

THE EFFECT OF LEAF ORIENTATION TO SUNLIGHT ON STOMATAL PARAMETERS OF *QUERCUS RUBRA* AROUND THE BELGRADE LAKES, CENTRAL MAINE

RACHEL G. DALY and ROBERT A. GASTALDO*

Department of Geology, Colby College, Waterville, Maine 04901, USA

e-mail: ragastal@colby.edu

ABSTRACT

Stomatal frequencies of fossil-plant species are used to estimate past $p\text{CO}_2$ levels based on the physiological functions of living taxa. There is a demonstrable inverse relationship between increasing $p\text{CO}_2$ and stomatal frequency parameters, in which there is a decrease in both stomatal density (SD) and stomatal index (SI). Concentration of CO_2 is not the only factor known to affect SD and SI values, which are a product of leaf development and expansion, as studies have shown a positive correlation between SD and light intensity. The present study tests the hypothesis that SD and SI are not influenced by a leaf's physical orientation relative to the sun during the growing season. Sun leaves of northern red oak, *Quercus rubra*, were collected from trees around the margins of six lakes of the Belgrade Lakes Region, central Maine, United States, in 2007. Lakes in NE–SW, NW–SE, and E–W orientations allowed for sampling of trees exposed to varying light intensities throughout the growing-season day. The SD and SI of each tree were calculated, and statistical comparisons were made between populations exposed to predominant morning or afternoon light intensities for each lake and between populations on lakes of differing orientations. There is no statistically significant difference in either SD or SI between populations growing under different orientations to growing-season sunlight. These data indicate that exposure to various sunlight regimes on opposite sides of a lake does not play a role in the stomatal response as reflected in SD and SI of plants during a single growing season.

INTRODUCTION

In light of recent predictions of global climate change, it is important to utilize a range of methods when estimating past climate variation in order to understand the long-term trends experienced by Earth systems. The use of plant stomata has been adopted as a paleoatmospheric proxy for the concentration of carbon dioxide ($p\text{CO}_2$) after it was demonstrated that a relationship exists between $p\text{CO}_2$ and leaf stomatal parameters in several taxa (e.g., Woodward, 1987; Beerling et al., 1993; van der Burgh et al., 1993). As a positive relationship exists between changes in $p\text{CO}_2$ and temperature (Crowley and Berner, 2001; Royer et al., 2001), a more comprehensive knowledge of $p\text{CO}_2$ variation in space and time will increase our understanding of global temperature change. Maximizing the effectiveness of plant stomatal analysis may allow for more accurate estimates of $p\text{CO}_2$ variation in the Quaternary and in deep time, which in turn may allow us to estimate future fluctuations in climate more accurately.

Several methods have been established to estimate past atmospheric $p\text{CO}_2$. The application of techniques based on degassing and weathering rates coupled with Sr and $\delta^{13}\text{C}$ isotope data have resulted in geochemical models, such as GEOCARB (Berner, 1991, 1994; Berner and Kothavala, 2001), to estimate $p\text{CO}_2$ variation over the Phanerozoic. Results of GEOCARB III models hypothesize that $p\text{CO}_2$ fluctuated up to 25 times present atmospheric levels over the last

600 myr (Berner and Kothavala, 2001). Another method for measuring past atmospheric gas concentrations is provided by the analysis of air bubbles trapped in ice cores (Petit et al., 1999). Yet, ice-core records exist for just the last 800 kyr of the late Quaternary (Lüthi et al., 2008) and these studies show that $p\text{CO}_2$ has only fluctuated between 180 and 280 ppm over the last 100 kyr (Beerling, 2002). In the last quarter century, empirical research has established that the inverse relationship between $p\text{CO}_2$ and stomatal parameters allows for a higher resolution estimate of this atmospheric gas (e.g., McElwain et al., 1995; Wagner et al., 1996; but see Eide and Birks, 2006).

Stomata are epidermal pores, either on the abaxial or adaxial leaf surface, that regulate gas exchange. A reduction in stomatal density (SD, measured in stomata/ mm^2) as $p\text{CO}_2$ increases allows for greater water conservation while ensuring the uptake of an adequate amount of the gas for photosynthesis (Royer, 2001). Other factors are known to influence the SD metric, including soil-moisture supply, atmospheric humidity, and temperature, making this relationship difficult to use solely as a $p\text{CO}_2$ proxy. Salisbury (1927) first introduced the concept of using stomatal index ($\text{SI} = (\text{SD}/[\text{SD} + \text{ED}]) \times 100$) (ED = epidermal cell density) as a more accurate measure of stomatal parameters. This is because CO_2 has a larger effect on the initiation of stomatal cells than it does on the size of epidermal cells (Royer, 2001). As such, SI is less sensitive than SD to confounding factors (Beerling, 1999).

Soon after the stomatal and $p\text{CO}_2$ relationship was recognized, it was tested for use as a paleoatmospheric proxy. Woodward (1987) first interpreted decreasing trends in both SD and SI since the beginning of the industrial revolution as plant responses to increasing $p\text{CO}_2$. Subsequently, this technique was applied to the Holocene (Beerling et al., 1993; Rundgren and Beerling, 1999), the Cenozoic (van der Burgh et al., 1993; Kürschner et al., 2008) and deeper time (McElwain and Chaloner, 1996; Retallack, 2001, 2009) records to evaluate variance in paleoatmospheric gas concentration. It is impossible, however, to account for the likely confounding factors that may have influenced the growth of the parent plants responsible for contributing leaf debris to the stratigraphic record (DiMichele and Gastaldo, 2008). Hence, factors that affect stomatal parameters in modern taxa need to be evaluated to determine the influence they may exert on stomatal development, reflecting growth conditions as much as $p\text{CO}_2$ in leaf expansion and maturity.

There are several factors that confound the effect of $p\text{CO}_2$ on stomatal parameters, including irradiance level, soil-moisture levels, sex of the plant, leaf position within the crown, and adjustments in stomatal development to CO_2 and climate gradients (Ashton and Berlyn, 1994; Kürschner, 1997; Beerling, 1999; Chen et al., 2001; Lake et al., 2001; Sun et al., 2003; Boyce, 2007). For example, SD and SI are influenced significantly by temperature and water supply during the growing season (Beerling, 1999; Fraser et al., 2008), and SD has been shown to vary between female and male individuals of a taxon (Chen et al., 2001). Morphological differences exist between sun (growing in direct sunlight on the edge of the tree crown) and shade leaves (growing inside the crown away from direct sunlight), with higher SD and SI values found in sun leaves of the same tree (Kürschner, 1997). More

* Corresponding author.

recent studies intimate, however, that sun-shade differences may be due to leaf hydration during expansion (soil moisture availability) rather than light intensity (Boyce, 2007).

The quality and quantity of sunlight during leaf expansion are documented to influence stomatal development (e.g., Lee et al., 2007) which indicates that orientation to sunlight during growth may also influence stomatal parameters. Both water availability (Boyer, 1968) and light intensity during growth affects leaf expansion (Dale, 1988), with high light intensities resulting in an increase in the stomatal frequency (Bjorkman et al., 1972) of *Atriplex patula*. Although an increase in this stomatal parameter occurs under increased irradiance, Bjorkman et al. (1972) reported no significant changes either in the length of the stomatal pore or the size of the guard cells. Dale (1988) noted that the red:far-red ratio (R:FR) of sunlight is an important photo-morphogenetic factor, and although it is constant for most of the day, there is a 20%–30% fall in the ratio accompanied by a substantial increase in the blue component of the spectrum at dawn and dusk. When light quality has been kept constant, though, leaf number and final size of individual leaves often are correlated positively with the rate of photon flux (Wilson, 1966). Hence, leaf expansion on riparian trees growing under the influence of varying quantitative and qualitative light conditions on opposite sides of lakes may, in principle, impact stomatal frequency.

Many Quaternary and pre-Quaternary fossil-leaf assemblages from which epidermal features can be assessed are preserved in lacustrine deposits (e.g., Gastaldo et al., 1998; Rundgren et al., 2005). Lakes are good sites to evaluate the responses of riparian trees because they are the principal contributing vegetation, along with lianas, to these depositional settings (Spicer and Wolf, 1987; Spicer, 1989; DiMichele and Gastaldo, 2008). Although it is posited that solar irradiation may affect leaf expansion and development (Ashton and Berlyn, 1994; Kürschner, 1997), no study has evaluated whether leaf expansion in a taxon is affected when grown under the prevailing influence of morning or afternoon sunlight, or by orientation of the water body along which the tree is established. The present study is an attempt to fill this gap.

Northern red oak (*Quercus rubra*) is used to test the hypothesis that stomatal parameters of this taxon are not affected by the orientation of trees growing on the margins of lacustrine systems to growing-season sunlight. If orientation to sunlight has no effect on stomatal parameters developed during a growing season, future studies that attempt to reconstruct paleoatmospheric conditions based on lacustrine fossil assemblages will be able to disregard it during data analysis. If the position of a tree relative to irradiance has an effect, however, the geometry of the depositional setting and the contributory debris to a fossil assemblage will need to be considered as a potential confounding factor.

MATERIALS AND METHODS

Quercus rubra was chosen due to its abundance in north-temperate latitudes and to complement a coeval study of the genus in the southeastern United States (Cantor and Gastaldo, 2007). The taxon is easy to identify by its lobate leaves which are sinuately three-toothed and bristle-tipped; the bark is dark brown and scaly at the surface (York, 1995). Sun-leaf collections were made from trees along the margins of the Belgrade Lakes, central Maine, in 2007 (44°29'N). The lakes are the result of Pleistocene glaciation (Caldwell, 1998) and are oriented in three directions. Great and Long Ponds display a N–S orientation, East and North Ponds are oriented in a NW–SE direction, whereas Snow and Salmon-McGrath Ponds are oriented in a NE–SW direction (Fig. 1; Table 1). Forests directly adjacent to all ponds were sampled, and a small leaf collection of the taxon also was made in the Auburn University Arboretum, Alabama, United States (32°35'N), to test if latitudinal position may have an effect on stomatal parameters during the same growing season.

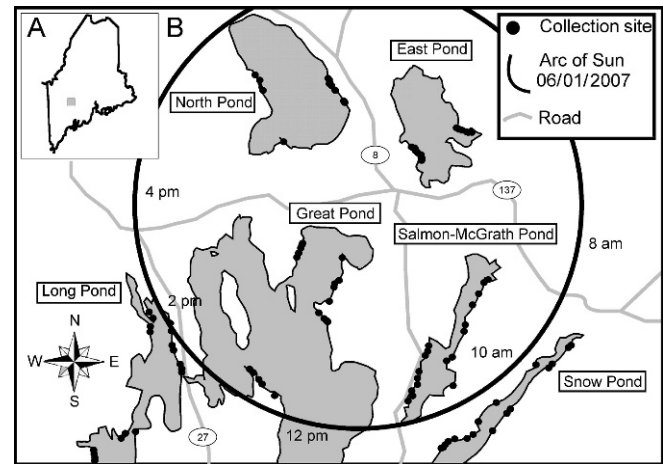


FIGURE 1—Collections of *Quercus rubra* (northern red oak) made during summer 2007. A) The location of Belgrade Lakes in central Maine. B) Leaf-collection sites along the lake margins of Belgrade Lakes. Solid line superimposed on map represents arc of sun at the beginning of the 2007 growing season (<http://usno.navy.mil/>).

Individual trees were chosen based on proximity to the water's edge, expediency of collection, and relationship to prevailing morning or afternoon sunlight. Sampling of trees along the lake margin ensured that nothing was blocking exposure of canopy leaves to the sun, increasing the probability that each leaf collected would be exclusively of a sun-leaf morphotype. Leaves were collected from the edge of the canopy hanging over the water, either by hand, picked directly off the tree, or by using an extendable 4.3 m Fiskars tree pruner.

Seven to ten trees were chosen along the sides of each lake that corresponded with their orientation to either direct morning or afternoon sunlight. Although a sample size of 10 trees per lake side was the collection goal, logistical problems along Snow and North Ponds and leaf processing with chromic acid limited this number in some cases. These factors resulted in an uneven number of samples for some lakes (Table 1).

The daily arc of the sun during the Maine growing season (May–June) was determined using data from the U.S. Department of the Navy astronomical applications (August 2007: <http://usno.navy.mil/>). This allowed for a qualitative assessment of the duration of sunlight each lakeside received during the growing season. Light-meter readings taken to assess the difference in light intensity at varying times throughout the day were done with an Extech EA30 (lux), along opposite sides of Snow Pond over five days during the summer (see Supplementary Data 1¹). Measurements, with the sensor held facing upwards, were taken every two hours on opposite sides of the lake from 8:00 to 16:00. Data were collected when there was little cloud cover, which would have reduced the amount of irradiance on the leaves. Climate data were acquired from the National Climatic Data Center (<http://www.ncdc.noaa.gov/oa/ncdc.html>, August 2007) to determine whether or not aspects of the 2007 growing season were abnormal (cooler or hotter, wetter or drier), potentially influencing leaf expansion.

Fresh leaves were processed by removing a one cm² section from between the second and third lateral vein adjacent to the midrib with the adaxial leaf surface facing up. Poole and Kürschner (1999) have shown that stomatal parameters vary across an individual leaf requiring standardization to eliminate the possibility that stomatal variance is due to the position sampled. Sections then were immersed in 10% by weight chromic acid solution and treated for durations of less than 24 to 48 hours to remove parenchymatous tissue. Abaxial and adaxial cuticles were recovered and separated manually under a dissecting

¹ www.paleo.ku.edu/palaios

TABLE 1—Localities and prevailing sunlight exposures of *Q. rubra* collected in the Belgrade Lakes, Central Maine, 2007.

Locality	Orientation of sampled lake margins	Sunlight exposure	GPS coordinates of lake margin	N
East Pond	Southwest	Morning	44° 35' 55.0" N; 69° 47' 14.4" W	10
	Northeast	Afternoon	44° 36' 29.1" N; 69° 46' 4.7" W	5
North Pond	Southwest	Morning	44° 37' 35.4" N; 69° 51' 16.7" W	5
	Northeast	Afternoon	44° 37' 28.6" N; 69° 49' 23.0" W	5
Salmon-McGrath Pond	Northwest	Morning	44° 31' 29.9" N; 69° 47' 16.0" W	9
	Southeast	Afternoon	44° 33' 7.2" N; 69° 45' 54.3" W	10
Snow Pond	Northwest	Morning	44° 30' 24.3" N; 69° 46' 34.5" W	9
	Southeast	Afternoon	44° 31' 52.7" N; 69° 45' 7.8" W	6
Great Pond	East	Afternoon	44° 33' 7.5" N; 69° 49' 3 4.3" W	4
	West	Morning	44° 31' 36.6" N; 69° 51' 12.7" W	4
Long Pond	East	Afternoon	44° 32' 21.6" N; 69° 53' 36.1" W	3
	West	Morning	44° 32' 35.8" N; 69° 54' 6.7" W	6

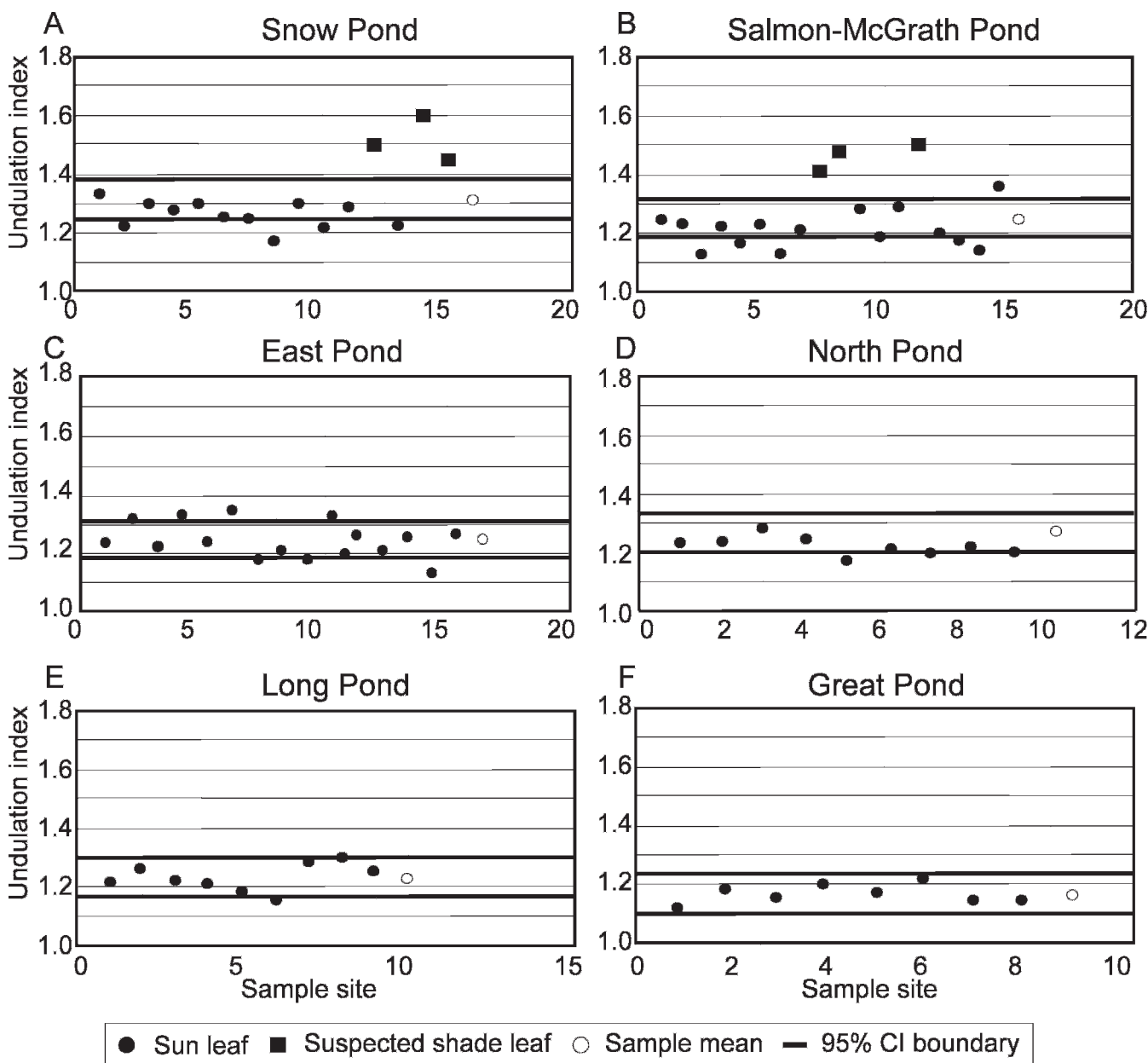


FIGURE 2—Calculated undulation indices (UI) and 95% confidence intervals (CI) for leaf samples from each of the Belgrade Lakes (A–F).

TABLE 2—Stomatal density (SD), epidermal cell density, and undulation index (UI) values determining shade-leaf morphology.

Pond Sample	Snow SE 14	Snow SE 16	Snow SE 17	Salmon NW 3	Salmon NW 4	Salmon NW 8
UI for possible shade leaves	1.50	1.60	1.45	1.42	1.48	1.51
Average sun-leaf SD for corresponding lake side	455.1			530.4		
SD of possible shade leaves (stomata/mm ²)	525.9	426.2	399.4	512.3	461.5	479.6
Percent sun-leaf SD higher than possible shade leaves (0.45 is level for shade leaf)	Sun lower	0.079	0.122	0.034	0.13	0.096
Average sun-leaf epidermal cell density for corresponding lake side	4184.7			4369		
Epidermal cell density of possible shade leaves (cells/mm ²)	4693.1	4067.3	4195.4	4496.1	4017.9	4442.1
Percent sun-leaf cell density higher than possible shade leaves (0.3 is level for shade leaf)	Sun lower	0.028	Sun lower	Sun lower	0.087	Sun lower

scope; abaxial cuticles were mounted on a glass slide using Biomedica aqueous gel mount. Cover slips were added and the slides were dried under a weight on a slide warmer for 12 hours.

Data were collected using a Zeiss Axioskop and analyzed with Axiovision software (www.zeiss.com/). Stomatal and epidermal cell counts were taken from twenty, 0.04 mm² fields of view on each slide preparation, with the total area (0.8 mm²) normalized to 1 mm². Previous studies have normalized data to 1 mm² based on 10 field-of-view counts (e.g., Beerling and Kelly, 1997; Beerling et al., 1998). Although leaf collections were made from the edge of the tree crown to ensure sun-leaf morphologies, the undulation index (UI = cell circumference [C_e]/circle circumference equal in cell area [C_o]; Poole and Kürschner, 1999) of each specimen was calculated to determine if any leaves may have developed a shade-leaf morphology. Shade leaves have epidermal cell margins that are more sinusoidal than sun leaves. Recently, Lake et al. (2001) have shown that the stomatal parameters in leaves that bud and expand later in the growth of juvenile plants are influenced by changes in atmospheric gas concentration in the immediate area. They infer that coding for SD and SI originate with mature leaves that could be anywhere on the plant. Such a mechanism may control stomatal parameters in trees that continuously produce leaves over the growing season, but may not have much influence on trees in which all leaves expand from an overwintering bud at the beginning of the growing season. By quantifying the relative sinuosity of the epidermal cells, UI can help to distinguish between sun and shade leaves.

Statistical tests that were used to evaluate the data included a two-tailed Student's t-test and ANOVA (Stata/IC 10.0; SPSS 16.0 for Windows; Minitab version 15.1.0.0; www.stata.com). The Student's t-test was employed to evaluate the statistical differences between the stomatal parameters from collections on (1) the opposing sides of each lake and (2) corresponding sides of each lake within a "lake pair." A lake pair was defined as the two lakes with similar geographic orientations. A Bonferroni adjustment was used to account for the presence of false positives due to uneven and small sample size. One-way ANOVA was used to evaluate statistical differences between the (1) stomatal parameter (SD, SI) and lake side, (2) stomatal parameter (SD, SI) and lake, and (3) stomatal parameter (SD, SI) versus lake orientation. An initial test was done to determine if any inherent variability exists between lakes, with subsequent analyses comparing all the lake sides to each other and then lake orientation. A two-way ANOVA was not used because each lake orientation can have only two lakesides. As such, lakeside and lake orientation are not separate factors but describe each other, negating the analysis (M. Wiest, personal communication, 11/09).

RESULTS

Climate data from central Maine during the 2007 growing season were assessed against 30-year averages for the region (see Supplementary Data 2–3¹). The monthly average temperatures were within the

95% confidence intervals (CI) for the 30-year averages. April 2007 was significantly wetter, however, and May, June, July, and September were significantly drier than average. Leaf expansion ceased in mid-June. Irradiance values along the margins of Snow Pond (see Supplementary Data 1¹) are lower on the SE side of the lake illuminated by afternoon sunlight than those of the NW side illuminated by morning sunlight.

The 2007 SD responses of *Q. rubra* on the margins of the Belgrade Lakes ranged from 353 to 641 stomata/mm², whereas SI responses ranged from 8.2% to 12.9%. The limits of the 95% CI for the SD values for all lakes were 456–488, whereas the 95% CI for SI values fell between 9.9% and 10.3% (see Supplementary Data 2¹).

Kürschner (1997) demonstrated empirically that a difference exists in both SD and SI response during leaf expansion depending upon the position of the leaf in the tree crown. To test whether all leaves in this study represented sun-leaf morphologies, the UI and epidermal cell density of each leaf were calculated. Undulation indices in *Q. rubra* ranged from 1.12–1.59, with the 95% CI ranging from 1.23 to 1.27 (see Supplementary Data 2¹; Fig. 2). Sun leaves of *Q. petraea* (the sessile oak) display SD values 45% higher than shade leaves, with slightly increased SI values (SI 12%–14% = a 16% difference; Kürschner, 1997). Six leaves of *Q. rubra* were found to have UI values beyond the

TABLE 3—Results of Student's t-test of SD and SI comparisons from opposing sides of each of the Belgrade Lakes, Central Maine, 2007. The threshold of significance was lowered from p = 0.05 to p = 0.00625 using a Bonferroni adjustment.

Lake	Parameter	Sides compared	Average	p
East Pond	SD	NE	420.5	0.57
		SW	443.7	
	SI	NE	9.2%	0.83
		SW	8.8%	
North Pond	SD	NE	477.6	0.82
		SW	467.2	
	SI	NE	10.9%	0.48
		SW	9.5%	
Salmon-McGrath Pond	SD	NW	515.1	0.036
		SE	448.4	
	SI	NW	10.5%	0.18
		SE	10.1%	
Snow Pond	SD	NW	486	0.41
		SE	453	
	SI	NW	10.2%	0.12
		SE	9.6%	
Great Pond	SD	E	504.3	0.25
		W	450.5	
	SI	E	10.0%	0.88
		W	10.1%	
Long Pond	SD	E	524.6	0.69
		W	500.6	
	SI	E	10.6%	0.58
		W	10.2%	

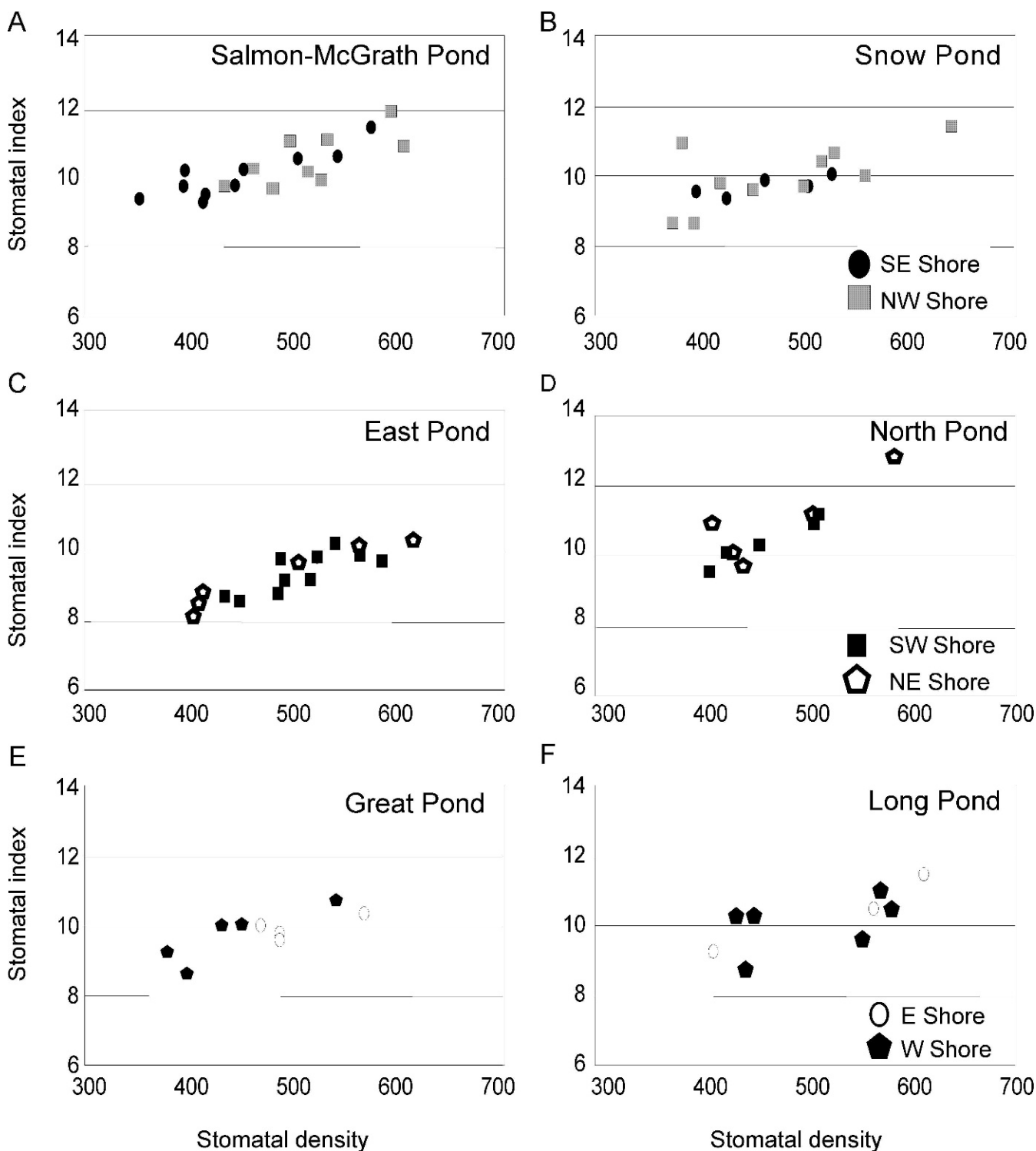


FIGURE 3—Stomatal index versus stomatal density for leaves collected on opposite sides of the Belgrade Lakes. A) NW and SE shores of Salmon-McGrath Pond. B) NW and SE shores of Snow Pond. C) NE and SW shores of East Pond. D) NE and SW shores of North Pond. E) E and W shores of Great Pond. F) E and W shores of Long Pond.

95% CI limits when compared with the sun-leaf population collected from the same side of the lake (Fig. 2). All SD values for sun leaves were <45% greater than the values for the suspected shade leaves (based on UI index). Similarly, SI values of the suspected shade leaves were less than the difference reported by Kürschner (1997; Table 2) for *Q. petraea*. Epidermal cell density in *Q. petraea* is 30% higher in sun than in shade leaves (Kürschner, 1997). The average sun-leaf epidermal cell density in the suspected shade leaves of *Q. rubra* were <13% higher

(Table 2). Hence, the population of UI values in *Q. rubra* sun-leaf morphologies are either due to (1) phenotypic variation rather than attribution of these leaves to a shade morphology, or (2) a shade-leaf morphology, although the differences between these two leaf types is not as pronounced as in other taxa (e.g., *Q. petraea*).

Of the tree populations surrounding the six lakes, only those around Salmon-McGrath Pond showed a statistically significant difference in SD when opposing sides of each lake were compared using the

TABLE 4—Results of Student's t-test of SD and SI for corresponding sides of similarly oriented lakes. Asterisks (*) show a statistically significant result ($p = 0.05$) was a false positive when the threshold of significance was lowered to $p = 0.00625$ using a Bonferroni adjustment.

East and North Ponds					
SW vs. SW (SD)	average	451.6	NE vs. NE (SD)	average	449.05
	p	0.33		p	0.283
SW vs. SW (SI)	average	9.75%	NE vs. NE (SI)	average	9.87%
	p	0.021*		p	0.011*
Snow and Salmon-McGrath Ponds					
SE vs. SE (SD)	average	450.05	NW vs. NW (SD)	average	500.71
	p	0.89		p	0.413
SE vs. SE (SI)	average	9.92%	NW vs. NW (SI)	average	10.39%
	p	0.13		p	0.335
Great and Long Ponds					
E vs. E (SD)	average	513	W vs. W (SD)	average	480.59
	p	0.736		p	0.299
E vs. E (SI)	average	10.27%	W vs. W (SI)	average	10.16%
	p	0.335		p	0.729

Student's t-test (Table 3). This statistical difference proved to be a false positive when a Bonferroni adjustment was applied to the data set. Hence, there is no statistical difference in SD values for trees grown on opposite sides of any lake. Similarly, there is no difference in SI values for trees on any lake evaluated. It can be seen that stomatal parameters of trees from opposite lake sides are commingled when SD:SI crossplots are generated (Fig. 3).

Leaf populations from paired lakes were tested to determine whether statistical differences exist between sites at different lakes with different orientations. When leaf populations from the three lake pairs were evaluated using the Student's t-test (Table 4), no statistical difference in SD values between the corresponding sides of any lake pair was found. Similarly, SI values for all lake pairs show no statistically significant difference (after application of a Bonferroni adjustment to North Pond and the SW sides of the lake pair). ANOVA results indicate that there is no statistically significant difference either in SD ($p = 0.069$) or SI ($p = 0.336$) when compared against lake sides, and when stomatal parameters are compared versus all lakes to determine if any inherent variability exists, there was no statistically significant difference for SD ($p = 0.214$), but a significant difference was found for SI ($p = 0.001$). When the three lake pairs were evaluated against each other using ANOVA to test for an effect of water-body orientation on stomatal parameters, though, no statistically significant differences were found within either SD ($p = 0.11$) or SI ($p = 0.337$) values.

The Belgrade Lakes data were compared against a 2007 datum of *Q. rubra* collected in the Auburn University Arboretum (Fig. 4). The Alabama datum plots outside of the 95% CI ellipse for the SD:SI crossplots of Maine data. There is a statistically significant difference between the Belgrade and Auburn data when the Auburn datum was used as a hypothetical population mean tested against the Belgrade data in a single sample t-test ($p = <0.001$).

DISCUSSION

The ability to use stomatal parameters as paleoatmospheric proxies in the recent and extrapolate the technique back into deeper time (e.g., McElwain, 1998) is dependent on an array of variables, several of which have been investigated (e.g., Ashton and Berlyn, 1994; Kürschner, 1997; Beerling, 1999; Chen et al., 2001; Boyce, 2007) while others still remain to be evaluated (Cantor et al., 2006). There are many confounding physical parameters that may have influenced the growth and stomatal characteristics of subfossil or fossil plants, including temperature, soil moisture, and prevailing wind velocities. These are difficult to evaluate in the deep time record and factor into whether or not one or more of these (or some combination thereof) may be responsible for the observed epidermal patterns rather than attributing the configuration

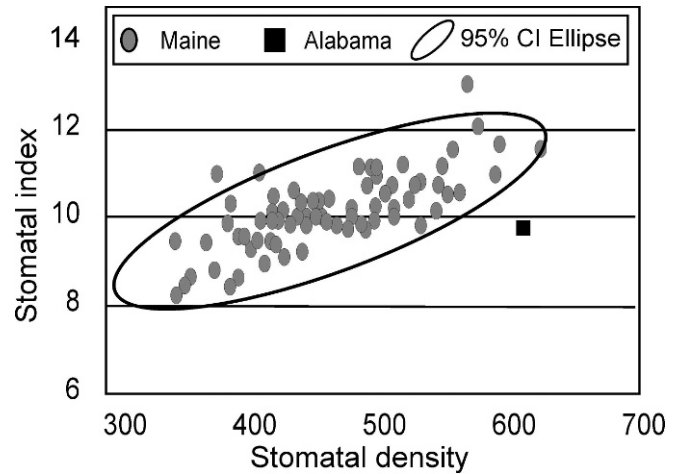


FIGURE 4—Stomatal index versus stomatal density of *Q. rubra* in central Maine (44°29'N) compared to the Auburn University Arboretum sample (32°35'N). There is a statistically significant difference between 2007 SD and SI values of the Alabama material and the Maine population, indicating that growing-season latitude has an influence on these proxies.

to a $p\text{CO}_2$ response. Hence, it is essential to understand the variance of stomatal features that one can expect within various biomes before extrapolating into the past.

The present study demonstrates that the orientation of shoreline trees to growing-season sunlight is not a confounding factor and plays no role in the development of stomatal parameters within the north-temperate species *Q. rubra*. Leaf populations assessed from opposing shores of each of the six water bodies in the Belgrade Lakes, central Maine, show no statistical difference between either SD or SI values for any lake examined (Table 3). Although a significant difference was identified in the ANOVA analysis when SI was tested against all lake data, the fact that this result was not repeated when SI was tested against each lake side may be due to the unevenness of data. Hence, stomatal parameters are neither affected by exposure to prevailing morning nor afternoon sunlight. Similarly, there is no statistically significant difference in SD or SI values from trees in the same geographic location within lake pairs and the orientation of the water body to sunlight has no effect on stomatal parameters (Table 4). Plants from around each contemporaneous lake provide the same signal, and minor physical changes within climatically consistent latitudes appear to have no effect on stomatal parameters within a single taxon.

These findings have positive implications for stomatal proxy studies that use subfossil or fossil-leaf assemblages from lacustrine environments to reconstruct paleoatmospheric conditions (Wagner et al., 2002, 2004; Rundgren and Björck, 2003; Rundgren et al., 2005; van Hoof et al., 2006a, 2006b). Because the orientation of a riparian tree to preferential morning or afternoon sunlight has no effect on the development of stomatal parameters, future studies will be able to assume a statistically homogenous sample when a leaf population is recovered from a single or from coeval lacustrine deposits.

This study also demonstrates that other factors can influence stomatal parameters and need to be considered when applying the technique to deep time deposits. The comparison of the Belgrade data set against the Auburn Arboretum datum for *Q. rubra* (Fig. 4) demonstrates the problem. The 2007 arboretum sample plots outside of the 95% CI ellipse and is statistically different, adding to the body of emerging literature that stomatal parameters for individual taxa are latitudinally dependent. For example, Garcia-Amorena et al. (2006) have shown that stomatal parameters for *Q. robur* varied with latitude when sample populations were compared between Iberia and NW Europe. Hence, any study that incorporates multiple fossil assemblages

originating from different latitudes would have to account for this effect (Wagner et al., 2004).

SUMMARY

Stomatal index data from *Quercus rubra* along the margins of six lakes in the Belgrade Lakes region, central Maine, during the 2007 growing season demonstrate that exposure of trees to either a prevailing morning or afternoon light regime does not have an effect on stomatal parameters. All SD and SI values are statistically equivalent for the population of sun leaves (confirmed using UI) collected during this growing season typified by normal temperatures and less than normal precipitation. When the Belgrade data set is compared to a 2007 sample collected in Auburn, Alabama, the present findings support the results of previous studies (e.g., Garcia-Amorena et al., 2006) showing that latitude affects stomatal parameters of trees within the same taxon. Hence, care is required when interpreting the meaning of stomatal proxies in pre-Quaternary investigations where paleogeographies have changed over time. The positive implications of these data relate to future stomatal studies using lacustrine fossil assemblages. Paleatmospheric estimates based on a single taxon (e.g., Cantor et al., 2006) from deposits in one or several contemporaneous lakes may provide for a high resolution record of changing $p\text{CO}_2$ conditions because it can be assumed that sun leaves from riparian vegetation represent a statistically homogenous population of that taxon.

ACKNOWLEDGMENTS

This study was supported by the Jana C. Rudnick Research Fellowship in Environmental Science at Colby College. Colby faculty including B.F. Rueger (Geology), D.W. King (Chemistry), and B.M. Mundy (Chemistry) are thanked for logistical support, J. Stone (Biology) for her assistance with tree systematics in the Belgrade Lakes region, and L. O'Brien (Mathematics) and M. Wiest (University of Idaho) for assistance with the statistical tests. A draft manuscript, submitted as a Senior Honors Thesis, was critiqued by B.F. Rueger and D.H. Firmage (Biology, Colby College).

REFERENCES

- ASHTON, P.M.S., and BERLYN, G.P., 1994, A comparison of leaf physiology and anatomy of *Quercus* (section *Erythrobalanus*-Fagaceae) species in different light environments: *American Journal of Botany*, v. 81, p. 589–597.
- BEERLING, D.J., 1999, Stomatal density and index: Theory and application, in Jones, T.P., and Rowe, N.P., eds., *Fossil Plants and Spores: Modern Techniques*: Geological Society, London, p. 251–256.
- BEERLING, D.J., 2002, Low atmospheric CO_2 levels from the Permo–Carboniferous glaciation inferred from fossil lycopsids: *Proceedings of the National Academy of Sciences of the United States of America*, v. 99, p. 12567–12571.
- BEERLING, D.J., CHALONER, W.G., HUNTLEY, B., PEARSON, J.A., and TOOLEY, M.J., 1993, Stomatal density responds to the glacial cycle of environmental change: *Proceedings of the Royal Society of London, Series B, Biological Sciences*, v. 251, p. 133–138.
- BEERLING, D.J., and KELLY, C.K., 1997, Stomatal density responses of temperate woodland plants over the past seven decades of CO_2 increase: A comparison of Salisbury (1927) with contemporary data: *American Journal of Botany*, v. 84, p. 1572–1583.
- BEERLING, D.J., McELWAIN, J.C., and OSBORNE, C.P., 1998, Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations: *Journal of Experimental Botany*, v. 49, p. 1603–1607.
- BERNER, R.A., 1991, A model for atmospheric CO_2 over Phanerozoic time: *American Journal of Science*, v. 291, p. 339–376.
- BERNER, R.A., 1994, GEOCARB II: A revised model of atmospheric CO_2 over Phanerozoic time: *American Journal of Science*, v. 294, p. 56–91.
- BERNER, R.A., and KOTHAVALA, Z., 2001, GEOCARB III: A revised model of atmospheric CO_2 over Phanerozoic time: *American Journal of Science*, v. 301, p. 182–204.
- BJORKMAN, O., BOARDMAN, N.K., ANDERSON, J.M., THORNE, S.W., GOODCHILD, D.J., and PYLIOTIS, N.A., 1972, Effect of light intensity during growth of *Atriplex patula* on the capacity of photosynthetic reactions, chloroplast components and structure: *Carnegie Institute of Washington Yearbook*, v. 71, p. 115–135.
- BOYCE, C.K., 2007, Seeing the forest with the leaves—Clues of canopy placement from leaf venation characteristics: *Geological Society of America, Abstracts with Programs*, v. 39, no. 6, p. 23.
- BOYER, J.S., 1968, Relationship of water potential to growth of leaves: *Plant Physiology*, v. 43, p. 1056–1062.
- CALDWELL, D.W., 1998, *Roadside Geology of Maine*, Mountain Press: Missoula, Montana, 317 p.
- CANTOR, B.M., AIGLER, B.V., PACE, D.W., REID, S.B., THOMPSON, C.Y., and GASTALDO, R.A., 2006, Intra- and Interspecific Variation in Stomatal Proxies for *Quercus* and *Nyssa* in the Subtropical Southeastern USA: *Geological Society of America, Abstracts with Program*, v. 38, n. 7, p. 487.
- CANTOR, B.M., and GASTALDO, R.A., 2007, Historical records of stomatal indices from *Quercus* and *Nyssa* from the southeastern US: *Geological Society of America, Abstracts with Programs*, v. 39, n. 6, p. 301.
- CHEN, L., LI, C., CHALONER, W.G., BEERLING, D.J., SUN, Q., COLLINSON, M.E., and MITCHELL, P.L., 2001, Assessing the potential for the stomatal characters of extant and fossil *Ginkgo* leaves to signal atmospheric CO_2 change: *American Journal of Botany*, v. 88, p. 1309–1315.
- CROWLEY, T.J., and BERNER, R.A., 2001, CO_2 and climate change: *Science*, v. 292, p. 870–872.
- DALE, J.E., 1988, The control of leaf expansion: *Annual Review of Plant Physiology and Plant Molecular Biology*, v. 39, p. 267–295.
- DiMICHELE, W.A., and GASTALDO, R.A., 2008, Plant paleoecology in deep time: *Annals of the Missouri Botanical Gardens*, v. 95, p. 144–198.
- EIDE, W., and BIRKS, H.H., 2006, Stomatal frequency of *Betula pubescens* and *Pinus sylvestris* shows no proportional relationship with atmospheric CO_2 concentration: *Nordic Journal of Botany*, v. 24, p. 327–339.
- FRASER, L.H., GREENALL, A., CARLYLE, C., TURKINGTON, R., and ROSS FRIEDMAN, C., 2008, Adaptive phenotypic plasticity of *Pseudoroegneria spicata*: Response of stomatal density, leaf area and biomass to changes in water supply and increased temperature: *Annals of Botany*, v. 103, p. 769–775, doi: 10.1093/aob/mcn252.
- GARCIA-AMORENA, I., WAGNER, F., VAN HOOF, T.B., and MANZANEQUE, F.G., 2006, Stomatal responses in deciduous oaks from southern Europe to the anthropogenic atmospheric CO_2 increase; refining the stomatal-based CO_2 proxy: *Review of Palaeobotany and Palynology*, v. 141, p. 303–312.
- GASTALDO, R.A., RIEGEL, W., PUTTMANN, W., LINNEMANN, U.G., and ZETTER, R., 1998, A multidisciplinary approach to reconstruct the later Oligocene vegetation in central Europe: *Review of Palaeobotany and Palynology*, v. 101, p. 71–94.
- KÜRSCHNER, W.M., 1997, The anatomical diversity of recent and fossil leaves of the durmast oak (*Quercus petraea* Lieblein/*Q. pseudocastanea* Goepfert)—Implications for their use as biosensors of paleoatmospheric CO_2 levels: *Review of Palaeobotany and Palynology*, v. 96, p. 1–30.
- KÜRSCHNER, W.M., KVAČEK, Z., and DILCHER, D.L., 2008, The impact of Miocene atmospheric carbon dioxide fluctuations on climate and the evolution of terrestrial ecosystems: *Proceedings of the National Academy of Sciences of the United States of America*, v. 105, p. 449–453.
- LAKE, J.A., QUICK, W.P., BEERLING, D.J., and WOODWARD, E.I., 2001, Signals from mature to new leaves: *Nature*, v. 411, p. 154.
- LEE, S.-H., TEWARI, R.K., HAHN, E.-J., and PAK, K.-Y., 2007, Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania somnifera* (L.) Dunal. plantlets: *Plant Cell, Tissue and Organ Culture*, v. 90, p. 141–151.
- LÜTHI, D., LE FLOCH, M., BEREITER, B., BLUNIER, T., BARNOLA, J.-M., SIEGENTHALER, U., RAYNAUD, D., JOUZEL, J., FISCHER, H., KAWAMURA, K., and STOCKER, T.F., 2008, High-resolution carbon dioxide concentration record 650,000–800,000 years before present: *Nature*, v. 453, p. 379–382.
- McELWAIN, J.C., 1998, Do fossil plants signal paleoatmospheric CO_2 concentration in the geologic past?: *Philosophical Transactions of the Royal Society B: Biological Sciences*, v. 353, p. 83–96.
- McELWAIN, J.C., and CHALONER, W.G., 1996, The fossil cuticle as a skeletal record of environmental change: *PALAIOS*, v. 11, p. 376–388.
- McELWAIN, J.C., MITCHELL, F.J.G., and JONES, M.B., 1995, Relationship of stomatal density and index of *Salix cinerea* to atmospheric carbon dioxide concentrations of the Holocene: *The Holocene*, v. 5, p. 216–219.
- PETTIT, J.R., JOUZEL, J., RAYNAUD, D., BARKOV, N.I., BARNOLA, J.M., BASILE, I., BENDER, I., CHAPPELLAZ, J., DAVIS, M., DELAYGUE, G., DELMOTT, M., KOTLYAKOV, V.M., LEGRAND, M., LIPENKOV, V.Y., LORIUS, C., PEPIN, L., RITZ, C., SATZMAN, E., and STIEVENARD, M., 1999, Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica: *Nature*, v. 399, p. 429–426.
- POOLE, I., and KÜRSCHNER, W.M., 1999, Stomatal density and index: The practice, in Jones, T.P., and Rowe, N.P., eds., *Fossil Plants and Spores: Modern Techniques*: Geological Society, London, 251–256.
- RESTALLACK, G.J., 2001, A 300-million year record of atmospheric carbon dioxide from fossil plant cuticles: *Nature*, v. 411, p. 287–289.

- RETALLACK, G.J., 2009, Greenhouse crises of the past 300 million years: Geological Society of American Bulletin, v. 121, p. 1441–1455.
- ROYER, D.L., 2001, Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration: Review of Paleobotany and Palynology, v. 114, p. 1–28.
- ROYER, D.L., BERNER, R.A., and BEERLING, D.J., 2001, Phanerozoic atmospheric CO₂ change: Evaluating geochemical and paleobiological approaches: Earth-Science Reviews, v. 54, p. 349–392.
- RUNDGREN, M., and BEERLING, D.J., 1999, A Holocene CO₂ record from the stomatal index of subfossil *Salix herbacea* L. leaves from northern Sweden: The Holocene v. 5, pp. 509–513.
- RUNDGREN, M., and BJÖRCK, S., 2003, Late glacial and early Holocene variations in atmospheric CO₂ concentration indicated by high-resolution stomatal index data: Earth and Planetary Science Letters, v. 213, p. 191–204.
- RUNDGREN, M., BJÖRCK, S., and HAMMARLUND, D., 2005, Last interglacial atmospheric CO₂ changes from stomatal index data and their relation to climate variations: Global and Planetary Change, v. 49, p. 47–62.
- SALISBURY, E.J., 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, Series B, v. 216, p. 1–65.
- SPICER, R.A., 1989, The formation and interpretation of plant fossil assemblages: Advances in Botanical Research, v. 16, p. 96–191.
- SPICER, R.A., and WOLFE, J.A., 1987, Plant taphonomy of late Holocene deposits in Trinity (Clair Engle) Lake, northern California: Paleobiology, v. 13, p. 227–245.
- SUN, B., DILCHER, D.L., BEERLING, D.J., ZHANG, C., YAN, D., and KOWALSKI, E., 2003, Variation in *Ginkgo biloba* L. leaf characters across a climatic gradient in China: Proceedings of the National Academy of Sciences of the United States of America, v. 100, p. 7141–7146.
- VAN DER BURGH, J., VISSCHER, H., DILCHER, D.L., and KÜRSCHNER, W.M., 1993, Paleoatmospheric signatures in Neogene fossil leaves: Science, v. 260, p. 1788–1790.
- VAN HOOF, T.B., BUNNIK, F.P.M., WAUCOMONT, J.G.M., KÜRSCHNER, W.M., and VISSCHER, H., 2006a, Forest re-growth on medieval farmland after the Black Death pandemic—Implications for atmospheric CO₂ levels: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 237, p. 396–411.
- VAN HOOF, T.B., KÜRSCHNER, W.M., WAGNER, F., and VISSCHER, H., 2006b, Stomatal index response of *Quercus robur* and *Quercus petraea* to the anthropogenic atmospheric CO₂ increase: Plant Ecology, v. 183, p. 237–247.
- WAGNER, F., AABY, B., and VISSCHER, H., 2002, Rapid atmospheric CO₂ changes associated with the 8,200-years-B.P. cooling event: Proceedings of the National Academy of Sciences of the United States of America, v. 99, p. 12011–12014.
- WAGNER, F., BELOW, R., DE KLERK, P., DILCHER, D.L., JOOSTEN, H., KÜRSCHNER, W.M., and VISSCHER, H., 1996, A natural experiment on plant acclimation: Lifetime stomatal frequency response of an individual tree to annual atmospheric CO₂ increase: Proceedings of The National Academy of Sciences of the United States of America, v. 93, p. 11705–11708.
- WAGNER, F., KOUWENBERG, L.L.R., VAN HOOF, T.B., and VISSCHER, H., 2004, Reproducibility of Holocene atmospheric CO₂ records based on stomatal frequency: Quaternary Science Reviews, v. 23, p. 1947–1954.
- WILSON, G.L., 1966, Studies on the expansion of the leaf surface. V. Cell division and expansion in a developing leaf as influenced by light and upper leaves: Experimental Botany, v. 17, p. 440–451.
- WOODWARD, F.I., 1987, Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels: Nature, v. 327 617–618.
- YORK, H.H., 1995, 100 Forest Trees of Alabama, Second Edition: Alabama Forestry Commission and Division of Vocational Education, Alabama State Department of Education, Montgomery, Alabama, 111 p.

ACCEPTED DECEMBER 8, 2009