The role of stomata in sensing and driving environmental change

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Stomata, the small pores on the surfaces of leaves and stalks, regulate the flow of gases in and out of leaves and thus plants as a whole. They adapt to local and global changes on all timescales from minutes to millennia. Recent data from diverse fields are establishing their central importance to plant physiology, evolution and global ecology. Stomatal morphology, distribution and behaviour respond to a spectrum of signals, from intracellular signalling to global climatic change. Such concerted adaptation results from a web of control systems, reminiscent of a 'scale-free' network, whose untangling requires integrated approaches beyond those currently used.

Stomata (Fig. 1) are small pores on the surfaces of leaves and stems, bounded by a pair of guard cells, that control the exchange of gases—most importantly water vapour and CO_2—between the interior of the leaf and the atmosphere. In this capacity they make major contributions to the ability of the plant to control its water relations and to gain carbon. Gas exchange is regulated by controlling the aperture of the stomatal pore and the number of stomata that form on the epidermis. Environmental signals such as light intensity, the concentration of atmospheric carbon dioxide and endogenous plant hormones control stomatal aperture and development. The acquisition of stomata and an impervious leaf cuticle are considered to be key elements in the evolution of advanced terrestrial plants[1], allowing the plant to inhabit a range of different, often fluctuating environments but still control water content. Here, we describe how the application of knowledge from cognate disciplines is providing new insights into how stomata evolve and are able to process information from simultaneous, often conflicting and sometimes rapidly changing signals. Although it is too early to say whether these recent advances will result in paradigm shifts in our understanding of how plants both respond to and drive environmental change, it is quite clear that stomata are a key experimental tool to investigate these phenomena.

Before considering specific aspects of stomatal biology it is important to reflect on the impact of stomata at the global level. Although the total stomatal pore area may be only 5% of a leaf surface[2], the rate of water vapour loss may reach as high as 70% of a similar structure without a cuticle. Stomata exert major controls on both the water and carbon cycles of the world. Annual precipitation over the land is about 110,000 km^3, or 110×10^{15} kg (ref. 3) and evaporation and transpiration total about 70×10^{15} kg. The contribution of stomatal transpiration alone to the global water cycle can be determined by using a dynamic vegetation model[4] (Fig. 2). The greatest rates of transpiration occur in the uniform and warm forested areas between the tropics with 32×10^{15} kg yr^{-1} of water vapour passing through stomata. This is double the water vapour content of the atmosphere (15×10^{15} kg yr^{-1}). Terrestrial gross photosynthesis annually fixes about 120×10^{15} g C (440×10^{15} g CO_2) from the atmosphere's 730×10^{15} g C (ref. 5). The global distribution of this flux parallels the distribution of transpiration, indicating the closely coupled controls of stomata on CO_2 and water vapour diffusion.

Stomatal evolution

Stomatal control of water loss allows plants to occupy habitats with fluctuating environmental conditions and so it can be predicted that stomata must be important contributors to speciation and evolutionary change. Stomata first appeared in terrestrial land plants over 400 million years ago (Myr)[6] and since then have changed markedly in size and density on plant surfaces. There are two broad morphological types of stomata, the dumb-bell-shaped stomata typical of the grasses and the kidney-shaped form found in other species (Fig. 1).

Is there any evidence that stomata are involved in speciation?

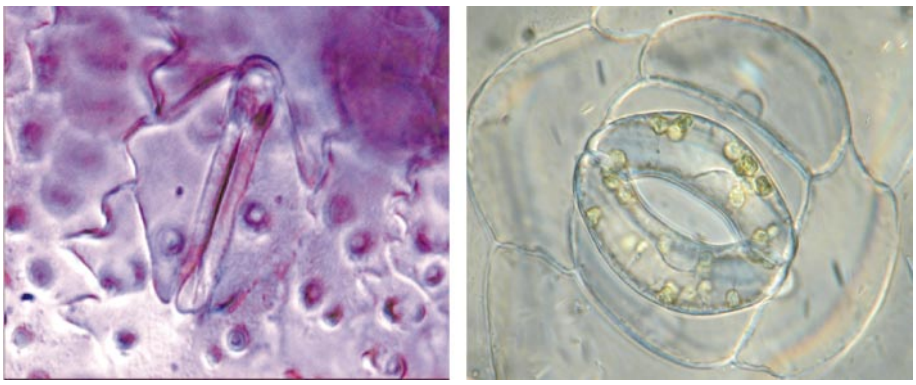

Figure 1 Dumb-bell-shaped stoma of rice typical of the grasses (left) and the kidney-shaped stoma typical of other species such as *Arabidopsis* and *Commelina* (right).

Intriguingly, over the 400 million years of the Phanerozoic era, periods of low atmospheric CO_2 concentrations are associated not only with high stomatal densities[7] but also the emergence of new plant groups such as the ferns, pteridosperms and angiosperms[8]. Whether this involves some interaction and indeed causation requires further investigation. Correlations between changes in global environmental conditions and stomatal evolution can be demonstrated for species in the Proteaceae and for the evolution of dumb-bell stomata in the Poaceae. These changes occurred in the Cenozoic era of the last 66 million years when there were profound changes in global climate and terrestrial flora and fauna[9].

Chloroplast DNA has been used to derive a phylogeny for species of *Banksia* and *Dryandra* in the Proteaceae of Australia[10]. Two clades of species are currently recognized by differences in stomatal distributions. In the clade Cryptostomata, stomata occur in shallow pits or in crypts, whereas they have a more superficial distribution in the clade Phanerostomata. The superficial occurrence appears to be the primitive state and species in the clade Phanerostomata occur in moist climates (such as the most recent common ancestor). Species of the clade Cryptostomata occur in much drier climates and probably diverged from the Phanerostomata clade 55–35 Myr, at a time when the climate was becoming more arid[11]. Although stomatal differences are not the only differences between the clades, the marked differences in stomatal location would have exerted differential capabilities for the spread and survival of the two clades in moist and arid climates[11]. The environmental correlates of the differences in stomatal distribution seen for the Proteaceae are nicely demonstrated in *Cistus incanus*, for which similar differences in stomatal distribution occur, but between the summer and winter of a Mediterranean climate[12]. Leaves produced in the cool and wet winter are large and flat with frequent stomata on the abaxial leaf surface; however, leaves developed in the hot and dry summer are crimped and partially rolled, forming a crypt on the lower surface, the only location of stomata.

The Poaceae consists of about 10,000 species for which the macro-evolutionary history has recently become established by the analysis of chloroplast and nuclear DNA[13]. The linear dumb-bell-shaped stomata of grasses (Fig. 1) are generally believed to represent a more evolutionary advanced form than their kidney-shaped counterparts. This is supported by the observation[14] that during development, Timothy grass guard cells adopt a transient kidney-shaped phase before assuming their typical (mature) dumb-bell shape. The linear dumb-bell design magnifies small changes in width to cause large openings, and maximizes the potential of the stomata to track changes in environmental conditions, probably with little energetic cost. Smaller changes in guard and subsidiary cell turgor lead to greater increases in stomatal aperture[15] in the dumb-bell-shaped stomata than occur for kidney-shaped stomata. This efficiency and speed of stomatal opening in grasses enhances photosynthesis and water use efficiency compared with non-grass species[16]. A rapid stomatal response to blue light augments photosynthesis in early morning and in intermittent sunlight, in which light has an enhanced blue light content[16] and which would have characterized the understorey environment during the early evolution of grasses. The low aerodynamic conductance of a grassland canopy could reduce the impact of changes in stomatal aperture on gas exchange[8]. However, field observations[17] demonstrate a limited impact of aerodynamic conductance on stomatal dynamics.

Grasses originated between about 55 and 70 Myr (ref. 13), leading to lineages that were understorey plants of tropical forests. Their spread and diversification, during global aridification 30–45 Myr, would have been enhanced by the dumb-bell stoma, capable of responding quickly and efficiently to the enhanced light conditions of newly open habitats, but with the capacity to avoid the increased likelihood of drought. This period just preceded the diversification of grazing animals[9,18] and was well before the origin of the C_4 pathway of photosynthesis. Animal grazing and browsing of grazing-intolerant shrubs and trees would have enhanced the spread of grazing-tolerant grasses, particularly into areas of open woodland, whereas the higher albedo of the grasslands may have enhanced regional aridity[18].

Environmental control of stomatal development

We shall not discuss here the details of stomatal development but rather the control of stomatal distribution and size by environmental factors. Depending on the species and the environmental conditions stomata range in size from about 10 to 80 μm in length and occur at densities between 5 and 1,000 mm^{-2} of epidermis[2] (Fig. 3a). In spite of this wide variability there is a strong and general relationship between density and size (Fig. 3a) for different plant

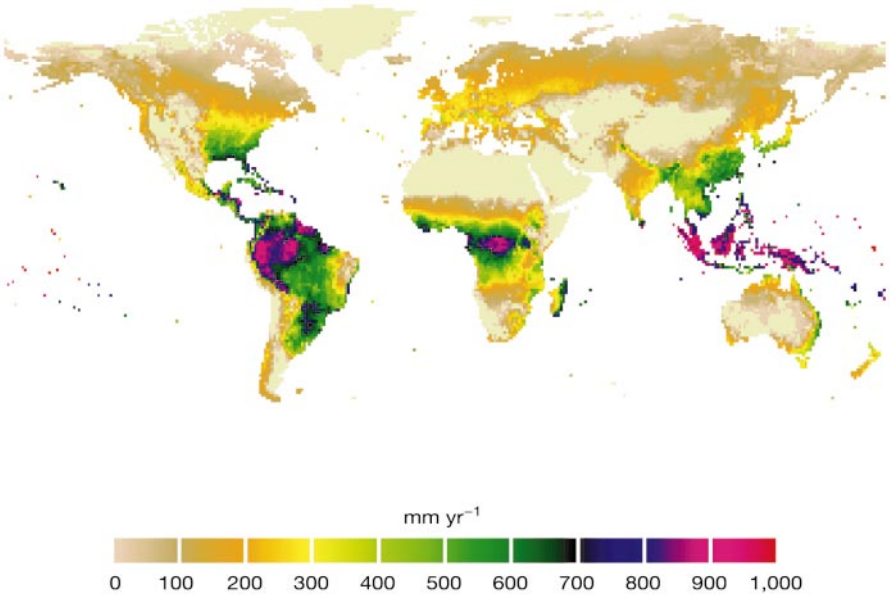

Figure 2 Transpiration (mm yr^{-1}) from terrestrial vegetation simulated with the Sheffield Dynamic Global Vegetation Model (SDGVM)[4] and averaged for the 1990s.

groups (grasses and non-grasses), fossil leaves and for different stomatal distributions on either one or both leaf surfaces. Simulations with a stomatal model of gaseous exchange[7] indicate that the average relationship in Fig. 3a has virtually the same trend to one in which changes in density and size are exactly compensatory, in terms of CO_2 and water vapour exchange. Apart from the earliest land plants of the Silurian and Devonian periods, with very low stomatal densities and small sizes, it also seems that this relationship has existed for the last 300 million years. On this basis it is not obvious how selection favours any particular species' characteristic of stomatal density and size.

The plant must maintain movement of water from the soil to the leaf, and rapid stomatal responses to environmental change are a major feature of this maintenance[1]. One study[19] has demonstrated that stomatal size has a key role in this control and for six forest trees there is a clear negative relationship between the length of the stomatal pore and sensitivity to increasing drought. In these species larger stomata (species with kidney-shaped stomata) were slower to close and demonstrated a greater potential for hydraulic dysfunction under drought. Ferns from deep shade possess large stomata at low densities[20] and in this natural environment, which may be cool and humid, it is found that truly shade-tolerant species often retain open stomata, even in deep shade, at least for early parts of the day[21]. The constancy of the open stomata will minimize the impact of what would otherwise be slow opening limitations to photosynthesis during short-lived periods of sunlight, which are critical for enhancing photosynthesis in this light-limited environment. Therefore the limited available information suggests that large kidney-shaped stomata seem to be an important feature for plants of humid and deep shade conditions but their slow dynamic behaviour could lead to problems under dry conditions. Small stomata can open and close more rapidly and their general association with high densities (Fig. 3a) provides the capacity for rapid increases in the stomatal conductance of a leaf, maximizing CO_2 diffusion into the leaf during favourable conditions for photosynthesis[19].

The effect of growth at elevated concentrations of CO_2 on stomatal density and stomatal index (the fraction of epidermal cells that are stomata) is one of the most intensively studied environmental controls on stomatal development. CO_2 enrichment changes the stomatal density of different species and different accessions (ecotypes) of *Arabidopsis thaliana* (Fig. 3b and ref. 22). With stomatal densities ranging from 45 to 720 mm^{-2} the mean response is an 11% reduction in density with a doubling of the CO_2 concentration (Fig. 3b), and which is insensitive to the basal

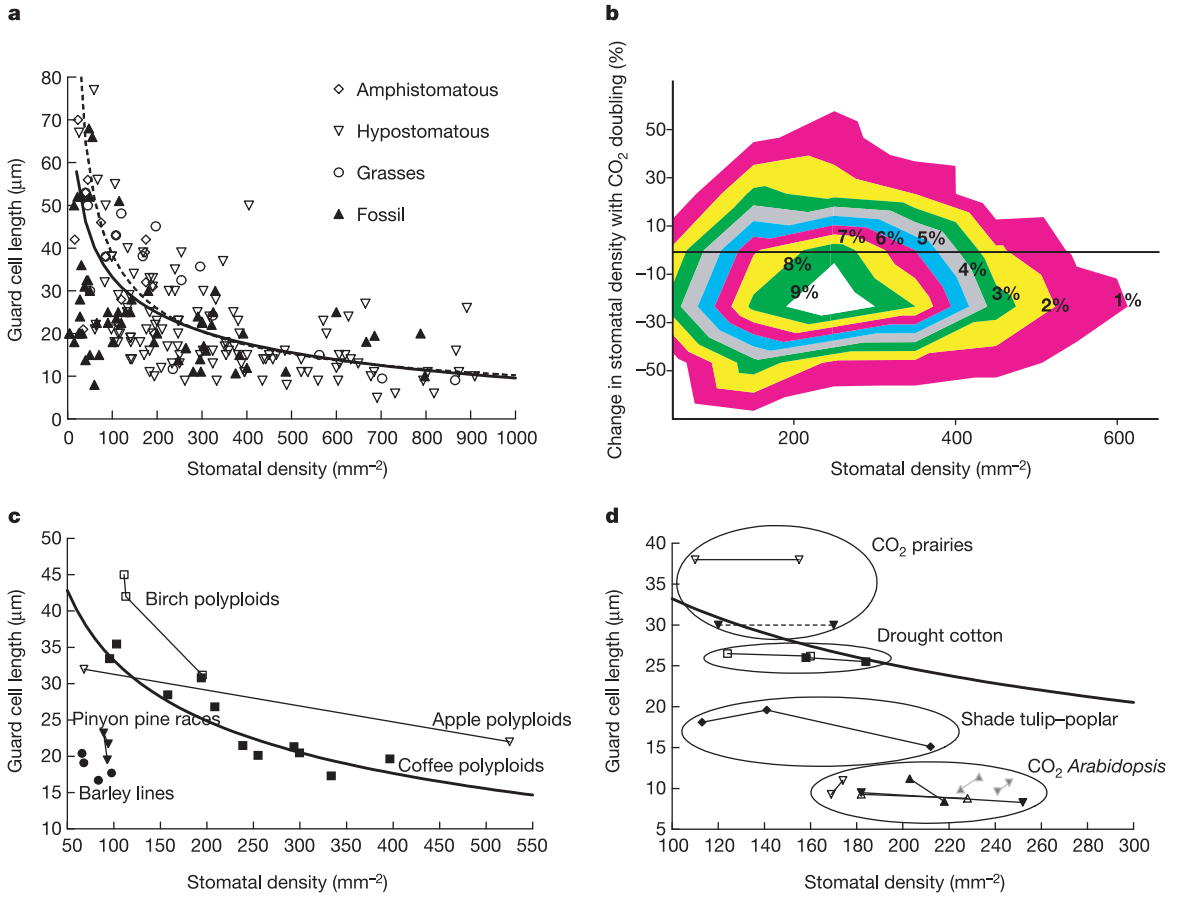

Figure 3 Control of stomata by the environment. **a**, Relationship between stomatal width and density. Data from amphistomatous species, hypostomatous species, grasses and fossil leaves are shown. The data are from refs 2, 7, 67–69 and F.I.W. (personal observations). The solid curve is log-normal, $y = -28.75 + 162x^{-0.2086}$, $r^2 = 0.5$; the dashed line shows equal stomatal conductance with variable stomatal density. **b**, Responses (per cent change) of stomatal density to CO_2 doubling for 125 species and 63 accessions of *A. thaliana* (from ref. 22 and new observations). Smoothed contours indicate fraction of genotypes (%) with particular values of stomatal density and per cent response to enrichment. **c**, Variations in stomatal characteristics with different genotypes. Curve as for **a**. Birch polyploids (2*n*, 5*n*, 6*n*)[70], apple polyploids (3*n*, 4*n*)[71], different races of pinyon pine[72], drought-selected lines in barley[73] and different polyploids in coffee[74] are shown. **d**, Stomatal responses to different environmental conditions. Curves as for **a**. CO_2 enrichment experiments, prairies[75]; *Arabidopsis* (our own unpublished data) where adaxial surface (filled triangles) and abaxial surface (inverted filled triangles) are indicted; tulip–poplar changes through a canopy[76]; and drought responses of cotton[77] are shown. Different ecotypes are represented by different colours within groups.

stomatal density. The reduction in stomatal density with CO_2 enrichment leads generally to a decrease in maximum stomatal conductance but an increase in the maximum rate of photosynthesis, at the elevated CO_2 concentration[7,8,22].

Royer[23] established that the reductions of stomatal density and stomatal index associated with growth under increased concentrations of atmospheric CO_2 were recorded more frequently in observations from longer-term measurements of stomatal responses (herbarium material with a decade to century timescale and from fossil leaves with a millennial timescale) than in short-term field or growth chamber seasonal experiments. Royer suggested that this increased frequency of response was due to the transition from a variable plastic response by species in short-term experiments to a genetic response, as a result of selection on longer timescales. Figure 3c supports the suggestion that genetic change can alter the stomatal density–size relationship (Fig. 3a), whereas environmental changes seem primarily to move the position of a species along the stomatal size–density curve (Fig. 3d). Changes in the degree of ploidy can also change stomatal density and size (Fig. 3c). Notably, in *Arabidopsis*, an unresponsive accession (C24) for the stomatal density response to CO_2 enrichment is actually regulated to show this minimum response because the mutation of a single gene leads to a very significant response to CO_2 (ref. 24).

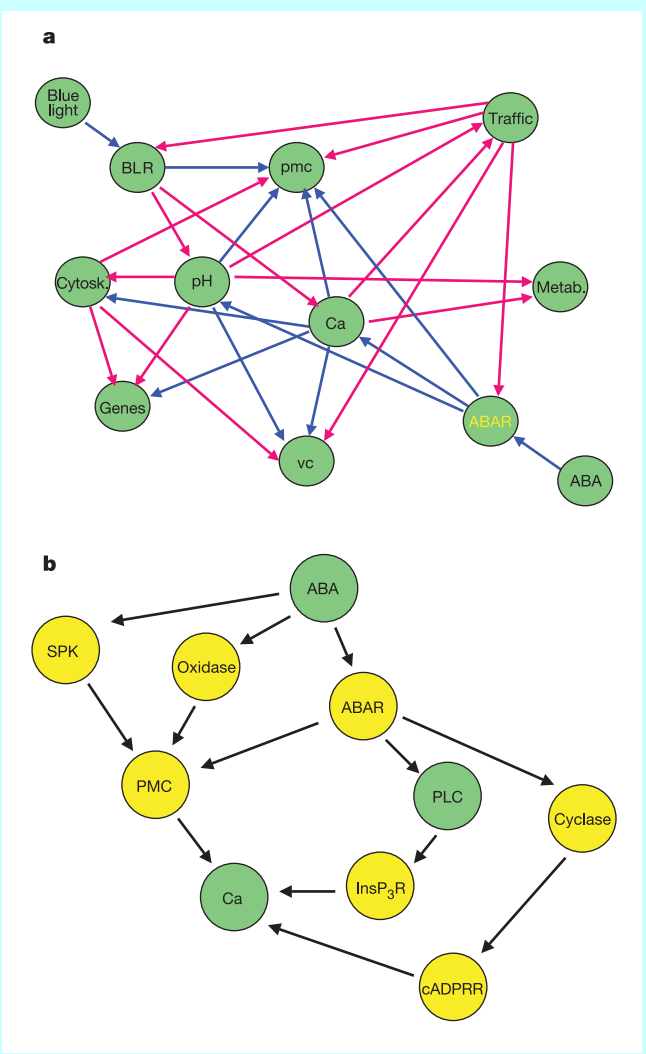

Figure 4 Model of guard cell signalling. **a**, Guard cell ABA and blue light signalling represented by a network-based model. In this model the nodes represent modules or groups of functionally related processes or second messengers. Modules linked by blue connections indicate that these links exist in guard cells. Red connections indicate that these links occur in other plant cells but their existence has not been investigated in guard cells. Yellow font indicates that an ABA receptor has not been isolated in guard cells. BLR, blue light receptor; pmc, plasma membrane ion channels; traffic, membrane trafficking; cytosk., cytoskeleton; vc, vacuolar ion channels; metab., metabolism of starch, sucrose, malate; ABAR, ABA receptor. Data are from refs 32, 33, 42. **b**, Guard cell signalling is robust. Multiple routes for generating increases in cytosolic Ca^{2+} concentration. Intracellular messengers ($InsP_3$, cADPR) and local messengers (sphingosine-1-phosphate, H_2O_2) are not shown. Components shaded in green have been isolated and characterized in guard cells. Other components (yellow) are predicted to exist on the basis of physiological or pharmacological evidence. For simplicity, possible feedback links are not shown. SPK, sphingosine kinase; oxidase, NADPH oxidase activity; PMC, plasma membrane calcium-permeable channels; ABAR, ABA receptor; PLC, phospholipase C; cyclase, ADP ribosyl cyclase; cADPRR, cyclic ADP ribose receptor; $InsP_3R$, $InsP_3$ receptor. Data are from refs 32, 33.

Currently we know rather little about the signalling pathways by which environmental signals control stomatal development. Recent work has shown that in *Arabidopsis* the *HIC* gene, which encodes a putative 3-ketoacyl CoA synthase (KCS), is involved in the control of stomatal development by elevated concentrations of CO_2 (ref. 24). As KCS is involved in the synthesis of wax components found in the cuticle it has been suggested that in *hic* an alteration to cuticular structure and properties interferes with the diffusion of an endogenous inhibitor that controls stomatal development[24]. This suggestion receives support from the observation that some *Arabidopsis* wax-deficient mutants display abnormal stomatal patterning[24,25]. Analyses of other *Arabidopsis* mutants[26] indicate that the CO_2 response of stomatal index is absent in *fad-4*, a jasmonic acid mutant, whereas in *ein-2*, an ethylene-insensitive mutant, the CO_2 response is absent only from the adaxial leaf surface. The jasmonate and ethylene transduction pathways are also involved in defence responses to pathogens[27] and the *ein-2* mutant is susceptible to attack by pathogens. It is remarkable that mechanisms for addressing pathogen attack are also central in the responses of stomatal development to CO_2 concentration. Accessions of *Arabidopsis* differ widely in resistance both to powdery mildew disease[28] and to CO_2 concentration[22], and it is tempting to link these two major responses, but experimental support is still wanting. Any connection between the two processes would influence strongly the processes of selection for the stomatal response to CO_2, in the long term, supporting the notion that the response is more reliable the longer the period of study[23]. More recent work[29] shows that, similar to responses to pathogen attack[30] there is also systemic control of stomatal development during growth under elevated CO_2 as mature leaves both detect CO_2 and produce a signal to influence development of younger expanding leaves.

The work on the *ein-2* mutant of *Arabidopsis*[26] suggests that ethylene differentially controls stomatal development on the upper and lower leaf surfaces. Comparing stomatal densities among *Solanum pennellii*, *Lycoperscicon esculentum* and a graft-induced periclinal chimaera, having an *S. pennellii* epidermis and an *L. esculentum* mesophyll, showed[31] that the two surfaces could behave independently. Both donors had more stomata on the lower than on the upper surface and *L. esculentum* had a greater stomatal density than *S. pennellii*. The stomatal density on the upper epidermis of the chimaera was similar to that of the epidermal donor *S. pennellii*. However, the density on the chimaeral abaxial epidermis was significantly lower than its donor. These data suggest that stomatal differentiation is subject to different controls on each surface, and at least in the case of the lower epidermis a role for the mesophyll seems possible.

Control of stomatal aperture by environmental signals

Recent work shows that the control of stomatal aperture by environmental signals depends on coordinated alterations to guard cell turgor (ionic fluxes and sugars), cytoskeleton organization, membrane transport and gene expression (see refs 32 and 33 and references therein). A number of lines of evidence suggest that

stand-alone, stimulus-specific signalling pathways might be an inadequate means of controlling stomatal aperture. First, and most importantly, the recent recognition that the control of stomatal aperture requires the coordinated control of multiple cellular processes must at the very least require repeated pathway bifurcation. Factoring in the requirement for feedback and temporal coordination of these different processes indicates that the architecture must become increasingly reticulate. In addition the presence of intracellular signalling components that feature in multiple signal pathways suggests that the existence of truly stand-alone pathways are highly unlikely. A good example of this phenomenon[34] is cytosolic free calcium ($[Ca^{2+}]_{cyt}$), others are the *Arabidopsis ABI1* and *ABI2* genes that encode type PP2C serine threonine phosphatases[32]. These phosphatases are involved in guard cell abscisic acid (ABA)[35], $[CO_2]$[36–38] and (lack of light) darkness[38] signalling. It is difficult to imagine how a signalling component could be isolated to the extent that it only affected a single pathway. The presence of common signalling components argues strongly for interaction between pathways. Although these potential problems do not rule out the possibility that guard cells contain stand-alone signalling pathways, they do provide the justification for examining whether there are alternative organizational structures. Is there any evidence that guard cell signalling is organized as a network and specifically as a type of network known as a scale-free network?

Guard cell signalling: an example of a scale-free network?

Scale-free networks are robust and flexible and are ideally suited for discriminating and processing multiple signals simultaneously. These properties arise from the network itself rather than from individual components[39–41]. Scale-free networks are composed of many interconnected nodes. In the context of this review, nodes equate to signalling components. The network contains a small number of highly connected nodes, known as hubs, which are central to the functioning of the scale-free network. Scale-free networks are characterized by a power law distribution of nodes (many sparsely and few highly connected nodes) and exhibit emergent properties of robustness against errors but fragility against the removal or damage to the hubs[39]. Defining whether a network is scale-free requires a large network map of at least 1,000 nodes, which is analysed for a power law distribution. Unfortunately, this analysis is not possible in guard cells because the current guard cell signalling map is both too small and incomplete. However, it is possible to arrange the elements in guard cell signalling as a network (Fig. 4a). It is also possible to ask whether guard cell signalling displays properties similar to the emergent properties of scale-free networks. Even though, as we shall see, guard cell signalling does exhibit properties reminiscent of scale-free networks, it must be stressed that to define the guard cell signalling system as scale-free with any rigour requires that the distribution of nodes must be measured and described by a power law.

Guard cell signalling is robust

Scale-free networks are robust, which means that they exhibit a high degree of tolerance to node removal. There are data showing that guard cells continue to function despite the loss of certain signalling components. For example *Arabidopsis* plants carrying mutations in either of the blue light receptor genes *PHOT1* or *PHOT2* exhibit wild-type blue-light-induced stomatal opening. Only when both receptor genes in the *phot1*, *phot2* double mutant are disrupted do the stomata fail to respond to blue light[42]. A second example of robust behaviour comes from ABA signalling. ABA can generate increases in $[Ca^{2+}]_{cyt}$ using plasma membrane calcium-permeable channels, the PI-PLC/inositol-1,4,5-trisphosphate ($InsP_3$) pathway, cyclic ADPribose, sphingosine-1-phosphate and possibly inositol hexakisphosphate (reviewed in ref. 40; see also Fig. 4b). Recent studies using tobacco, in which the levels of PI-PLC protein have been reduced in guard cells, reveal a very modest effect of interfering with this signalling system on the ability of stomata to respond to ABA[43]. It is possible to conclude from these investigations that when PI-PLC levels are reduced, the guard cell is able to compensate for this loss by making use of other calcium mobilizing systems or other PI-PLCs. These are two examples in which guard cells exhibit behaviour reminiscent of the robust properties of a scale-free network.

What advantage might guard cell robustness confer on the plant? One possibility is that it will protect, to some extent, the vital process of carbon acquisition and the regulation of water loss from the (possibly) deleterious effects of mutation in genes encoding guard cell signalling components. In addition the presence of multiple copies of components or mechanisms for generating a similar outcome provides the raw material on which evolution could operate.

Guard cell signalling is fragile to hub removal

Fragility to hub removal is a well-characterized property of scale-free networks[39]. Does guard cell signalling exhibit similar behaviour? In the signalling context a hub can have a central role in coupling stimulus responses. On the basis of our current knowledge of guard cell signalling perhaps the best candidate for a hub is the increase in the concentration of guard cell $[Ca^{2+}]_{cyt}$ (Fig. 4b) that occurs in ABA, extracellular calcium ion, hydrogen peroxide, CO_2 and IAA signalling[32,33]. As mentioned above, there are multiple mechanisms for generating an increase in guard cell $[Ca^{2+}]_{cyt}$. Interfering with one of these results in very small phenotypic effects, which is reminiscent of the robust properties of scale-free networks. However, when the increase in $[Ca^{2+}]_{cyt}$ is totally prevented by microinjection of the calcium chelator BAPTA into the guard cell cytosol there is no ABA-mediated loss of turgor[44]. This is an example where interfering with the activity of a hub results in the failure of the system. Taken together, these results show that guard cell signalling exhibits similar properties to scale-free networks, however, as pointed out above, testing whether this is an accurate description of the architecture of the guard cell signalling system requires much more data and a rigorous mathematical analysis.

Acclimation and rhythmic behaviour in guard cells

If guard cell signalling is organized as a network then a striking property of the network is that it acclimates to external signals. We are not aware whether acclimation is an emergent property of scale-free networks. Stomata of *Vicia faba* grown in a growth chamber are markedly more sensitive to CO_2 than stomata from plants grown in a greenhouse[45]. These alterations in CO_2 sensitivity were also observed for stomata present in isolated epidermal preparations, suggesting that guard cell behaviour, rather than the properties of the leaf as a whole, had been modified.

The response of stomatal conductance to CO_2 doubling is highly variable but observations on trees, lasting up to 4 yr (ref. 46), demonstrated an average reduction of 21%. Some of the response may also include changes in stomatal density[47], although this was not measured directly.

Although we do not yet know the cellular basis of acclimation in guard cells the issue does have a bearing on the organization of guard cell signalling components. If CO_2 signalling were a stand-alone pathway we would predict that acclimation would have no effect on the response of guard cells to other signals. However, if the effects of CO_2 on stomatal aperture are brought about through a signalling network then alterations in sensitivity to this signal should have effects on other pathways. Is this the case? When *Arabidopsis* is grown under ambient concentrations of CO_2 and then exposed to elevated CO_2, stomatal conductance decreases[48]. This was transient and conductance returned to pre-treatment levels quickly, suggesting that stomata are able to acclimate rapidly to elevated CO_2. Plants were then grown at ambient and elevated CO_2 for two days and stomatal conductance measured after exposure to

osmotic stress or treating them with ABA. Enhanced responses to both treatments were observed in the plants grown at elevated CO_2 relative to their ambient controls[48]. These data suggest that acclimation to one signal leads to alterations in sensitivity to another signal and are consistent with a network-based organization. However, it should be noted that effects were not obvious, at least in the case of ABA applied to isolated epidermal strips.

Stomata are influenced by rhythms. These either control stomatal aperture directly or modulate the response of stomata to other signals. The most thoroughly investigated of these are the circadian rhythms that in C_3 and C_4 plants result in stomatal opening during the day and closure at night[49]. The circadian clock can also modulate the sensitivity of stomata to exogenous signals such as light[50]. Studies using *V. faba* revealed that stomatal opening at the start of the day was primarily associated with K^+ accumulation whereas later in the day sucrose was the dominant solute. Differential circadian regulation of the components responsible for the accumulation or loss of these solutes might underlie this phenomenon. There is also evidence, which would be worthy of re-investigation using contemporary controlled growth facilities, of an annual rhythm affecting stomatal apertures[51]. This annual variation in aperture is mirrored to some extent by the physiological properties of guard cells, where there is seasonal variation in membrane potential[52] and plasma membrane (H^+)ATPase activity[53].

Autonomous units and collective behaviour

Early in development guard cells lose plasmodesmatal connections with each other and their neighbours[54]. This symplastic isolation explains why injecting a single guard cell from a stoma with cyclic ADP ribose causes reductions in turgor in the injected cell while the uninjected partner remains unchanged[55]. Although symplastic isolation makes it possible for an individual stoma to behave as an autonomous unit, is such behaviour likely to have physiological significance? A stoma is unlikely on its own to have a significant impact on leaf processes. However, groups of stomata may exhibit localized coordinated behaviour and this can benefit the plant. Indeed, this is what probably underlies the phenomenon of patchiness in which some areas of the leaf display relatively open stomata while other parts of the same leaf may exhibit reduced apertures[56]. The benefits of localized semi-autonomous behaviour are most readily seen in plants with relatively large leaves that inhabit habitats characterized by localized unpredictable variations in environmental signals. Perhaps the best-investigated example is the response of leaves in the forest understorey to sunlight penetrating through the canopy and illuminating areas of the leaf in a highly localized manner. The adaptive significance here is fairly obvious. Opening of the stomata in the illuminated region of the leaf, while maintaining the remaining stomata in a relatively closed state in the dim light of the understorey would allow the plant to exploit the available light efficiently without losing unnecessary amounts of water[21]. There will also be attendant energy savings to opening in restricted sectors of the leaf. In this context semi-autonomous behaviour can be seen to support the opportunistic capture of resources. However, it should be pointed out that localized collective behaviour has recently been interpreted as evidence for non-autonomous behaviour of stomata[57]. Of course in other situations where the plant has small leaves or the leaf experiences homogeneous exposure to the dominant environmental variable the result will be uniformity of stomatal aperture. However, even emergent collective behaviour is still the result of signals being perceived by and operating on individual stomata.

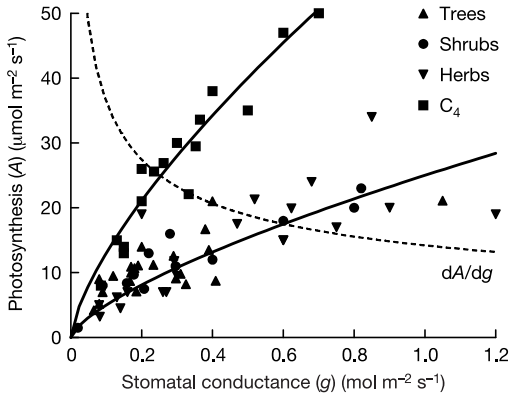

Figure 5 Field observations of maximum photosynthesis (*A*) and stomatal conductance to water vapour (*g*). Data are from refs 78–100. The dotted line is the differential of the line of best fit between *A* and *g* for the C_3 species, and indicates d*A*/d*g* (μmol mol^{-1}).

Stomatal impact on photosynthesis and transpiration

The diffusion of CO_2 into the leaf during photosynthesis and the outward diffusion of water vapour are both controlled by the opening and closing of stomata, although the diffusion rate of water vapour is 1.6 times greater than CO_2. There is also another measure of stomatal control, the relationship between the maximum conductance of stomata (determined by the opening and density of stomata) and the maximum rate of photosynthesis measured under optimal conditions (Fig. 5). Short-term field measurements demonstrate a wide range of rates of photosynthesis (*A*) and stomatal conductance (*g*). However, leaves of different species and life forms fall, with some scatter, on either of two curvilinear relationships. Plants with the C_4 pathway of photosynthesis show greater rates of photosynthesis than those with the C_3 pathway. There is also a close to constant ratio between the substomatal or intercellular CO_2 concentration and the CO_2 concentration in the ambient air. The ratio falls between about 0.65 and 0.8 for C_3 species and between 0.4 and 0.6 for C_4 species. This indicates a close matching of the diffusional supply of CO_2 to the chloroplasts of the leaf and the demand from the photosynthetic enzymes, a central feature of the photosynthetic process[58]. The mechanism that maintains this close relationship is still unclear, and reflects the fact that unlike photosynthesis, a fully mechanistic model of stomatal activity does not exist[59].

The slope of the relationship between *A* and *g* (d*A*/d*g*) (Fig. 5) is shown for a C_3 species and is a measure of water use efficiency (the rate of carbon gain per unit increase in stomatal conductance). The greatest efficiency occurs at low stomatal conductances but flattens quickly at conductances above about 0.4 mol m^{-2} s^{-1}. The relationship between transpiration, *E*, and conductance (d*E*/d*g*) has the same form as d*A*/d*g*, whereas the response of leaf temperature, *T* (d*T*/d*g*) is inversely related to d*E*/d*g*. This means that stomata can exert significant control over both leaf temperature and gaseous diffusion. Small changes in *g* exert the greatest impacts on leaf temperature when *g* is small, with a flattening of response above 0.4 mol m^{-2} s^{-1}. Plants with higher stomatal conductances show minimal gain of photosynthesis and minimal changes in leaf temperature for large changes in conductance, reflecting high water usage and little limitation on water supply. By contrast plants with low values of *g* may occur in water-limited environments, where small reductions in stomatal conductance significantly increase water use efficiency. The continued increase in atmospheric CO_2 concentration will enhance photosynthesis, at least in C_3 species, and decrease stomatal conductance[46] and so plants will become more efficient at water use and may use less water[60]. However, species differ significantly in these CO_2 responses, with some species being more favoured than others, indicating that differing species-specific responses of stomatal conductance and photosynthesis to CO_2 have the capacity to change community composition[61].

Field experiments have demonstrated that differences in water supply can select for a wider range of plant responses than suggested from Fig. 5. About half of the plants with stomatal conductances less than 0.4 mol $m^{-2} s^{-1}$ occur in dry conditions, whereas about 40% of those with conductances greater than 0.4 mol $m^{-2} s^{-1}$ are also found in dry habitats. Plants with high *g*, in dry conditions, demonstrate opportunistic and rapid growth during short periods of water availability, in which high conductances and photosynthetic capacity will maximize growth. This contrasts with other plants from dry conditions with less active periods of growth, and low values of *g*, but with a more extended period of activity.

In cotton and wheat, yields are positively correlated with maximum stomatal conductance, but not with photosynthesis[62]. In this case high stomatal conductances lead to significant evaporative cooling of irrigated crops, to temperatures below the threshold for yield reduction. Experiments on *Cakile edentula* growing in cooler conditions demonstrated that fitness in a dry environment was greatest for individuals with the highest water use efficiency[63], indicating selection at the leaf level on the balance of carbon uptake and water loss. In the dry environment stomatal conductance was negatively correlated with fitness, whereas water use efficiency was not selected in a wet environment. An example of differential selection in nature for stomatal behaviour with water supply has been demonstrated for hermaphrodite and dioecious populations of the species *Wurmbea dioica*[64]. These populations occur sympatrically, but the hermaphrodite populations occur in wetter microsites. Even though they are found at wetter sites, the hermaphrodite populations adjust stomatal conductance to minimize water loss, which allows greater photosynthetic allocation to leaves and reproductive structures. The dioecious populations, from the drier sites, maximize CO_2 uptake and water loss, with maximal stomatal conductances, in parallel with increased allocation to storage organs.

Future prospects

In this review we have advanced the hypothesis that guard cell signalling is organized as a scale-free network. Before we can rigorously test whether this is an accurate description we need to identify more components that contribute to guard cell signalling. An increase in the application of forward and reverse genetic strategies to guard cell function and development combined with protein–protein interaction studies are likely to produce the data sets required to test whether the power law distribution applies to guard cell signalling components. It is worth pointing out that if a network or more specifically, as we suggest here, a scale-free network does reflect the organization of the signalling components in the guard cell rather than a collection of linear pathways, we shall need to re-evaluate how we interpret the 'positioning' of signalling components relative to one another using epistasis analysis. Avery and Wasserman[65] identified a set of conditions that need to be satisfied before it is possible to draw meaningful conclusions from epistasis analyses. One of these is that "the signal and two genes under study are the sole determinants of the phenotype under the conditions of the experiment". Scale-free networks with their inherent redundancy and robustness would seem to contravene this rule. Similarly, the application of this rule would make the interpretation of data gained by using stomatal aperture as the phenotype problematic, as this is clearly controlled by multiple signals acting simultaneously. Accordingly, until we know more about the architecture of the guard cell signalling system, we suggest that the positioning of components relative to one another using epistasis analysis is treated with some caution. Finally, in the context of signalling it would be interesting to determine whether defined scale-free networks exhibit properties such as acclimation that are typical of guard cell signalling.

We also need to know much more about the underlying cell biology of the dumb-bell-shaped stomata of the grasses. The evolution of the dumb-bell stomata may have been a major component not only in the geographical spread of grasses but also in the coevolution of grazing animals and regional climate. Geological evidence indicates a three-way interaction between the evolution of grazing tolerance in grasses, the rise of grazing ungulates and the decline in browsers since the end of the Eocene epoch, 38 million years ago[18]. The end of the Eocene epoch was characterized by a drier climate, which would have favoured the spread of drought-tolerant grasses into vegetation that had been dominated by trees and shrubs[66]. In this respect the evolution of the efficient dumb-bell stomata of grasses and their impact on the ability of the grasses to dominate new habitats can be seen as a prerequisite for the evolution and subsequent domestication of grazing animals by man. Given the importance of grasses ecologically and in the evolutionary context and also as commercial crops we need to understand much more about their development and function. In the past efforts to investigate these subjects have been impeded by the tough lignified guard cell wall that resists protoplasting and impedes microinjection. With the completion of the rice genome it seems likely that we are set to gain many insights into the mysteries of grass stomatal development and evolution. □

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