Insight
Ancient cell structural traits and photosynthesis in today’s environment

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Mesophyll conductance to CO2 – a key factor in plant photosynthesis – is strongly influenced by leaf anatomy. In this issue, Verömann-Jürgenson et al.

pages 1639–1653 provide evidence of the conservation of ancient structural traits (extremely thick cell walls) in evolutionarily old taxa that suggest apparent

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evolutionary constraints on CO₂ fixation. This opens the way for integrated approaches combining evolutionary constraints of diffusive, structural and biochemical factors on plant photosynthesis.

For many decades, the rate of CO₂ diffusion through stomata (stomatal conductance, gs) and the capacity of photosynthetic machinery to convert light to biochemical energy and fix CO₂ into sugars (biochemical capacity) were considered the only two factors constraining plant photosynthesis. However, pioneer studies already suggested that CO₂ diffusion from sub-stomatal cavities to carboxylation sites inside chloroplasts (mesophyll conductance, gm) could also limit photosynthesis (Nobel, 1970). There is now an increasing interest among plant physiologists in studying the role of gm as the third major player involved in controlling the rate of photosynthesis, and this is reflected in the number of studies recently published addressing the ecophysiological significance of gm and its regulatory mechanisms (see Flexas et al., 2012, and references therein).

Large variations in gm among species or plant groups can be explained through the existence of several barriers to CO₂ diffusion across the mesophyll (including air, cell walls, lipid membranes, cytoplasm and chloroplasm stroma) differing in nature and size (Evans et al., 2009; Terashima et al., 2011). Recently, a small number of studies have quantified the importance of different leaf anatomical traits in determining the variability in gm and photosynthesis among species (Tomás et al., 2013; Peguero-Pina et al., 2016a; Peguero-Pina et al., 2017) or even within the same species growing under contrasting environmental conditions (Terashima et al., 2011; Tosens et al., 2012a; Peguero-Pina et al., 2016b, c). These analyses showed that gm was most strongly correlated with the chloroplast surface area facing intercellular air spaces (S/S), thickness of the mesophyll cell walls (Tcw), and chloroplast size; however, depending on foliage structure, the overall importance of gm in constraining photosynthesis and the importance of different anatomical traits in the restriction of CO₂ diffusion varied (Evans et al., 2009; Terashima et al., 2011; Tosens et al., 2012b).

### Ancient structural traits constrain photosynthesis in old taxa

Mesophyll conductance has been estimated for more than 100 species from all major plant groups, but mainly spermatophytes (angiosperms and gymnosperms), with little data for ferns, liverworts and hornworts (Flexas et al., 2012; Carriquí et al., 2015; Tosens et al., 2016). Considerable variations in gm

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**Box 1. Mesophylls of evolutionarily old or modern species which have evolved under different CO₂ concentrations**

The schematic representation shows the mesophyll of (A) an evolutionarily old species which evolved under high CO₂ concentration and (B) an evolutionarily modern species which evolved under low CO₂ concentration. Photosynthesis in evolutionarily old species at current CO₂ concentrations could be constrained by low values of stomatal conductance (gs) (due to larger stomatal size but lower stomatal density: Franks and Beerling, 2009), low values of mesophyll conductance (gm) (due to extremely thick cell walls, Tcw: Veromann-Jürgenson et al., 2017), and lower carboxylase catalytic efficiency (kcat/Kc) (Galmés et al., 2014).
and its underlying traits among different plant groups have supported the hypothesis that an evolutionary trend exists towards higher \( g_m \) together with the diversification of embryophytes (Flexas et al., 2012; Carriquí et al., 2015). However, there is still a significant knowledge gap concerning phylogenetic/evolutionary trends in \( g_m \).

The number of studies concerning \( g_m \) in gymnosperms is surprisingly limited, in spite of the great importance of coniferous forests throughout the world (Breckle, 2002). Specifically, \( g_m \) had only been estimated in 13 conifer species before the study by Veromann-Jürgenson et al. (2017; see also references therein). Although gymnosperms show the lowest \( g_m \) values across spermatophytes (Flexas et al., 2012), available data show a high degree of interspecific variation and suggest the primary role of \( g_m \) as a limiting factor for net CO\(_2\) assimilation in conifers. However, as pointed out by Veromann-Jürgenson et al. (2017), information about \( g_m \) with its underlying structural traits is especially limited in conifers, and only Peguero-Pina et al. (2012, 2016b) had previously correlated \( g_m \) with ultrastructural needle anatomy in species belonging to this plant group.

In this context, Veromann-Jürgenson et al. (2017) have characterized the structural traits (i.e. \( S_d/S \), chloroplast size and \( T_{cw} \)) that are mainly responsible for low \( g_m \) and photosynthetic performance in several evolutionarily old gymnosperms and herbaceous species with contrasting phylogenetic age. These authors have found, for the first time, striking evidence about the effect of divergence time on structure and physiology, and specifically a negative correlation between estimated evolutionary age of the plant genus and area-based photosynthesis (\( A_N \)). However, as they recognize, this statement should be treated with caution because species’ evolutionary adaptation to prevailing environmental conditions can actually drive photosynthetic capacity more strongly than their evolutionary age (Tosens et al., 2016). Regarding CO\(_2\) diffusion across the mesophyll, although \( g_m \) itself was not related to plant evolutionary age, the lowest \( g_m \) values (which scaled positively with \( A_N \) regardless of evolutionary age) were observed for the oldest genera.

The most significant conclusion emerging from the study of Veromann-Jürgenson et al. (2017) is that the preservation of old traits suggests constraints on evolution due to the co-occurrence of low \( g_m \) and \( A_N \) and the corresponding high \( T_{cw} \) for species with widely contrasting ecological strategies. Thereby, these authors hypothesize that (i) the high-CO\(_2\) atmosphere when several of these thick-cell-walled species evolved (about 65–200 million years ago) suggests a lower control of diffusional limitations on the rate of photosynthesis, and (ii) the preservation of this ancient trait in spite of the gradual CO\(_2\) decrease through evolution has led to stronger control of foliage assimilation rates by \( g_m \) (Box 1).

**Integrated approaches: the way forward**

The phylogenetic trend consisting of a reduction of the cell wall thickness through evolution from bryophytes to angiosperms was recently considered by Carriquí et al. (2015), who suggested that this reduction was probably crucial to allow plants to achieve larger photosynthetic rates albeit at the expense of a reduction in desiccation tolerance. Increased values of cell wall thickness have been related to a greater ability to preserve the structure of the cells under water stress (Proctor and Tuba, 2002; Carriquí et al., 2015). Related to this, Corcuerà et al. (2002) suggested that cell wall thickness may be associated with the maximum bulk modulus of elasticity (\( \varepsilon_{\max} \)), one of the main physiological traits related to the functional role of the cell wall. Higher \( \varepsilon_{\max} \) values are seen as an efficient mechanism for plant performance under dry climates, as low cell-wall elasticity (i.e. high \( \varepsilon_{\max} \)) would allow a rapid recovery after a decrease in soil water content (Corcuerà et al., 2002). To the best of our knowledge, there are no published studies empirically relating higher \( \varepsilon_{\max} \) values with increasing cell wall thickness. However, there does seem to be a positive trend between both parameters when values of cell wall thickness are plotted against \( \varepsilon_{\max} \) for several oak species (Box 2). Additional studies including simultaneous measurements of both parameters in a larger number of species from different genera are required for understanding the ultimate causal factors involved in this trade-off.

Besides \( g_m \), Veromann-Jürgenson et al. (2017) found that \( A_N \) also depended strongly on \( g_m \), which correlated negatively with the age of the genus. This empirical result is supported by Franks and Beerling (2009), who stated that periods of falling atmospheric CO\(_2\) challenged plants with diminished CO\(_2\) availability, inducing a selection for higher maximum

**Box 2. Cell wall thickness and maximum bulk modulus of elasticity**

The graph shows the relationship between cell wall thickness (\( T_{cw} \)) and the maximum bulk modulus of elasticity (\( \varepsilon_{\max} \)) for several Quercus species. Mean values of \( \varepsilon_{\max} \) are from Corcuerà et al. (2002); mean values of cell wall thickness are from Peguero-Pina et al. (2016a, 2017).
$g_s$ through a trend towards smaller stomatal size and higher density, thereby alleviating the negative impact of diminishing CO$_2$ on photosynthesis (Box 1). This co-regulation between $g_m$ and $g_s$ is to some extent expected (Flexas et al., 2012) because CO$_2$ and water vapour share, in part, diffusion pathways in the mesophyll (Evans et al., 2009; Terashima et al., 2011).

Beyond diffusive components (i.e. $g_s$ and $g_m$), other factors also determine the rate of plant photosynthesis, such as the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Galmés et al. (2014) found evolutionary trends in relation to atmospheric CO$_2$ when analyzing the variability in Rubisco kinetics in different plant species. These authors confirmed that evolution of Rubisco towards increased affinity for CO$_2$ ($K_c$ falling) and increased carboxylase catalytic efficiency ($k_{cat}/K_c$) in land plants is likely to have been complementary to falling CO$_2$/O$_2$ ratios, as well as to adaptations in leaf architecture, morphology and conductance (Beering et al., 2001; Franks and Beering, 2009; Haworth et al., 2011) (Box 1).

Veromann-Jürgenson et al. (2017) provide an interesting starting point for further studies on the role of phylogenetic aspects in plant physiological performance (i.e. the influence of the age on photosynthesis associated with the preservation of ancient traits in evolution, such as extremely thick cell walls). Currently, the way forward is through the implementation of integrated approaches that combine evolutionary constraints of diffusive, structural and biochemical factors on plant photosynthetic performance, together with other functional traits (e.g. plant hydraulics).

Key words: Cell wall elasticity, cell wall thickness, evolutionary constraints, gymnosperms, leaf anatomy, mesophyll conductance, photosynthesis, Rubisco, stomatal conductance.

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