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HISTORICAL PERSPECTIVE

Stomatal control as a driver of plant evolution

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Stomata are the pores on a leaf surface through which plants regulate the uptake of carbon dioxide (CO_2) for photosynthesis against the loss of water via transpiration. Turgor changes in the guard cells determine the area of stomatal pore through which gaseous diffusion can occur, thus maintaining a constant internal environment within the leaf (Gregory *et al.*, 1950). Stomata first occurred in the fossil record ~400 million years ago (Ma), and are largely identical in form to the stomatal complexes of many extant plants, illustrating their effectiveness and importance to terrestrial plants (Edwards *et al.*, 1998). Stomatal control is critical to a plant's adaptation to its environment; it is this fundamental importance that has led to a wealth of stomatal research ranging in scale from biomolecular analysis to landscape processes (e.g. Gedney *et al.*, 2006; Hu *et al.*, 2010).

The first issue of *Journal of Experimental Botany*, published 60 years ago, contained four papers relating to stomatal function. These included an analysis by Heath of the effects of atmospheric CO_2 concentration ($[CO_2]$) on stomatal aperture and conductance; an area of research that is increasingly relevant to our understanding of the past and prediction of future vegetation responses to atmospheric composition. Heath (1950) was the first to observe that reductions in $[CO_2]$ below ambient levels induced stomatal opening, an ecophysiological response of great interest, and that the site of CO_2 sensing was most probably in the substomatal cavity and not the guard cells. Stomatal research has become vastly important to crop production, biodiversity responses, and hydrology (particularly in terms of 'run-off') with respect to rising atmospheric $[CO_2]$, changing water regimes, and growing populations. As our understanding of stomatal physiology develops, the role of stomata in the evolution of terrestrial vegetation and development of the terrestrial landscape and atmospheric composition is becoming increasingly evident, alongside the use of fossil stomata as palaeo-proxies of past atmospheres (e.g. McElwain *et al.*, 2004; Berry *et al.*, 2010; Smith *et al.*, 2010).

The stomatal control responses of plants consist of 'short-term' stomatal aperture changes in response to availability of water, light, temperature, wind speed, and carbon dioxide, and also 'longer term' changes in stomatal density that set the limits for maximum stomatal conductance in response to atmospheric $[CO_2]$, light intensity/quality, and root-to-shoot signals of water availability (Schoch *et al.*, 1980, 1984; Davies *et al.*, 2000; Casson *et al.*, 2009). Stomatal control determines the water use efficiency (WUE) of a plant by optimizing water lost against carbon gained. Additionally, the stomatal control mechanisms employed by a plant species will determine: the risk of xylem embolism by reducing the probability of cavitation through stomatal closure during episodes of high transpirative demand (Brodribb and Jordan, 2008; Meinzer *et al.*, 2009); leaf temperature and resistance to heat stress (Srivastava *et al.*, 1995; Jones *et al.*, 2002); tolerance of toxic atmospheric gases (Mansfield and Majernik, 1970); nutrient uptake via promotion of root mass flow (Van Vuuren *et al.*, 1997); and the maximum rate of photosynthesis (Körner *et al.*, 1979). Those plant species with more effective stomatal control will be expected to be more successful than those with less effective stomatal control. However, not all plant species, or individuals within a species, possess equally effective stomatal control, in the setting of either stomatal numbers or the regulation of stomatal aperture (i.e. speed and 'tightness' of closure). Given that any trait that confers a selective advantage is likely to become universal within a population (McNeilly, 1968), it may be reasonable to assume that stomatal control incurs certain 'costs', and that these costs have played a significant role in plant evolution over the last 400 million years.

The origination of major plant groups, and morphological advances such as the development of planate leaves, coincide with periods of 'low' atmospheric $[CO_2]$ (Fig. 1) (Woodward, 1998; Beerling *et al.*, 2001). The reduced availability of the substrate for photosynthesis is predicted to be compensated by increases in the carboxylation efficiency of RubisCO and enhanced stomatal conductance to maintain CO_2 uptake during periods of low $[CO_2]$ (Woodward, 1998; Franks and Beerling, 2009). This elevated stomatal conductance incurs higher rates of water loss and associated risks of desiccation and xylem embolism, in addition to the metabolic costs of enhanced construction of stomatal complexes. It is these costs during periods of low $[CO_2]$ that may serve as evolutionary tipping points, where species with more efficient and effective stomata and hydraulic systems are favoured (Robinson, 1994; Brodribb
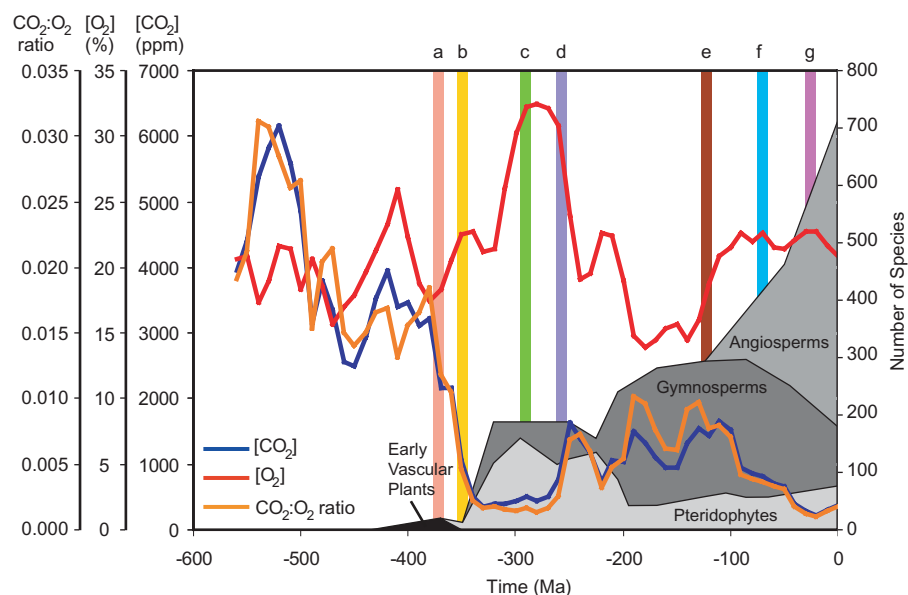
Fig. 1. Levels of atmospheric [CO_2] (Berner, 2006) and [O_2] (Berner, 2009) over Phanerozoic time and the diversification of plant species (Niklas *et al.*, 1983). Origination of: (a) planate leaf; (b) ferns; (c) cycads and Ginkgoaceae; (d) conifers; (e) angiosperms; (f) grasses, and (g) C_4 grasses.
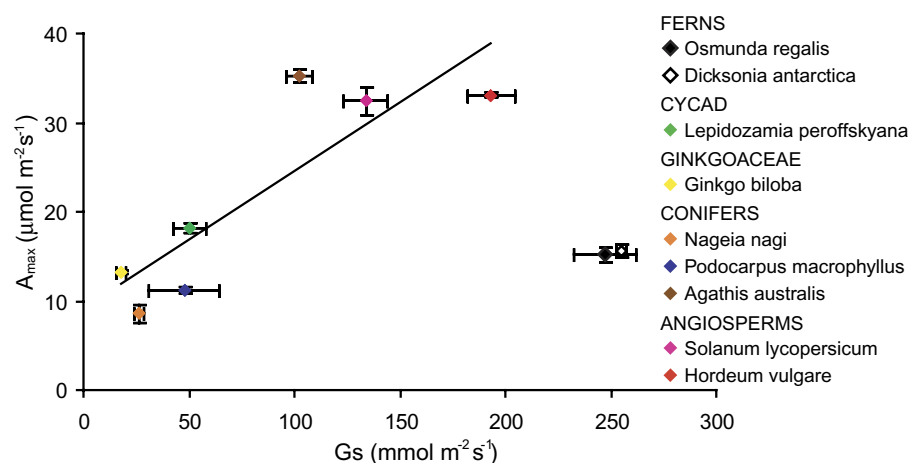

Fig. 2. Maximum rate of photosynthesis (A_{max}) and stomatal conductance (Gs) of an evolutionary range of plants. The line of best fit relates to angiosperms and gymnosperms and does not include pteridophytes (R^2=0.748; linear regression, P=0.012). Error bars indicate the standard error either side of the mean.

and Jordan, 2008; Brodribb and Feild, 2010). The localized availability of water may also have played a determining role during these periods of low [CO_2]. A high water availability aquatic habitat is considered a likely ecological niche for early angiosperms, prior to their later diversification and colonization of lower water availability habitats in the Late Cretaceous (100–65 Ma) (Feild *et al.*, 2004; Saarela *et al.*, 2007).

In extant plants, photosynthetic capacity is associated with maximum stomatal conductance (Körner *et al.*, 1979; Hetherington and Woodward, 2003), and this is observed across an evolutionary range of gymnosperms and angiosperms (cycads, Ginkgoaceae, conifers, dicotyledons, and monocotyledons) (Fig. 2), suggesting that maximal photosynthetic rates sustained by higher stomatal conductance

may have served as a driver of plant evolution throughout earth history (Robinson, 1994; Franks and Beerling, 2009). It is unclear whether this evolution is driven exclusively by [CO_2] or the photosynthetic availability of CO_2 determined by the atmospheric ratio of CO_2 to oxygen (O_2). RubisCO displays an affinity for both CO_2 and O_2 as part of the competing processes of photosynthesis and photorespiration (Miziorko and Llorimer, 1983). The origination and radiation of key plant groups such as ferns, cycads, Ginkgoaceae, and angiosperms occur during periods where the CO_2:O_2 ratio is less favourable towards photosynthesis, and atmospheric O_2 concentration ([O_2]) is high or rising (Fig. 1). Levels of atmospheric O_2 may also have driven plant evolution via respiratory effects on stomatal control. Stomatal opening is an energetically expensive process

requiring O_2 to fuel the ion transport required to raise guard cell turgor (Humble and Hsiao, 1970; Shimazaki *et al.*, 1983; Schwartz and Zeiger, 1984; Mawson, 1993*a*). Indeed, higher maximum stomatal conductances in *Gossypium barbadense* are associated with higher guard cell respiratory demands (Srivastava *et al.*, 1995). However, maintenance of stomatal aperture via guard cell chloroplast photophosphorylation and production of osmotically active sugars may reduce the consumption of oxygen (Walker and Zelitch, 1963; Vavasseur *et al.*, 1988; Mawson, 1993*b*). Oxygen is also required for effective stomatal control, increasing the speed of guard cell turgor loss during stomatal closure, and the ability of guard cells to respond to changes in atmospheric [CO_2] (Akita and Moss, 1973). During periods of 'high' atmospheric [O_2] the respiratory demands of stomatal control will be reduced, favouring those species with a high degree of stomatal control. Thus the combined effects of O_2 on photosynthesis relative to photorespiration, and the energetic requirements of stomatal control, may have acted as a selective driving force in plant evolution in conjunction with [CO_2] starvation.

An evolutionary trend is also evident in the stomatal responses of plant groups to [CO_2]. Unlike conifers and ferns, angiosperms exhibit reductions in stomatal conductance to an increase in atmospheric [CO_2] (Brodribb *et al.*, 2009). This optimization of WUE through short-term stomatal control confers a selective advantage to angiosperms over more ancient evolutionary plant groups in a 'low' [CO_2] world (Robinson, 1994; Brodribb *et al.*, 2009). The ability to sustain higher stomatal conductance rates and stomatal sensitivity in angiosperms may be due to the possession of higher stomatal densities of smaller stomata than gymnosperms and pteridophytes (Hetherington and Woodward, 2003; Franks *et al.*, 2009), permitting the operation of a higher diffusible area of stomatal pore relative to the total leaf area, and greater exploitation of the edge effect (Jones, 1992). This selective advantage of greater stomatal control is also apparent in the 'dumb-bell' stomata of grasses that permit greater and more responsive

changes in stomatal aperture than kidney-shaped stomatal complexes (Hetherington and Woodward, 2003; Franks and Farquhar, 2007). Nonetheless, this greater degree of 'short-term' stomatal control and maximum conductance rates accrues costs in terms of hydraulic constraints (Brodribb *et al.*, 2003) and the operation of effective mechanisms to sense environmental conditions such as light or [CO_2] and signal to individuals or groups of stomata (Heath, 1950; Pospisilova and Santrucek, 1994; Hetherington and Woodward, 2003; Hu *et al.*, 2010).

The evolutionary significance of the costs associated with stomatal control may be observed by comparing the stomatal responses of plant species from volcanic CO_2 degassing vents, that have experienced multigenerational growth at high [CO_2], with individuals of the same species that have not experienced [CO_2] above current ambient ($\sim$380 ppm CO_2) (Fig. 3). In the grass species *Agrostis canina*, this is manifested in higher rates of stomatal conductance in the plants adapted to 'high' [CO_2], but identical photosynthetic rates to individuals adapted to 'lower' [CO_2] when grown at current ambient levels of [CO_2]. The *Agrostis* population adapted to 'low' [CO_2] displays instantaneous WUE ratios $\sim$30% greater than their 'high' [CO_2] adapted counterparts when both are grown under current ambient atmospheric [CO_2]. However, when grown in atmospheres of enriched [CO_2] (1500 ppm), those plants adapted to 'high' [CO_2] maintain stomatal conductance rates and exhibit pronounced rates of assimilation relative to individuals adapted to 'low' [CO_2] that reduce stomatal conductance by $\sim$77% (Haworth *et al.*, 2010*a*). This suggests that differing strategies of stomatal control have developed to suit the prevailing atmospheric conditions experienced by both populations.

The evolutionary cost of stomatal control may also be apparent in the differences observed in the regulation of stomatal aperture and optimization of stomatal numbers between angiosperms and conifers. Many conifer species alter their stomatal frequency over a larger and higher range of [CO_2] values than many angiosperms (Kouwenberg *et al.*,
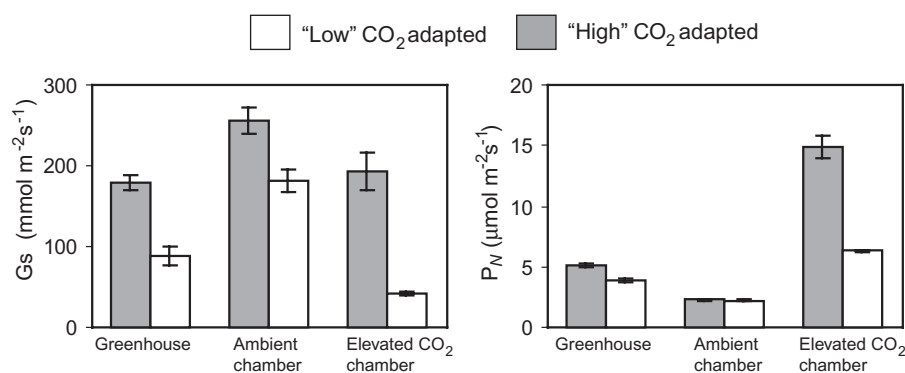

Fig. 3. Stomatal conductance (G_s) and photosynthesis rate (P_N) of individuals of *Agrostis canina* adapted to 'high' and 'low' [CO_2]. Atmospheric [CO_2] levels were $\sim$400 ppm in the greenhouse, 380 ppm in the ambient growth chamber, and 1500 ppm in the elevated [CO_2] treatment. Individuals adapted to 'high' [CO_2] were collected from the volcanic CO_2 degassing vent of Mefite di Ansanto, Italy, where vegetation experiences mean atmospheric [CO_2] values of $\sim$3500 ppm. Error bars indicate the standard error either side of the mean (Haworth *et al.*, 2010*a*).

2003; Haworth *et al.*, 2010*b*). However, angiosperms exhibit greater proportional changes in stomatal number over a narrower [CO_2] range, and often do not alter stomatal initiation to fluctuations above current ambient levels of CO_2 (Woodward, 1987; Kürschner *et al.*, 1997), possibly as a result of their greater exploitation of short-term stomatal control via changes in stomatal aperture (Franks and Farquhar, 2007; Brodribb *et al.*, 2009). An understanding of the role of evolutionary selective pressures in shaping both the strategies and the degree of stomatal control exerted by modern plants would greatly assist predictions of likely future crop, biodiversity, and landscape process responses. The exploitation of controlled environment studies, and technological advances such as infra-red thermography, synchronous leaf gas exchange, chlorophyll fluorescence measurements, and compound-specific carbon and hydrogen isotopic analysis will build upon the significant achievements in stomatal research conducted over the past 60 years.

Acknowledgements

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