Stomatal Length Correlates with Elevation of Growth in Four Temperate Species†

NICHOLAS HOLLAND¹ and ANDREW D. RICHARDSON²

¹Mass Audubon, Lincoln, Massachusetts, USA
²University of New Hampshire, Complex Systems Research Center, Durham, New Hampshire, USA

Stomatal size and density are considered two key ecophysiological parameters, because they jointly influence stomatal conductance. In the present study, we examine trends in these anatomical traits along a 660-m elevational gradient on Mt. Moosilauke, in the White Mountains of New Hampshire. Samples were collected from two broadleaf tree species (Betula papyrifera var. cordifolia and Sorbus americana) and two herbaceous understory species (Cornus canadensis and Dryopteris carthusiana). Guard cell length increased with elevation in all four species (all p ≤ .10), but there were no clear elevational trends in stomatal density in any of the four species (all p ≥ .10). A “potential conductance index” [= (guard cell length)² × stomatal density × 10⁻⁴] was positively correlated with elevation for all species, but this trend was significant only for the two understory species (both p ≤ .10). Results are discussed in the context of prevailing theories to explain changes in stomatal traits with elevation.

KEYWORDS alpine, elevation, guard cell length, leaf anatomy, montane forests, stomatal density, potential conductance index, Betula papyrifera var. cordifolia, Sorbus americana, Cornus canadensis, Dryopteris carthusiana

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Address correspondence to Andrew D. Richardson, University of New Hampshire, CSRC/ EOS, Morse Hall, 8 College Road, Durham, NH 03824, USA. E-mail: andrew.richardson@unh.edu
INTRODUCTION

Stomata regulate the exchange of gases between leaves and the atmosphere, and thus control the water use efficiency of photosynthesis, i.e., the balance between water loss and CO₂ uptake. The development of stomata (about 400 million years ago) is therefore considered a key event in the evolution of advanced land plants (Hetherington & Woodward, 2003). Stomatal diffusion resistance, and hence conductance, is directly related to the size and spacing of stomata on the leaf surface (Jones, 1992), and so guard cell length and stomatal density can both be considered important eco-physiological parameters (Beerling, Chaloner, Huntley, Pearson, & Tooley, 1993). There is a general negative correlation between guard cell length and stomatal density (Carpenter & Smith, 1975). Across functional groups, and even including fossil plants, this relationship is almost exactly compensatory: Although stomatal densities ranging from 5 to 1,000 mm⁻² are observed, the concurrent changes in mean guard cell length result in a nearly constant (on the mean) stomatal conductance (Hetherington & Woodward, 2003).

Guard cell length and stomatal density are both sensitive to growth environment, and there is considerable genotypic variation and phenotypic plasticity for these two traits. In a common-garden experiment, stomatal density and guard cell length varied among provenances of *Azadirachta indica*, and stomatal density correlated with leaf-level net photosynthesis and whole-plant dry weight (Kundu & Tigerstedt, 1998). Other comparative studies have indicated that xeric species typically have higher stomatal densities than mesic species (Carpenter & Smith, 1975), and sun leaves typically have higher stomatal densities than shade leaves (Hanson, 1917; Lichtenthaler, 1985). Sun/shade plasticity for stomatal density varies among species: In one study, the capacity for phenotypic plasticity correlated with drought tolerance in three *Quercus* spp. (Ashton & Berlyn, 1994). Stomatal density appears to be relatively plastic compared to stomatal length (either guard cell length or stomatal aperture length), which often does not vary as much, or as consistently, across crown positions (Ashton & Berlyn, 1994; Richardson, Ashton, Berlyn, Mcgroddy, & Cameron, 2001; Richardson, Berlyn, Ashton, Thadani, & Cameron, 2000).

Studies show a consistent inverse relationship between stomatal density and atmospheric CO₂ concentration; this is thought to be an adaptive response to maximize water-use efficiency (Woodward, 1987). Meta-analysis of enrichment experiments indicates that stomatal density generally decreases by about 9% in response to enrichment from 350 to 700 ppm CO₂ (Woodward & Kelly, 1995), and stomatal density has been shown to vary predictably in response to past changes in atmospheric CO₂ concentration at a range of timescales (hundreds, thousands, and tens of thousands of years) (Woodward, 1987; Beerling & Chaloner, 1993; Beerling et al., 1993; but see Körner, 1988).
Here we look at patterns in guard cell length and stomatal density of four temperate species (two deciduous tree species and two herbaceous species) along a 660-m elevational gradient (from 730 to 1390 m) on Mt. Moosilauke, in the White Mountains of New Hampshire. We investigate the hypothesis that these diverse but co-occurring species will display similar modes of adaptation along the elevational gradient.

Elevational studies can be seen as “natural experiments” from which we can learn about plant adaptation or response to different environmental conditions (Körner, 1999). Previous anatomical studies show a general trend towards increased stomatal density at higher elevations, but this trend is by no means universal, and a variety of different hypotheses (e.g., decreases in the partial pressure of CO₂ with elevation, increases in drought stress with elevation, or changes in the amount of intercepted solar radiation along the elevational gradient), have been advanced to explain the different patterns (Körner, 1999). In a previous study of *Abies balsamea* leaf structure, DeLucia and Berlyn (1984) reported that stomatal length decreased with increasing elevation, whereas stomatal density increased, between 730 and 1402 m on Mt. Moosilauke.

**METHODS**

**Study Site**

Study sites at three elevations (730, 1020 and 1390 m ASL) were located along the Gorge Brook Trail on the eastern slope of Mt. Moosilauke (44° 01′ N, 71° 51′ W, summit elevation 1463 m), one of the ten highest peaks of New Hampshire’s White Mountains. Between 800 and 900 m, the mountain’s forests show a transition from northern hardwood species (*Fagus grandifolia*, *Acer saccharum*, and *Betula alleghaniensis*) to spruce/fir (*Picea rubens* and *Abies balsamea*). The alpine treeline is located at about 1400 m. Above this, vegetation is dominated by prostrate *Krumholz* and *Carex* spp.

Although the White Mountains have low relief (1000 m to 1500 m from valley floor to summit), the climatic gradient is exceptionally steep (Reiners & Lang, 1979). This is evidenced by the overall pattern of vegetational zonation, and, in particular, the extremely low-elevation treeline. The only climatic or meteorological traits generally characteristic of montane environments worldwide are altitude-dependent decreases in atmospheric pressure (and therefore the partial pressures of O₂ and CO₂) and mean temperature (Körner, 1999). Atmospheric pressure decreases by ≈ 1% for every 100-m increase in elevation. The mean lapse rate of air temperature in the White Mountains (0.58°C/100 m) is comparable to that reported for other mountain ranges around the world (Barry, 1992; Richardson, Lee, & Friedland, 2004). Relative humidity also increases with elevation, and the frequency of very high (≥90%) relative humidity values at 1425 m on Mt. Moosilauke is roughly
twice that at 748 m (Richardson et al., 2004). Although White Mountain summits are frequently engulfed in clouds (Reiners & Lang, 1979), incident photosynthetically active radiation does not appear to change substantially along the elevational gradient (Richardson et al., 2004). Precipitation increases with increasing elevation, and Reiners, Hollinger, and Lang (1984) used soil moisture data combined with models of potential and actual evapotranspiration to demonstrate that water limitation decreases with increasing elevation, and is typically rare above elevations of about 800 m, on Mt. Moosilauke.

Sample Collection and Preparation

Outer canopy or “sun leaves” were collected from two deciduous tree species (*Betula papyrifera* var. *cordifolia* (Regel) Fern., mountain paper birch, and *Sorbus americana* Marsh., American mountain-ash), while shade leaves were collected from two herbaceous understory species (*Cornus canadensis* L., bunchberry, and *Dryopteris carthusiana* (Vill.) Fuchs, spinulose wood-fern). Leaf samples (1 cm²) were collected from at least six leaves from each of three individuals of each species at each elevation, and were immediately fixed in FAA (formalin, acetic acid and alcohol; Berlyn & Miksche, 1976). In the laboratory, leaf samples were dehydrated in an ethanol series, cleared in a sodium hydroxide solution at 50°C, stained with toluidine blue, and then temporarily mounted on slides with Karo light corn syrup. Three leaf samples from each individual were used for anatomical studies.

Electronic image analysis equipment (Leica Q500MC and QWin V1.00 software) was used for all anatomical measurements. Stomatal counts were conducted for each of 10 different fields of view for each leaf sample. Guard cell length was measured on 15 different stomata for each leaf sample. Objectives of 20 × and 40 × (depending on the size and density of stomata) were used in conjunction with a 5 × ocular. The camera-computer setup provided an additional 8 × magnification.

A significance level of \( p \leq .10 \) was used for all statistical tests. The sample size was \( n = 9 \) (3 elevations × 3 individuals at each elevation) for each species.

**RESULTS**

Both guard cell length and stomatal density varied among species (Table 1). *D. carthusiana* guard cells were longer than those of any other species, but stomatal density of *D. carthusiana* was less than that of the other species. *C. canadensis* had the shortest guard cells, and *S. americana* the highest stomatal density. There was a significant negative correlation between guard cell length and stomatal density in *D. carthusiana* and *S. americana* (both \( p \leq .10 \)).
Guard cell length generally increased with elevation. There was a significant linear correlation between elevation and guard cell length in all species (all \( p \leq .10 \); Table 1a). The effect was modest for *C. canadensis* (4% difference between 730 and 1390 m), but much more pronounced for *D. carthusiana* (26% difference) and *S. americana* (24% difference).

Stomatal density tended to decrease with increasing elevation in *D. carthusiana* and *S. americana*, but increase with elevation in *B. papyrifera* (Table 1b). However, the correlation between elevation and stomatal density was not statistically significant (all \( p \geq .10 \)) for any species.

Stomatal conductance depends on both stomatal density, and the size (area) of the stomatal aperture. If we assume that the stomatal aperture area is proportional to the guard cell length squared, then a unitless “potential conductance index” (PCI) can be calculated as:

\[
PCI = (\text{guard cell length})^2 \times \text{stomatal density} \times 10^{-4}
\]

For the two herbaceous species, PCI was positively correlated with elevation (both \( p \leq .10 \)), and increased by \( \approx 30\% \) between 730 m and 1390 m (Table 1c). For the two deciduous tree species, the data exhibited the same pattern, but the trend was not statistically significant (both \( p \geq .10 \)).

**TABLE 1** Changes in guard cell length, stomatal density, and potential conductance index \( [= (\text{guard cell length})^2 \times \text{stomatal density} \times 10^{-4}] \) with increasing elevation. Results are mean \( \pm 1 \) SE, based on \( n = 3 \) individuals of each species at each elevation (mean for each individual based on three leaf samples, with 15 guard cell length measurements, and 10 stomatal density counts, per leaf). Pearson’s correlation statistic, \( r \), is reported for the linear correlation between elevation and the mean measurement for each individual (\( n = 9 \)); \( p \) values are considered significant at \( p \leq .10 \)

<table>
<thead>
<tr>
<th>Elevation</th>
<th><em>C. canadensis</em></th>
<th><em>D. carthusiana</em></th>
<th><em>B. papyrifera</em></th>
<th><em>S. americana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>730 m</td>
<td>25.0 ± 0.2</td>
<td>43.2 ± 1.3</td>
<td>29.0 ± 0.1</td>
<td>31.9 ± 1.0</td>
</tr>
<tr>
<td>1070 m</td>
<td>26.1 ± 0.3</td>
<td>53.8 ± 0.9</td>
<td>30.2 ± 1.3</td>
<td>36.9 ± 1.3</td>
</tr>
<tr>
<td>1390 m</td>
<td>26.0 ± 0.4</td>
<td>54.5 ± 2.6</td>
<td>31.1 ± 0.3</td>
<td>39.7 ± 0.4</td>
</tr>
<tr>
<td>correlation ( r )</td>
<td>0.62 (( p = .08 ))</td>
<td>0.80 (( p = .01 ))</td>
<td>0.60 (( p = .09 ))</td>
<td>0.91 (( p = .01 ))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Elevation</th>
<th><em>C. canadensis</em></th>
<th><em>D. carthusiana</em></th>
<th><em>B. papyrifera</em></th>
<th><em>S. americana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>730 m</td>
<td>115 ± 7</td>
<td>48 ± 6</td>
<td>162 ± 30</td>
<td>239 ± 16</td>
</tr>
<tr>
<td>1070 m</td>
<td>99 ± 9</td>
<td>42 ± 2</td>
<td>174 ± 17</td>
<td>197 ± 41</td>
</tr>
<tr>
<td>1390 m</td>
<td>139 ± 13</td>
<td>42 ± 3</td>
<td>175 ± 26</td>
<td>175 ± 9</td>
</tr>
<tr>
<td>correlation ( r )</td>
<td>0.45 (( p = .11 ))</td>
<td>-0.41 (( p = .27 ))</td>
<td>0.14 (( p = .70 ))</td>
<td>-0.58 (( p = .11 ))</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Elevation</th>
<th><em>C. canadensis</em></th>
<th><em>D. carthusiana</em></th>
<th><em>B. papyrifera</em></th>
<th><em>S. americana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>730 m</td>
<td>7.2 ± 0.5</td>
<td>8.9 ± 0.7</td>
<td>13.7 ± 2.5</td>
<td>24.5 ± 3.1</td>
</tr>
<tr>
<td>1070 m</td>
<td>6.8 ± 0.8</td>
<td>12.0 ± 0.2</td>
<td>15.7 ± 0.8</td>
<td>26.3 ± 4.3</td>
</tr>
<tr>
<td>1390 m</td>
<td>9.5 ± 1.1</td>
<td>12.3 ± 0.9</td>
<td>17.0 ± 2.7</td>
<td>27.5 ± 1.4</td>
</tr>
<tr>
<td>correlation ( r )</td>
<td>0.55 (( p = .06 ))</td>
<td>0.78 (( p = .01 ))</td>
<td>0.40 (( p = .29 ))</td>
<td>0.26 (( p = .50 ))</td>
</tr>
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We cannot reject the hypothesis that all four species respond similarly to the elevational gradient: Trends in guard cell length were of the same sign, and significant in all four species, whereas trends in stomatal density, although varying in sign among species, were not statistically significant. These data are also consistent with the idea that stomatal conductance increases with elevation.

**DISCUSSION**

**Comparison with Previous Studies**

The statistically significant trends in guard cell length seen in all four species are notable. If anything, one would have expected that cell size would decrease with increasing elevation (e.g., *Abies balsamea*; see DeLucia & Berlyn, 1984). It could be hypothesized, for example, that dwarfism in high-elevation species or genotypes is attributable to reductions in cell size. Cells in high-elevation leaves generally have thicker walls than those in low-elevation leaves, and high-elevation leaves themselves are generally smaller than low-elevation leaves (Körner, Neumayer, Menendez-Riedl, & Smeets-Scheel, 1989), but, in fact, there is no strong evidence that cell size is reduced at high elevations. For example, Körner et al. (1989) found no significant differences in cell size between leaves of high- (≈ 3000 m) and low-elevation (≈ 600 m) plants. That being said, some particular high-elevation specialist species, such as *Ranunculus glacialis*, have been shown to have unusually large cells (Körner et al., 1989), and species in a high altitude mossy forest were found to have larger stomata than species in a lower montane forest (reviewed in Doley, 1981). However, we are not aware of previously published reports that, within a species, cell size tends to increase with elevation. In a study of two conifers at Newfoundland’s Gros Morne National Park, we found that guard cell length of *Picea mariana* increased by 10% between low (10 m) and high (770 m) elevation sampling sites, but guard cell length of *A. balsamea* did not show any trends with regard to elevation (Richardson, unpublished data). Because both stomatal size and density affect stomatal conductance, the results presented here would tend to suggest that it may be just as important to quantify elevation-related changes in guard cell length as it is to measure changes in stomatal density, although the focus tends more often to be on the latter trait.

In the present study, there were no clear elevational trends in stomatal density in any of the four species examined. Previous reports of changes in stomatal density with elevation are not unequivocal. Körner and colleagues, who conducted numerous studies of the anatomical characteristics of high elevation plants (e.g., see reviews in Körner et al., 1989; Körner, 1999), concluded that although there is a general trend towards increased stomatal density at high elevation, and although this pattern is strong and consistent
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in many mountain ranges around the world, there are nevertheless a number of locations where the pattern is reversed or nonexistent. In *Lobelia teleki* from Mt. Kenya, for example, stomatal density increased by 20% between 3750 m and 4190 m, then decreased by nearly 50% between 4190 m and 4740 m; on some wet tropical mountains, such as Mt. Wilhelm in Papua New Guinea, there did not appear to be any elevational trend in stomatal density (Körner et al., 1989).

Other investigators have reported similarly mixed results. DeLucia and Berlyn (1984) found that there was a trend towards increased stomatal density at higher elevations on Mt. Moosilauke in *Abies balsamea*. In the mountains of Greece, stomatal density on both upper and lower leaf surfaces of *Origanum vulgare* was shown to increase with increasing elevation (Kofidis, Bosabalidis, & Moustakas, 2003). McElwain (2004) found that relationship between stomatal density and elevation are nonlinear in *Quercus kelloggii* across an elevational range from ≈500–2500 m in the mountains of California. However, when the analysis was restricted to samples from above 1000 m, there was a clear positive correlation, i.e., stomatal density increased with increasing elevation.

By comparison, Sharma (1975) found that stomatal density of *Cannabis sativa* was higher at a hot, dry low-elevation site (250 m) than at a more mesic high elevation (2000 m) site in the Himalaya. In the Rocky Mountains, stomatal density decreased with increasing altitude in *Pinus contorta* and *Abies lasiocarpa*, but did not vary significantly with altitude in *Pseudotsuga menziesii* or *Picea engelmannii*, despite significant relationships between foliar δ13C and elevation in all four species (Hultine & Marshall, 2000). The authors concluded that, across species and sites, leaf mass to area ratio (LMA), rather than stomatal characteristics, explained the variation in δ13C. This brings into question the idea that stomatal density is, by itself, an important ecophysiological trait. Studying *Pinus flexilis*, another Rocky Mountain conifer, Schoettle and Rochelle (2000) reported that the stomatal density decreased with increasing elevation.

Although they did not measure stomatal density directly, Richardson and Berlyn (2002) found that stomatal conductance of *B. papyrifera* on Mt. Mansfield (in the Green Mountains of Vermont, ≈100 km northwest of Mt. Moosilauke) decreased along an elevational gradient from 550–1160 m. By comparison, our PCI index suggests the potential for increased stomatal conductance with increasing elevation in all four study species, including *B. papyrifera*, on Mt. Moosilauke. This is especially interesting considering that the PCI index, calculated from data presented by DeLucia and Berlyn (1984), suggests a 15% decrease in potential conductance of *A. balsamea* along an almost identical elevational gradient on the same mountain. A possible explanation may be that the small (2–4 m in height), young *B. papyrifera* and *S. americana* trees used in the present study came from more sheltered (and thus mesic) microsites than the large, mature trees from which DeLucia and Berlyn’s samples were collected.
Explanations for Elevation-Related Changes in Stomatal Traits

These apparently contradictory results demand a discussion of the various theories to explain relationships between stomatal traits and elevation. Three main theories have been advanced: (a) reduced CO₂ availability; (b) drought stress; and (c) solar radiation. These will theories will be discussed in order. Arguments are typically framed in terms of stomatal density, because that is the trait most frequently measured, but as has already been shown, stomatal size effects may potentially be as important as density effects if conductance is the key underlying trait.

The molar fraction of CO₂ in the atmosphere does not change with elevation, but because barometric pressure decreases quite predictably with increasing elevation, there is a corresponding decrease in the partial pressure of CO₂ (\(p_{\text{CO}_2}\)) (McElwain, 2004). Some authors have argued that, because this may act as a limitation on photosynthesis, it is to be expected that plants will respond by increasing stomatal density, and the resulting increase in stomatal conductance should offset the decrease in \(p_{\text{CO}_2}\) such that CO₂ availability is not a limiting factor (Körner, 1988; McElwain, 2004). This argument ignores the fact that the product of stomatal density and stomatal aperture size together determine stomatal conductance. As the present study has demonstrated, changes in cell size with elevation may be as important in this regard as changes in stomatal density. The reduced CO₂ theory would also predict a universal increase in stomatal density with increasing elevation (since \(p_{\text{CO}_2}\) universally decreases with elevation); as has been discussed above, there are clearly many situations where this pattern is not observed. It has also been pointed out that the diffusion coefficient of CO₂ also increases with altitude. Thus, on theoretical grounds there is no reason for photosynthesis to be CO₂-limited at altitude in the first place (Gale, 1972; Terashima, Masuzawa, Ohba, & Yokoi, 1995; Johnson, Smith, & Silman, 2005). In the present study, elevation-related trends in stomatal density were not significant (all \(p > .10\)) for any of the four species, but the increase in guard cell length with elevation (significant at \(p \leq .10\) in all four species), and the increase in potential conductance as indicated by PCI (significant at \(p \leq .10\) for two of four species) are at least consistent with the ideas underlying the CO₂ availability theory, namely that stomatal traits should respond to reduced \(p_{\text{CO}_2}\) at higher elevations. Clearly, however, these responses need not be restricted solely to changes in stomatal density.

An alternative theory proposes that increases in altitude may affect leaf structure more through drought effects: Not only does the air generally become drier with increasing elevation, but the diffusion coefficient of water vapor in air is also increased at high elevation; both of these would tend to promote transpiration and desiccation (Terashima et al., 1995; Johnson et al., 2005). Changes in stomatal traits with elevation may therefore be a response...
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to changes in water relations, rather than a CO₂ signal. A comprehensive modeling study demonstrated that even in the tropics, where high-elevation regions are generally cool and perennially humid, the growth environment can be very xeric for plants (Leuschner, 2000). Schoettle and Rochelle (2000) proposed that reduction in stomatal density with increasing elevation was an adaptation by *Pinus flexilis* to minimize water loss in a dry, high elevation environment. However, this contradicts the general relationships for xeric vs. mesic species, and sun vs. shade foliage (sun leaves generally have smaller but more abundant stomata, e.g., Hanson, 1917; Lichtenthaler, 1985; Ashton & Berlyn, 1994). In any case, in the White Mountains of New Hampshire, water limitation is not considered to be a problem above ≈800 m in elevation (Reiners et al., 1984). Summertime precipitation is usually abundant, and the region is classified as having a cool, moist climate (Dfb by the Koppen-Geiger system; Reiners & Lang, 1979). Thus, it may not be a surprise that we do not see significant changes in stomatal density along the elevational gradient on Mt. Moosilauke. In fact, the observed increases in guard cell length with increasing elevation may be a direct result of water stress becoming less limiting. We acknowledge, however, that this explanation is not consistent with results (discussed above) presented by DeLucia and Berlyn (1984).

The third theory, advanced by Körner (1988, 1999), attributes the elevational patterns in stomatal density to changes in foliar light interception. On some mountains, insolation increases with increasing elevation because the shorter atmospheric path length reduces scattering and absorption. On other mountains, the frequency of cloud immersion increases with elevation, and insolation decreases with increasing elevation. On mountains falling into the first category, stomatal density typically increases with increasing elevation (e.g., the Alps). This pattern is consistent with what would be expected based on sun/shade dimorphism. On mountains falling into the second category (e.g., very wet tropical mountains), stomatal density typically remains unchanged, or possibly decreases, with increasing elevation. On Mt. Moosilauke, clear-sky mid-day fluxes of photosynthetically active radiation are only 4% higher at 1425 m than at 748 m (Richardson et al., 2004), but when cloudy days are included as well, fluxes are marginally higher at the lower elevation site. Thus a possible explanation for the overall absence of any significant elevation-stomatal density relationships in any of the four species studied could be that the differences in light environment between our low- (730 m) and high-elevation (1390 m) collection sites are not sufficiently large to trigger a phenotypic response. However, if changes in solar radiation determine the elevational patterns in stomatal density, then one might expect overstory species to exhibit a more pronounced response to the gradient than understory species. This hypothesis needs to be tested in a system where there is a strong elevational gradient in solar radiation.
REFERENCES


