

We next regressed D on leaf thickness, again fixing the slope but allowing intercepts to vary. The allometric slope was 0.302 (± 0.060 , $p < 0.0005$), thus increasing thickness is associated with an increased stomatal density with an allometry of $D \propto \text{thickness}^{0.302}$. Here the model explained 13.6% of the initial variation of D between species coming from the same family and 6.4% of this variation between the leaves of the same individual. However, the variance of D between families and between individuals belonging to the same species increased.

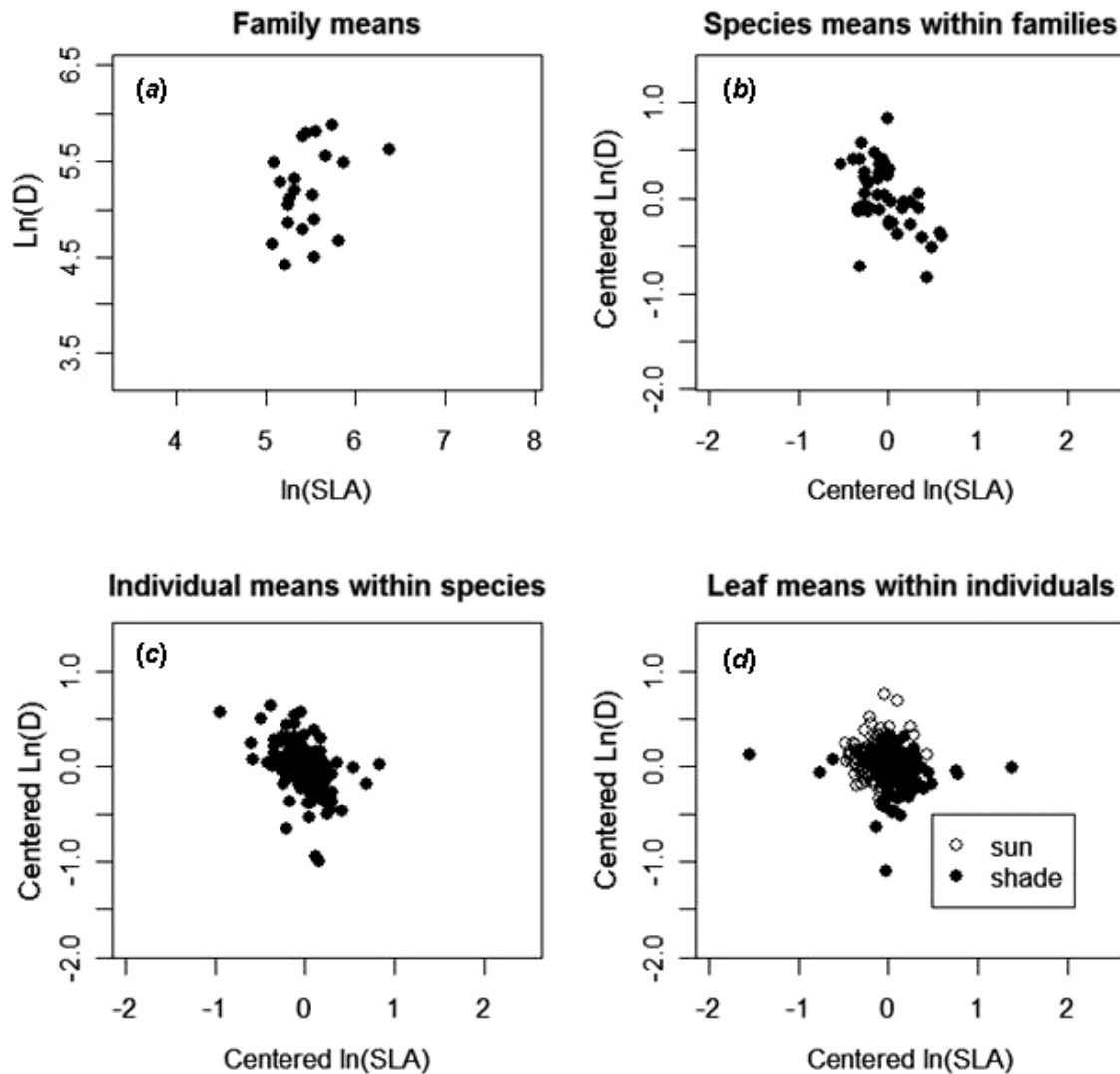
Finally, we regressed D on leaf chlorophyll concentration in a mixed model having the same characteristics as the previous one. Here the allometric slope was -0.233 (± 0.042 , $p < 0.0005$). Leaves with more chlorophyll per gram dry mass have a lower stomatal density and the allometric relationship is the same as for SLA: $D \propto 1/\text{chlorophyll}^{0.23}$. This model explained 4.2% of the initial variation of D between the species of the same family and 8.3% of this variation between the leaves of the same individual. Here again, we noticed that the variance of D between families and between the individuals of the same species increased.

Discussion

We have shown that D is negatively correlated to SLA at two hierarchical levels: between species, and between individuals within same species, but that this relation does not seem to exist between families and within a single individual (Fig. 1). If most of the covariation between these two variables existed at the family level then this would point to conservative evolutionary relationships that are not responsive to microevolution. Since this is not the case, then this suggests that the covariation between SLA and D can respond to selection over shorter evolutionary time scales — a necessary requirement for inferring environmental changes from interspecific comparisons of fossil leaves. Indeed, SLA and D seem to be linked even with respect to purely plastic changes generated by canopy position.

We observed, as did Al Afas et al. (2007), that SLA was higher and D lower for shade leaves than sun leaves. These results are also in agreement with Rijkers et al. (2000), who proposed that LMA (the inverse of SLA) could be a key variable in linking variation of the other leaf traits. Lomax et al. (2009) have shown that changing irradiance (as well as other environmental conditions) induced changes in guard cell length (a measure of cell size) and therefore in stomatal density (Beaulieu et al. 2008). Since sun leaves are also well known to increase in thickness and even in the number of layers of palisade mesophyll, this suggests that both cell number and size change with changes in irradiance. It is perhaps for this reason that in the regression of D with both leaf area and leaf dry mass, both partial allometric slopes were significant and similar in magnitude, but opposite in sign. Thus the negative correlation between D and SLA is unlikely to be only a result of a greater cell size via expansion of leaf cells in the bud after the end of the cell division. If this were the case, then variation in surface area would be much more important than leaf dry mass in the regression with D . According to our results, there is

Fig. 1. (a) Family means of abaxial stomatal density (no. mm^{-2}) as a function of specific leaf area ($\text{cm}^2\cdot\text{g}^{-1}$). (b) Species means centered about their respective family means. (c) Means of individual plants centered about their respective species means. (d) Means of individual leaves centered about their individual plant means; sun and shade leaves are differentiated.



probably a general link between D and morphological and physiological leaf traits at both the interspecific and intraspecific levels. D can also be linked to leaf thickness and leaf chlorophyll concentration, since thicker leaves with lower chlorophyll content tend to have a higher D . At the interspecific level and above, it is clear (Beaulieu et al. 2008; Knight and Beaulieu 2008) that differences in genome size are positively correlated with changes in the size of leaf cells, and that this presumably impacts stomatal density. However, since there are also similar scaling relationships between D and SLA at an intraspecific level, where genome size will not vary, environmental effects on leaf cell size and number must also come into play. This interplay is still poorly understood.

Given these results, we cannot simply measure D of leaves and then directly link those data to variation in $[\text{CO}_{2\text{atm}}]$, regardless of other leaf characteristics, because the variation in D could be due only (or in part) to the variation of those characteristics. As D seems to covary with many other leaf traits, it would be useful to experimentally

vary $[\text{CO}_{2\text{atm}}]$ in interspecific comparisons and simultaneously measure these other traits (D , SLA , light intensity available, leaf thickness, and chlorophyll content), as was done by Lomax et al. (2009) for guard cell length. In this way we could see whether $[\text{CO}_{2\text{atm}}]$ has an influence on variation in D independently of the links between D and other leaf traits, and, if so, we could quantify it.

Also, according to our results, there was no detectable relationship between D and the other leaf traits at the level of individuals after taking into account the sun–shade contrast. This conclusion must be considered very tentative. It has been shown that D of young leaves depends on the state of stomatal conductance of older leaves (Lake et al. 2001; Miyazawa et al. 2006). This could come from an individual plasticity that allows the plant to adapt quickly to its environment. According to Kürschner et al. (1998), the lack of this plasticity in some species, preventing stomatal acclimation of plants to their environment, could explain the fact that some studies don't observe significant results, but this remains uncertain.

Finally, based on our results, it is possible that interspecific variation in *D* is part of the worldwide economic spectrum of correlated leaf traits (Reich et al. 1997; Wright et al. 2004; Shipley et al. 2006). If so, this would represent a further generalization of this important evolutionary tendency in the design of leaves. This would help us to understand this “economic spectrum” and lead to an ability to correlate adequately *D* to variation in paleoclimates, as did Royer et al. (2007) with other leaf traits.

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Appendix A

Appendix A, Table A1, appears on the following pages.

Table A1. Number of individual per species (nb), adaxial and abaxial stomatal density (Adaxial *D* and Abaxial *D*, stomata-mm⁻²), petiole fresh and dry mass (PFM and PDM, g), leaf blade fresh and dry mass (LBFM and LBDM, g), leaf chlorophyll concentration ([chloro], μmol·g⁻¹), leaf thickness (LT, mm), surface area (SA, cm²) and specific leaf area (SLA, cm²·g⁻¹).

Family	Species	nb	Adaxial <i>D</i>	Abaxial <i>D</i>	PFM	LBFM	PDM	LBDM	[chloro]	LT	SA	SLA
<i>Aceraceae</i>	<i>Acer negundo</i>	4	0.00	412.33	0.2453	2.0784	0.0587	0.5266	2.12	0.18	121.39	262.12
<i>Aceraceae</i>	<i>Acer pennsylvanicum</i>	5	0.00	174.54	0.1510	1.3671	0.0298	0.3538	2.73	0.15	126.46	406.75
<i>Aceraceae</i>	<i>Acer rubrum</i>	5	0.00	227.06	0.2240	0.8746	0.0527	0.3026	2.67	0.14	73.75	238.19
<i>Aceraceae</i>	<i>Acer saccharinum</i>	4	0.00	100.04	0.2030	0.9811	0.0528	0.3654	2.44	0.17	64.25	186.66
<i>Aceraceae</i>	<i>Acer saccharum</i>	5	0.00	279.63	0.3523	1.4396	0.1054	0.5468	2.59	0.13	115.80	226.45
<i>Hippocastanaceae</i>	<i>Aesculus hippocastanum</i>	2	0.00	167.68	1.4375	6.1988	0.3616	1.9413	4.40	0.22	362.11	202.03
<i>Betulaceae</i>	<i>Alnus rugosa</i>	2	0.00	88.36	0.0513	0.6987	0.0125	0.2320	3.70	0.19	45.54	206.92
<i>Asclepiadaceae</i>	<i>Asclepias syriaca</i>	4	14.53	329.65	0.0664	1.9053	0.0123	0.3593	1.78	0.26	83.54	238.68
<i>Betulaceae</i>	<i>Betula alleghaniensis</i>	4	0.00	128.94	0.0343	0.5376	0.0099	0.1716	2.92	0.15	42.63	272.93
<i>Betulaceae</i>	<i>Betula papyrifera</i>	4	0.00	83.72	0.0421	0.6246	0.0128	0.2157	2.58	0.18	38.81	191.14
<i>Betulaceae</i>	<i>Betula populifolia</i>	5	0.00	129.18	0.0197	0.2215	0.0063	0.0791	2.91	0.13	18.15	237.15
<i>Fagaceae</i>	<i>Castanea sativa</i> Mill.	2	0.00	325.04	0.0160	0.4660	0.0059	0.2029	4.05	0.09	60.84	318.43
<i>Ulmaceae</i>	<i>Celtis occidentalis</i>	2	0.00	190.00	0.0274	0.7561	0.0089	0.3188	2.23	0.16	52.43	172.92
<i>Cornaceae</i>	<i>Cornus alternifolia</i>	2	0.00	124.92	0.0540	0.4524	0.0131	0.1721	2.59	0.15	33.49	200.01
<i>Cornaceae</i>	<i>Cornus stolonifera</i>	3	0.00	135.54	0.0374	0.4518	0.0092	0.1626	3.19	0.16	28.49	194.44
<i>Rosaceae</i>	<i>Crataegus</i> sp.	3	0.00	140.66	0.0225	0.2623	0.0071	0.1059	3.11	0.14	22.28	223.22
<i>Asteraceae</i>	<i>Eutrochium maculatum</i>	4	0.00	260.75	0.0653	1.3095	0.0106	0.2932	2.37	0.27	68.87	245.68
<i>Fagaceae</i>	<i>Fagus grandifolia</i>	4	0.00	227.74	0.0095	0.4742	0.0026	0.1539	2.77	0.09	71.79	503.56
<i>Oleaceae</i>	<i>Fraxinus americana</i>	5	0.00	97.92	0.2534	2.2838	0.0674	0.6944	2.48	0.14	181.97	369.34
<i>Oleaceae</i>	<i>Fraxinus pennsylvanica lanceolata</i> (Borkh.) Sarg.	2	0.00	194.65	0.1222	1.6263	0.0566	0.7693	2.72	0.13	113.82	158.30
<i>Ericaceae</i>	<i>Gaultheria hispidula</i>	3	0.00	367.48	0.0003	0.0085	0.0001	0.0026	1.26	0.24	0.40	153.14
<i>Ericaceae</i>	<i>Gaultheria procumbens</i>	4	0.00	210.40	0.0046	0.1245	0.0014	0.0439	1.33	0.26	5.14	119.02
<i>Balsaminaceae</i>	<i>Impatiens capensis</i>	4	89.04	288.97	0.1024	0.3202	0.0076	0.0518	3.90	0.15	32.64	617.87
<i>Juglandaceae</i>	<i>Juglans cinerea</i>	4	0.00	142.50	0.4205	4.4306	0.1137	1.4107	3.50	0.18	350.47	264.39
<i>Ericaceae</i>	<i>Kalmia angustifolia</i>	1	0.00	364.94	0.0035	0.0979	0.0015	0.0467	1.70	0.16	5.56	119.10
<i>Lamiaceae</i>	<i>Lycopus americanus</i>	3	0.00	317.38	0.0048	0.1423	0.0008	0.0305	2.16	0.13	10.72	359.94
<i>Lythraceae</i>	<i>Lythrum Salicaria</i>	3	5.63	203.69	NA	0.1275	NA	0.0321	2.56	0.19	7.89	234.11
<i>Rosaceae</i>	<i>Malus pumila</i>	3	0.00	167.47	0.0443	0.4378	0.0132	0.1519	2.28	0.20	24.65	170.68
<i>Lamiaceae</i>	<i>Mentha canadensis</i>	3	0.00	185.50	0.0071	0.1199	0.0013	0.0238	1.68	0.15	7.90	367.13
<i>Betulaceae</i>	<i>Ostrya virginiana</i>	5	0.00	65.50	0.0060	0.2429	0.0019	0.0908	2.46	0.13	32.58	379.79

Table A1 (concluded).

Family	Species	nb	Adaxial <i>D</i>	Abaxial <i>D</i>	PFM	LBFM	PDM	LBDM	[chloro]	LT	SA	SLA
<i>Vitaceae</i>	<i>Parthenocissus quinquefolia</i>	3	0.00	94.28	0.3206	1.9080	0.0528	0.4585	2.87	0.18	104.09	236.05
<i>Polygonaceae</i>	<i>Polygonum cuspidatum</i>	2	NA	85.03	0.1194	1.8457	0.0191	0.4302	1.13	0.32	76.99	190.47
<i>Salicaceae</i>	<i>Populus balsamifera</i>	4	0.00	130.65	0.1233	0.8294	0.0351	0.2906	2.34	0.20	45.53	165.14
<i>Salicaceae</i>	<i>Populus deltoides</i>	4	70.39	108.37	0.1546	0.9422	0.0424	0.2980	1.67	0.24	42.35	146.26
<i>Salicaceae</i>	<i>Populus grandidentata</i>	2	0.00	95.68	0.1309	0.6675	0.0315	0.2249	2.43	0.12	50.23	229.47
<i>Salicaceae</i>	<i>Populus tremuloides</i>	4	0.00	96.95	0.0655	0.4325	0.0227	0.1916	3.84	0.16	28.09	151.14
<i>Rosaceae</i>	<i>Prunus serotina</i>	3	0.00	178.77	0.0155	0.4082	0.0039	0.1260	2.59	0.13	33.02	273.71
<i>Fagaceae</i>	<i>Quercus macrocarpa</i>	2	0.00	472.37	0.1098	1.7745	0.0338	0.6736	2.67	0.27	98.16	157.63
<i>Fagaceae</i>	<i>Quercus robur</i> L.	2	0.00	352.85	0.0206	0.7425	0.0060	0.2493	2.71	0.16	49.82	216.04
<i>Fagaceae</i>	<i>Quercus rubra</i>	5	0.00	402.02	0.0690	1.1078	0.0201	0.3684	2.59	0.14	81.93	218.23
<i>Rhamnaceae</i>	<i>Rhamnus frangula</i>	3	0.00	356.66	0.0194	0.2792	0.0043	0.0723	2.56	0.14	22.72	318.33
<i>Anacardiaceae</i>	<i>Rhus typhina</i>	3	0.00	320.65	0.7862	6.4445	0.1882	1.8721	3.20	0.18	425.19	233.65
<i>Rosaceae</i>	<i>Rosa rugosa</i>	2	0.00	326.16	0.0663	0.8656	0.0221	0.3080	2.51	0.20	47.15	156.71
<i>Salicaceae</i>	<i>Salix nigra</i>	2	120.64	73.77	0.0078	0.2234	0.0022	0.0622	1.73	0.16	10.77	179.97
<i>Rosaceae</i>	<i>Sorbus × thuringiaca fastigiata</i> S.	1	0.00	192.12	0.0292	0.3688	0.0058	0.0991	2.40	0.17	28.90	296.96
<i>Oleaceae</i>	<i>Syringa vulgaris</i>	5	24.16	231.93	0.0541	0.9100	0.0194	0.3095	2.53	0.22	38.41	133.45
<i>Tiliaceae</i>	<i>Tilia americana</i>	3	0.00	84.94	0.1585	1.2690	0.0259	0.2909	3.54	0.17	124.32	438.40
<i>Tiliaceae</i>	<i>Tilia cordata</i> Mill.	3	0.00	151.95	0.0581	0.5373	0.0133	0.1616	3.61	0.18	46.45	279.95
<i>Ulmaceae</i>	<i>Ulmus americana</i>	4	0.00	190.78	0.0154	0.8542	0.0046	0.3017	4.09	0.14	70.97	251.82
<i>Ulmaceae</i>	<i>Ulmus rubra</i>	3	0.00	251.28	0.0212	0.8620	0.0074	0.3818	3.32	0.15	73.06	187.61
<i>Ericaceae</i>	<i>Vaccinium myrtilloides</i>	3	0.00	174.01	0.0006	0.0367	0.0002	0.0122	3.06	0.10	3.52	289.80
<i>Vitaceae</i>	<i>Vitis riparia</i>	2	0.00	170.00	0.1455	1.3990	0.0298	0.4641	2.49	0.16	99.05	226.60

Note: Nomenclature follows Marie-Victorin et al. (1995) unless otherwise indicated.