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# **Stomatal heterogeneity**

## Keith A. Mott<sup>1</sup> and Thomas N. Buckley

Biology Department, Utah State University, Logan, UT 84322-5305, USA

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## Abstract

Stomatal heterogeneity that is systematic at large scales and random at small scales is observed under most conditions. The reverse situation, distinguished by the co-ordination of stomatal behaviour at small scales and its disco-ordination at large scales, is known as 'patchy stomatal conductance'; it appears to be less common and has yet to be explained mechanistically. This phenomenon is reviewed in the context of possible mechanisms, and a variety of potential mechanisms are briefly described. Hydraulic interactions among stomata are proposed as a mechanism that may explain many aspects of patchiness and are discussed in detail in the context of recent modelling and experimental work in this area.

Key words: Stomata, patchy, conductance, heterogeneity.

#### Introduction

Heterogeneity among stomata on a single leaf exists at several spatial and temporal scales and has many facets. Several stomatal characters show apparent random variability over the leaf surface. At the smallest scale, these include the size of each individual guard cell pair and the steady-state pore aperture under a particular environment condition (for reviews see Weyers and Lawson, 1997; Spence, 1987). At larger scales, variability is seen in both stomatal density and average stomatal conductance among different regions of the leaf (Weyers and Lawson, 1997; Pospíšilová and Šantrůček, 1994). These aspects of stomatal heterogeneity have been apparent since the first microscopic examinations of stomata. More recently, a special case of stomatal heterogeneity has been reported in which stomata in small but distinct regions of a leaf are mostly closed while stomata in adjacent regions are mostly open (Terashima et al., 1988; Downton et al., 1988). In these studies the phenomenon, called 'patchy

stomatal conductance', was induced by applying ABA through the transpiration stream and was detected using starch accumulation and direct visual observation of stomatal apertures. Subsequent studies have found patchy stomatal conductance in response to many perturbations and have used various techniques to detect it (Beyschlag and Eckstein, 1998; Pospíšilová and Šantrůček, 1994; Terashima, 1992).

Patchy stomatal conductance is interesting for at least two reasons. Firstly, it can affect the calculation of intercellular CO<sub>2</sub> concentration and therefore has important implications for the interpretation of gas-exchange data. Secondly, it suggests the existence of previously unsuspected mechanisms, or unrecognized aspects of known mechanisms, for stomatal functioning. It is this second aspect of patchy stomatal conductance that provides the focus for this article. Patchy stomatal conductance has been the subject of several excellent reviews within the last few years (Beyschlag and Eckstein, 1998; Weyers and Lawson, 1997; Pospíšilová and Šantrůček, 1994, 1997; Terashima, 1992), and the reader is referred to these for more information on the subject. This article will address primarily the mechanism for patchy stomatal conductance, which has not been addressed to any substantial degree in the published literature and has, therefore, received less attention in previous reviews. The approach of this discussion will be first to review the phenomenology of patchy stomatal conductance in the context of possible mechanisms, then briefly to summarize general possibilities for mechanisms and, finally, to consider recent evidence concerning the role of hydraulic mechanisms.

### What is patchy stomatal conductance?

In the light of the different kinds of heterogeneity in stomatal apertures discussed above, the term 'patchy' clearly does not apply to all cases of heterogeneity. It is

<sup>1</sup> To whom correspondence should be addressed. Fax: +1 801 797 1575. E-mail: biomail@biology.usu.edu

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tempting to define the term based on scale, and conclude that any variation occurring at a scale larger than an areole qualifies as patchiness. However, this definition fails because stomatal size and density as well as xylem water potential and, therefore, the overall water status of adjoining tissues may vary among regions of the leaf (Yang and Tyree, 1996). The broad gradients in aperture caused by this type of variation are generally not considered as 'patchy' stomatal conductance, although they may cause errors in the calculation of  $c_i$  similar to those caused by patchy stomatal conductance if gas-exchange measurements are performed on a sufficiently large leaf area.

Patchiness could also be defined by requiring that the stomata within each patch behave more like one another than like the stomata in an adjacent patch. However, there are apparently no published data addressing the behaviour of individual stomata within a patch. Terashima et al. (1988) measured apertures on leaves with patchy stomatal conductance, but in that study it was only stated that areas with no starch accumulation had few open stomata and vice versa. There are numerous studies that show that stomata within a small area tend to behave similarly (Saxe, 1979; Kappen et al., 1987), and the data of Laisk et al. (1980) also suggest a certain level of local uniformity in stomatal apertures. However, none of these studies employed leaves that were known to be exhibiting patchy stomatal conductance, and since it seems possible that mechanisms causing heterogeneity at larger scales could increase variability at small scales, these data cannot be used with certainty to address heterogeneity of apertures within a patch.

The question of stomatal behaviour within a patch is further complicated by the fact that all of the methods used thus far for the detection of patchy stomatal closure probably average stomatal conductance over an area corresponding to many stomata and perhaps to a single areole for heterobaric leaves (leaves with bundle sheath extensions that block gas diffusion between adjacent areoles). This is true for starch accumulation and chlorophyll fluorescence imaging partly because the resolution of the techniques as applied thus far is not high enough and partly because lateral diffusion of CO<sub>2</sub> within the leaf will tend to average  $c_i$  for a given areole. It is true for vacuum infiltration of leaves because the resistance of an areole to infiltration will be determined by the most open stomata in that areole, that is, if one or two stomata are fairly wide open while all others in the areole are mostly closed, the entire areole will infiltrate. Thus, until data for the behaviour of neighbouring individual stomata on a leaf showing patchy stomatal closure are available, hypothetical mechanisms need not explain co-ordinated behaviour of all stomata within an areole, only enough stomata to influence the average conductance for the patch.

To address the problems raised above, patchiness may be defined as 'the appearance of co-ordinated behaviour below a threshold spatial scale, coupled with nonsystematic heterogeneity at larger scales.' This excludes random variability, which should be independent of spatial scale, and it allows for systematic gradients within areoles or across entire leaves. This definition also leads immediately to a more well-defined goal in the search for mechanisms of patchy stomatal conductance. Such mechanisms must cause the average stomatal behaviour of different patches to diverge, while allowing for co-ordinated stomatal behaviour within each patch.

## Prevalence of patchy stomatal conductance

The prevalence of patchy stomatal conductance is difficult to determine with accuracy, primarily because of the technical difficulty in detecting the phenomenon. The number of treatments that can cause patchy stomatal conductance has increased steadily since patchiness was first described in response to ABA (Terashima et al., 1988; Downton et al., 1988). Known effectors of stomatal patchiness now include water stress (Sharkey and Seemann, 1989; Gunasekera and Berkowitz, 1992), low humidity (Mott et al., 1993; Beyschlag et al., 1992), a sudden change from high to low light (Cardon et al., 1994; Eckstein et al., 1996), and a sudden change from darkness to high light (Bro et al., 1996). Several studies have shown that plants in natural environments. repeatably undergo patchy stomatal closure during specific periods of the day (Beyschlag and Pfanz, 1990; During and Loveys, 1996), and patchiness has been observed in a large number of species and families (Eckstein, 1997). All of the above suggests that patchy stomatal conductance could be fairly common.

Because heterogeneous stomatal conductance can cause overestimation of the average intercellular  $CO_2$  partial pressure  $(c_i)$  in leaf gas-exchange measurements, stomatal patchiness should be readily detectable as a transient or unexpected deviation from the steady-state relationship between CO<sub>2</sub> assimilation (A) and  $c_i$  (see Pospíšilová and Santrůček, 1997, for a review). However, this issue has been muddled by conflicting results. While modelling analyses show that many random distributions of stomatal aperture do not cause large changes in the A versus  $c_i$  relationship (Cheeseman, 1991), it has been experimentally shown that large deviations from the Aversus  $c_i$  curve can occur in conjunction with some patchy conductance distributions (Mott, 1995). The latter study prompted a more complete analysis of patchy stomatal conductance based on computer modelling (Buckley et al., 1997). That study showed that stomatal patchiness causes large changes in the measured relationship between A and  $c_i$  only for conductance distributions that are focused at low conductances, are relatively bimodal, have very

large ranges, or are right-skewed (meaning that more leaf area has a conductance below the mean than above it). Any distribution not meeting one of these criteria was shown to have a minimal effect on gas-exchange calculations.

Early observations of patchy stomatal conductance reported that the average conductance within each patch was either quite low or quite high, resulting in a bimodal distribution of conductances among patches (Terashima et al., 1988). Subsequent studies (Daley et al., 1989; Beyschlag et al., 1992; Mott, 1995) have shown that although bimodal distributions of conductance do occur, in many cases there is a wide range of conductance values among patches, and the distribution of conductance values is not bimodal. Thus, it appears that most patchy conductance distributions (and indeed, most stomatal heterogeneity) will not have an appreciable effect on measured gas-exchange parameters. Since patchiness is rarely tested for in the absence of gas-exchange effects, these results suggest that patchiness could go undetected in many cases, making the overall prevalence of the phenomenon difficult to estimate.

## Spatial and temporal characteristics

Patches can range in size from a single areole to at least several millimetres across, but they are usually bounded by veins (Beyschlag and Eckstein, 1998; Pospíšilová and Šantrůček, 1994; Terashima, 1992). It is possible that this aspect of patchiness is an artefact of the measurement techniques because both infiltrated water and CO<sub>2</sub> diffusion will be blocked by veins in heterobaric leaves (see above). However, if patches are really bounded by veins, it suggests that veins must present a barrier to any mechanism that co-ordinates stomatal behaviour. It also suggests a possible role for hydraulics, because water flow normally occurs centripetally in vein-bounded regions. In heterobaric plants, veins also represent a barrier to gasphase diffusion within the leaf, so the bounding of patches by veins also suggests a possible role for a gaseous signal such as intercellular  $CO_2$ .

The temporal characteristics of patchy stomatal conductance can be complex. Several studies have shown that the conductance of some patches may oscillate while other patches on the same leaf do not (Cardon *et al.*, 1994; Siebke and Weis, 1995). In these studies, the phase, period, and amplitude of the oscillations varied substantially among patches on a single leaf. In other studies there were apparently no oscillatory patches, but the pattern of patches was not always constant over time (Daley *et al.*, 1989; Mott, 1995). In addition to changes in pattern, the range of conductance values for patches was shown to be large during stomatal closure following ABA treatment, but the range declined as stomatal conductance stabilized at a lower value (Mott, 1995).

Stomatal oscillations have been hypothesized to be caused by lags between feedback loops controlling stomatal aperture (Cowan, 1972; Delwiche and Cooke, 1977; Haefner et al., 1997). Thus variability in the behaviour of patches could result from variability in the average characteristics of stomatal oscillations for each patch, or from the dominating influence of certain oscillating stomata within any given patch. It is, however, difficult to devise a mechanism that results in both oscillating and stable patches on the same leaf. One possibility is a variable distribution of some chemical or electrical signal which can cause either oscillations or complete closure of stomata. However, to explain the co-occurrence of stable, open patches with oscillating and closed patches, such a signal would have to be quite variable in its spatial distribution. It would have to be absent or present at insignificant levels in the neighbourhood of any stable open patches, while at the same time occurring at sufficient levels to cause oscillations in other patches, or complete closure in other patches.

Another spatial aspect of stomatal patchiness with important implications for its mechanism is that patterns of patchy stomatal conductance can be different for the two surfaces of amphistomatous leaves. This has been demonstrated for patchiness caused by low humidity using fluorescence imaging (Mott *et al.*, 1993), and it has been inferred from gas-exchange data for patchiness caused by ABA (Fall and Monson, 1992). This finding suggests that the mechanism for patchy stomatal conductance involves the epidermis or tissues close to epidermis and does not involve a general signal from the mesophyll in a localized area of the leaf.

## Possible mechanisms for stomatal patchiness

There are very few data that directly address mechanisms for patchy stomatal conductance, but from the discussion above it is possible to deduce some general characteristics of a possible mechanism. First of all, it seems unlikely that patchy stomatal conductance results directly from differences in average stomatal size or density for regions of a leaf because such patterns in conductance would most likely persist over wide ranges of conditions. In contrast, the patterns of patchy conductance can change rapidly, and patchiness appears suddenly in response to some environmental perturbations. The diversity of treatments causing patchy stomatal closure suggests that the mechanism is inherent in normal stomatal functioning and is not simply an aberration caused by a single unusual perturbation. It is conceivable that different mechanisms are responsible for the occurrence of patchiness in response to each perturbation, so any given mechanism does not necessarily have to explain, or even be consistent with all of these stimuli. Nevertheless, Occam's Razor suggests that the simplest case-namely, the smallest set

of mechanisms that can account for all of these observations—is the most likely case, and it is useful to begin a search for the mechanism with this assumption.

There are two distinct aspects of patchiness that require a mechanistic explanation: (1) the co-ordination of stomatal behaviour within a patch, and (2) the divergence of average stomatal behaviours among patches.

## Co-ordination within patches

Most evidence suggests that stomata are capable of responding individually to environmental perturbations (Spence, 1987; Saxe, 1979; Lange *et al.*, 1971), and it seems likely that much of the stomatal response observed at larger scales is a result of these individual responses. Nevertheless, the characteristics of patchy stomatal conductance discussed above strongly suggest that there is also some mechanism that tends to co-ordinate the behaviour of nearby stomata. Furthermore, since patchy stomatal conductance is not ubiquitous, under many conditions this mechanism must act uniformly or not at all.

There are at least two general types of mechanisms for producing co-ordination of stomatal behaviour within a patch. First, there could be some general signal that affects all stomata within a patch, causing them to behave more or less identically. Second, there could be direct interactions among adjoining stomata that tend to co-ordinate their behaviour. These two general scenarios are not mutually exclusive, nor are they always clearly distinguishable from one another.

Several candidates for a general signal exist. Local production and distribution of a diffusible chemical signal such as ABA, IAA, potassium ions, or other molecules that influence stomatal behaviour could account for many of the observed responses. Differences between areoles in the magnitude of a chemical signal will tend to be maintained by transpirational water flow, which is mostly centripetal (from the veins). Leaf intercellular  $CO_2$  ( $c_i$ ) could also serve as a co-ordinating signal, because a change in aperture of a single stoma or a few stomata within an areole could substantially change the  $c_i$  for the entire areole. However, the closure of a few stomata in an areole will decrease  $c_i$  for neighbouring stomata, causing them to open more and thereby increasing withinareole variability in aperture. It is unlikely that water vapour within the intercellular spaces could serve as a co-ordinating signal because most data show that the air in the intercellular spaces is saturated with water vapour (so the vapour pressure depends only on temperature), and the high thermal conductivity of water makes physiologically significant temperature gradients within a single areole unlikely.

Perturbations of the water potential of veins surrounding an areole could provide a general signal common to all stomata within the areole, assuming changes in the vein water potential are not localized to one part of the areole boundary. This assumption appears justified by the fact that both hydraulic resistance and capacitance within the xylem are very small (Malone, 1993), suggesting that any hydraulic perturbations or signals will be propagated rapidly and uniformly throughout the xylem.

Some of these candidates for general signals could also mediate local interactions between adjacent stomata. Chemical or electrical signals need not be uniformly or instantly propagated through an areole. For example, in the process of initiating pore closure, a guard cell pair may chemically alter the local apoplastic environment in some way (by slowing rates of proton extrusion, or releasing potassium ions or ABA). This change may be small in magnitude or spatial extent, and influence only that guard cell pair's immediate neighbours. Similarly, changes in the surface potential of a guard cell pair may only be strong enough to influence the nearest guard cells. Local signals of this nature could effect co-ordinating interactions among neighbouring stomata without being uniformly distributed throughout the areole. Hydraulic and mechanical interactions are also strong candidates for a local phenomenon that leads to co-ordinated behaviour within an areole, and they are discussed in detail below.

## Different behaviour among patches

The most simple explanation for differences in behaviour among patches is spatial and temporal variation in some local signal that co-ordinates stomatal behaviour within the patch. However, different behaviour of patches might also be caused by random variation in the responses of individual stomata, which when combined with local interactions (see above) causes variation in the emergent collective behaviour of small groups of stomata (Haefner et al., 1997; Mott et al., 1997; see below). There are many possible causes of random variation in stomatal responses. Guard cells may vary in their sensitivity to extrinsic factors such as ABA and  $CO_2$ , and if the average sensitivity to these factors differs between areoles, then the temporal aspects of stomatal responses will also differ. Variation in guard cell sensitivity to ABA may arise by interactions with other potentially variable factors. For example, the effects of ABA on stomata appear to be limited by both indole-3-acetic acid (IAA) (Snaith and Mansfield, 1982a), and increasing external potassium ion concentrations (Wilson et al., 1978). The stomatal response to CO<sub>2</sub> is also variable. Some plants grown without exposure to stress tend to be unresponsive to  $CO_2$ , but applied ABA sensitizes these plants to  $CO_2$ (Raschke, 1977). IAA may mediate this effect. Snaith and Mansfield (1982b) observed that IAA limits the CO<sub>2</sub>

response, whereas ABA reverses this limitation. Variation among areoles in the levels of either hormone may translate to variation in stomatal  $CO_2$  sensitivity.

Stomata may also vary in their mechanical interactions with epidermal cells. A theoretical analysis of guard cell mechanics (Sharpe et al., 1987) suggests that epidermal turgor may dominate the control of aperture, so that an equal increase of turgor pressure in epidermal and guard cells decreases stomatal aperture. The ratio of the sensitivities of aperture to epidermal and guard cell turgor, often called the 'mechanical advantage' (Sharpe et al., 1987), would then be greater than unity. Most available evidence suggests a range of values between 1 and 2.5 (Edwards et al., 1976; Meidner and Bannister, 1979; Peter Franks, personal communication; Tom Buckley, unpublished data). The mechanical advantage directly controls the response of aperture to changes in epidermal water relations, so stomatal behaviour will vary with this parameter. Variability in the mechanical advantage could involve differences in the geometrical relationships of guard and epidermal cells (Sharpe et al., 1987) or in the mechanical properties of guard cell walls. The mechanical advantage may therefore vary with levels of IAA, which loosens cell walls (Salisbury and Ross, 1992).

## Hydraulic mechanisms

Hydraulic mechanisms are an attractive starting point in the investigation of patchy stomatal conductance for several reasons. Firstly, the apparent delimitation of patches by veins strongly suggests a hydraulic mechanism (assuming it is not an artefact of the measurement techniques, as discussed above). Since veins are effectively ridges of high water potential surrounding an area of water potential drawdown (because of transpiration), the supply of water they provide may serve as a general, co-ordinating hydraulic signal within an areole. Secondly, hydraulic mechanisms could operate quickly enough to co-ordinate the relatively rapid movements associated with patches that oscillate (Malone, 1993), whereas diffusible chemical signals might not. Finally, there is a great deal of evidence (see below) that stomatal aperture is influenced by epidermal cells in addition to guard cells, and that transpiration affects epidermal water relations. The dynamic water relations of transpiring epidermal tissues could therefore form the basis of interactions among neighbouring stomata.

One possible mechanism for these interactions is based on the observations that (1) an increase in transpiration rate caused by a decrease in ambient humidity causes a rapid decrease in epidermal turgor pressure (Shackel and Brinckmann, 1985), and (2) guard and epidermal cell turgor act antagonistically in controlling stomatal aperture (Spence *et al.*, 1983; Edwards *et al.*, 1976; Franks *et al.*, 1995; Sharpe *et al.*, 1987). Therefore, if a single stoma or several neighbouring stomata were to open, the resulting decrease in epidermal turgor (caused by increased transpiration rate) could cause other nearby stomata to open slightly. This would further increase the rate of transpiration through those stomata, thereby further lowering local epidermal turgor and reinforcing the effect. More importantly, it would propagate the perturbation to yet other neighbouring stomata. The opposite set of events would occur in response to stomatal closure.

It has not been determined experimentally whether changes in transpiration of one stoma can actually influence the turgor of epidermal cells adjoining another stoma by a sufficient amount to change their aperture. However, changes in water potential should propagate along the epidermis because water flow occurs substantially along the epidermis in many species (Sheriff and Meidner, 1974), and there is no evidence for separate water pathways to each stoma from the xylem. Once propagated, these changes in water potential should influence the aperture of other stomata. This effect was observed by Sheriff and Meidner (1974), who reported propagation of water potential changes through the epidermis over a distance of several cm. Hydraulic interactions of this nature, when coupled with feedback loops for humidity and  $CO_2$ , may lead to the complex, co-ordinated stomatal responses that are characteristic of patchy stomatal conductance.

### Modelling

To investigate the possibility that hydraulic interactions among stomata could produce co-ordinated movements of stomata in a single areole, a spatially-explicit mathematical model of stomatal functioning in an intact leaf was constructed that included the hydraulic interactions discussed in the preceding section (Haefner *et al.*, 1997). The principal assumptions are that water evapourates from the epidermis or from a tissue in close hydraulic contact with the epidermis, that stomata are *not* supplied with water by unique pathways, and that stomatal aperture is determined by both guard cell and epidermal turgor pressure.

The model has two components. The first governs the behaviour of each individual 'stomatal unit', defined as a guard cell pair and the surrounding epidermal tissue, and is fundamentally similar to the model described by Delwiche and Cooke (1977). To produce a hyperbolic dependence of stomatal conductance on the mole fraction gradient for water vapour across the stomatal pore  $(\Delta w)$  and a linear dependence on transpiration (Monteith, 1995), guard cell osmotic pressure was regulated by epidermal turgor such that stomatal aperture increases with epidermal turgor and, therefore, decreases with  $\Delta w$ . The second component of the model links adjacent

stomatal units by modelling water flow through the adjoining epidermal tissues. An areole is represented by a grid of linked stomatal units surrounded by veins that supply water, but do not themselves experience changes in water potential (this simplification rules out interactions between areoles). The parameters and output lack rigorous physical units, so their numerical values should be considered as quantitative measures only relative to one another. The reader is referred to Haefner *et al.* (1997) for a detailed description of the model.

When all stomata were given identical characteristics and initial conditions, the model predicted stomatal responses to humidity similar to those observed in leaves, including a transition from stable apertures at low  $\Delta w$  to oscillating apertures at high  $\Delta w$ . Local interactions among stomata, and the implications of such interactions, became apparent in model runs that varied stomatal characteristics and/or initial conditions. With spatially randomized initial conditions for water potential and guard cell osmotic potential, the model predicted the emergence of a co-ordinated stomatal conductance time-course within each areole. The actual value of conductance varied only systematically within the areole (with proximity to veins), and stomata oscillated synchronously at high  $\Delta w$ (Fig. 1b). Thus, the model predicts that hydraulic interactions can co-ordinate stomatal behaviour within an areole.

When stomatal characteristics were spatially random-



Fig. 1. Stomatal conductance ( $g_s$ , arbitrary units) versus timestep for each of 16 stomata within an areole when the water potential and osmotic pressure for each guard cell pair were assigned random initial conditions. Both panels show  $g_s$  converging to a uniform pattern of behaviour throughout the areole as a result of stomatal interactions. The quantitative differences in  $g_s$  among stomata within an areole were due to random variation in stomatal characteristics (in panel (a), discussed below), and varying proximity to veins (in both panels; stomata with the same proximity to veins had similar values of  $g_s$ , and these groups are labelled with an open, grey or solid box corresponding to one of three possible positions as shown in the inset at top right). In (a) the mechanical advantage of the epidermis ( $\theta$ ) was spatially randomized according to a triangular probability density function with mode 1.6 and range [1.35, 2.0]. Although the maximum value for  $\theta$  occurring in this model run was 1.867, the prevailing lower values of  $\theta$  dominated stomatal behaviour in the areole, and early oscillations were damped out. In (b), the entire areole was assigned a value of 1.867 for  $\theta$ , the model run was repeated, and oscillations resulted. This indicates that in (a) the oscillatory tendencies of the high- $\theta$  stoma were masked by interactions with neighbouring non-oscillatory stomata. From Haefner *et al.* (1997); used with the permission of *Plant, Cell and Environment.* 

#### Stomatal heterogeneity 413

ized in addition to initial conditions, the model predicted that the behaviour of an individual stoma could not necessarily be predicted by its own characteristics because neighbouring stomata could exert a controlling influence. Model runs were performed in which the mechanical advantage of the epidermis ( $\theta$ ) was spatially randomized using a triangular probability density function with a mode of 1.6 and range from 1.35 to 2.0. (The mechanical advantage was chosen for randomization because preliminary results showed that stomatal behaviour was particularly sensitive to this parameter, and because theoretical analyses of guard cell mechanics suggest that small differences in guard and epidermal cell dimensions could cause this parameter to vary substantially.) If an entire areole was assigned a high value of  $\theta$  (1.867), every stoma oscillated (Fig. 1b), but if  $\theta$  was spatially randomized, an individual stoma with that same high value of  $\theta$  would not oscillate if its neighbours had been randomly assigned lower values of  $\theta$  (Fig. 1a). This means that the behaviour of each individual stoma was determined at the level of the areole, and the behaviour of each areole was determined by complex interactions among its component stomata.

This result had interesting implications for the behaviour of different areoles within a leaf. The oscillatory amplitude and period of stomatal oscillations were found to vary among areoles when  $\theta$  was spatially randomized. A given value of  $\Delta w$  caused some areoles to oscillate only



**Fig. 2.** Spatial and temporal dynamics of stomatal conductance associated with patchy stomatal movements induced by high  $\Delta w$  in (A) the model, and (B) a leaf of *Xanthium strumarium* L. at  $\Delta w = 20.8 \text{ mmol m}^{-1}$  (*PFD* = 1000  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, ambient [CO<sub>2</sub>]=350  $\mu$ mol mol<sup>-1</sup>, [O<sub>2</sub>]=20 mmol mol<sup>-1</sup>, leaf temperature = 25 ± 0.2 °C). Conductance timecourses are accompanied in (A) by graphical images of the spatial variation in conductance from the model, and in (B) by fluorescence images of the leaf for which gas-exchange measurements were being taken (the technique of inferring stomatal conductance from chorophyll fluorescence images is described elsewhere, Mott *et al.*, 1993). In both cases, brighter areas correspond to areas of higher stomatal conductance. The thin lines for both (A) and (B) represent average conductance for several selected areoles over time (determined explicitly for the model, and by gas-exchange measurements for the leaf). From Haefner *et al.* (1997); used with the permission of *Plant, Cell and Environment.* 

slightly, whereas other areoles oscillated with a large amplitude (Fig. 2a). Because the oscillatory period also varied among areoles, areoles began to oscillate out of phase. The resulting effect was very similar to patterns of stomatal patchiness observed in leaves (Fig. 2b).

Although the model reproduced some aspects of patchy stomatal conductance, other features were not predicted. First, differences in areole behaviour in the model were caused mostly by differences in their oscillatory periods, so it took a long time for these oscillations to go out of phase. Also, the model never predicted a steady, high conductance to occur for one areole at the same time that another areole had a steady, low conductance. It is possible that hydraulic interactions between areoles, mediated by changes in xylem water potential and therefore excluded from this model, could force areole oscillations out of phase more rapidly. Furthermore, although this model did not incorporate random variation in other critical parameters for stomatal functioning, such variation could increase the potential range of stomatal behaviour.

#### Experimental approaches

To test for the existence of the hydraulic mechanisms described above, Mott et al. (1997) used a small gas flow applied to the surface of a leaf of Xanthium strumarium L. with a needle (inside diameter =  $80 \mu m$ ) to control the humidity for a small area of an intact leaf independently of the humidity for the rest of the leaf. Stomatal apertures were observed with a light microscope during the experiments. Although it was impossible to perturb the humidity of a single stoma while maintaining constant humidity for the surrounding stomata (because of the high diffusional velocity of water vapour at the scale of an individual stoma), it was possible to produce humidity perturbations for small regions of a leaf containing four to eight stomata. Within this region, one or two stomata experienced a humidity that was approximately equal to that of the needle gas, while the others experienced humidity levels somewhere between that of the needle gas and that of the ambient air (Fig. 3).

These experiments showed that an increase or decrease in the humidity of the ambient air caused almost identical



Fig. 2. (continued)



Fig. 3. The effect on stomatal apertures in an intact leaf of Xanthium strumarium L. of decreasing ambient water vapour pressure  $(e_{wv})$  while maintaining a constant  $e_{wv}$  for the needle gas stream (see text). The black lines show the isobars for  $e_{wv}$  produced by the needle flow, and the numbers inside each isobar refer to the prevailing  $e_{wv}$  as a percentage of the difference in  $e_{wv}$  between the needle gas stream and ambient air. The graph shows stomatal aperture as a function of time;  $e_{wv}$  of the primary stream was decreased from 2.09 kPa to 1.10 kPa at time zero, while  $e_{wv}$  of the needle flow was kept at approximately 2.1 kPa. The picture shows the arrangement of the stomata that were measured, and the numbers on each stoma correspond to the numbers of the lines in the graph. The scale mark at the lower left of the picture is 50  $\mu$ m. From Mott *et al.* (1997); used with permission of *Plant, Cell and Environment*.

responses of all stomata in the viewing field, despite the fact that stomata closest to the needle were receiving little or no perturbation in humidity. All stomata showed an initial transient opening response to a decrease in humidity followed by a slower closing response (Fig. 3b). A similar response, but of smaller magnitude, was observed when the humidity of the needle flow was decreased and the ambient humidity was held constant (data not shown; see Mott *et al.*, 1997).

Lange *et al.* (1971) performed similar experiments in which neighbouring groups of stomata were exposed to streams of wet or dry air using microcapillaries and, in contrast to the results of Mott *et al.* (1997), reported that stomata appeared to respond only to the moisture of the air to which they were directly exposed. However, in

those experiments the wet and dry air capillaries were separated by much greater distances  $(600 \,\mu\text{m})$  versus  $200 \,\mu\text{m}$ ) than the spatial extent of the humidity gradients and stomatal interactions observed in Mott *et al.* (1997). Lange *et al.* (1971) also used epidermal peels floated on water; this set-up excludes any non-epidermal water pathways and provides an essentially infinite water supply at a very high water potential and low resistance, perhaps limiting the range of influence of local changes in epidermal water potential.

These data suggest the presence of some sort of signal among neighbouring stomata. Although there are several possibilities for this signal, hydraulic interactions are consistent with the observed response and offer the simplest explanation. To test whether hydraulic mechanisms

could produce the effects seen in these experiments, the model discussed above was reconfigured to simulate the experiments. In the new model  $\Delta w$  was perturbed by different amounts across an areole, and all stomata in the areole responded almost identically despite experiencing different perturbations (Haefner *et al.*, 1997). In the limiting case, where one stomatal unit was kept at constant  $\Delta w$  while the rest of the areole experienced a step increase in  $\Delta w$ , the unperturbed stoma responded in a manner indistinguishable from its neighbours (Haefner *et al.*, 1997). The only co-ordinating mechanisms that could have produced these results in our model were hydraulic interactions mediated by epidermal water potential.

## Conclusion

Although the diversity of responses associated with patchy stomatal conductance makes it difficult to devise a simple universal mechanism, the hydraulic interactions discussed above provide a useful starting point. Modelling studies suggest that these interactions will tend to co-ordinate stomatal movements within an areole under most conditions, but that this phenomenon does not usually manifest itself as patchy stomatal conductance because all areoles commonly have approximately the same average conductance. At low humidities, however, random variability in stomatal characteristics combines with instabilities inherent to the stomatal humidity response to produce variability in the collective behaviour of stomata within areoles. This results in a patchy distribution of apertures, and reveals the existence of strong hydraulic interactions within areoles.

One strength of the proposed mechanism is that novel processes for stomatal functioning are not required; co-ordination among stomata results from simple hydraulic interactions, and variability among areoles results from random spatial heterogeneity at the level of individual stomata. It is particularly interesting that random heterogeneity at this scale is the source for structured heterogeneity at the next scale, that is, in the emergent collective behaviour of each patch. Similar mechanisms may be responsible for patchiness in response to ABA and light, but in these cases, random variation in the response to ABA or light among stomata could produce differences in collective stomatal behaviour among areoles. Furthermore, the model described above and in Haefner et al. (1997) excluded dynamics in xylem water potential that may result from differences in areole transpiration rates, and such dynamics may give rise to larger-scale interactions and influence the spatial and temporal distribution patterns of chemical effectors such as ABA. These hypotheses remain to be tested.

## References

- Beyschlag W, Eckstein J. 1998. Stomatal patchiness. In: Progress in botany. Springer-Verlag (in press).
- Beyschlag W, Pfanz H. 1990. A fast method to detect the occurrence of nonhomogeneous distribution of stomatal aperture in heterobaric plant leaves. *Oecologia* 82, 52–5.
- Beyschlag W, Pfanz H, Ryel RJ. 1992. Stomatal patchiness in Mediterranean evergreen sclerophyllls. Phenomenology and consequences for the interpretation of the midday depression in photosynthesis and transpiration. *Planta* 187, 546–53.
- Buckley TN, Farquhar GD, Mott KA. 1997. Qualitative effects of patchy stomatal conductance distribution features on gasexchange calculations. *Plant*, *Cell and Environment* 20, 867–80.
- **Bro E, Meyer S, Genty B.** 1996. Heterogeneity of leaf CO<sub>2</sub> assimilation during photosynthetic induction. *Plant, Cell and Environment* **19**, 1349–58.
- Cardon ZG, Mott KA, Berry JA. 1994. Dynamics of patchy stomatal movements, and their contribution to steady-state and oscillating stomatal conductance calculated using gas-exchange techniques. *Plant, Cell and Environment* 17, 995–1007.
- **Cheeseman JM.** 1991. PATCHY: simulating and visualizing the effects of stomatal patchiness on photosynthetic CO<sub>2</sub> exchange studies. *Plant, Cell and Environment* 14, 593–9.
- **Cowan IR.** 1972. Oscillations in stomatal conductance and plant functioning associated with stomatal conductance: observations and a model. *Planta* **106**, 185–219.
- Daley PF, Raschke K, Ball JT, Berry JA. 1989. Topography of photosynthetic activity of leaves obtained from video images of chlorophyll fluorescence. *Plant Physiology* **90**, 1233–8.
- **Delwiche MJ, Cooke JR.** 1977. An analytic model of the hydraulic aspects of stomatal dynamics. *Journal of Theoretical Biology* **69**, 113–41.
- **Downton WJS, Loveys BR, Grant WRJ.** 1988. Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. *New Phytologist* **108**, 263–6.
- During H, Loveys BR. 1996. Stomatal patchiness of field-grown Sultana leaves: diurnal changes and light effects. *Vitis* 35, 7–10.
- Eckstein J. 1997. Heterogene Kohlenstoffassimilation in Blättern höherer Pflanzen als Folge der Variabilität stomatärer Öffnungsweiten. Charakterisierung und Kausalanalyse des Phänomens 'stomatal patchiness'. PhD-Dissertation, University of Würzburg.
- Eckstein J, Beyschlag W, Mott KA, Ryel R. 1996. Changes in photon flux induce stomatal patchiness. *Plant, Cell and Environment* 19, 1066–74.
- Edwards M, Meidner H, Sheriff DW. 1976. Direct measurements of turgor pressure potentials of guard cells. *Journal of Experimental Botany* 27, 163–71.
- Fall R, Monson RK. 1992. Isoprene emission rate and intercellular isoprene concentration as influenced by stomatal distribution and conductance. *Plant Physiology* **100**, 987–92.
- Franks PJ, Cowan IR, Tyerman SD, Cleary AL, Lloyd J, Farquhar GD. 1995. Guard cell pressure/aperture characteristics measured with the pressure probe. *Plant, Cell and Environment* 18, 795–800.
- Gunasekera D, Berkowitz GA. 1992. Heterogenous stomatal closure in response to leaf water deficits is not a universal phenomenon. *Plant Physiology* **98**, 660–5.
- Haefner JW, Buckley TN, Mott KA. 1997. A spatially explicit model of patchy stomatal responses to humidity. *Plant, Cell and Environment* 20, 1087–97.

- Kappen L, Andresen G, Losch R. 1987. In situ observations of stomatal movements. Journal of Experimental Botany 38, 126–41.
- Laisk A, Oja V, Kull K. 1980. Statistical distribution of stomatal apertures of *Vicia faba* and *Hordeum vulgare* and the *Spannungsphase* of stomatal opening. *Journal of Experimental Botany* 31, 40–58.
- Lange OL, Losch R, Schulze E-D, Kappen L. 1971. Responses of stomata to changes in humidity. *Planta* 100, 76–86.
- Lee J-S, Bowling DJF. 1995. Influence of the mesophyll on stomatal opening. Australian Journal of Plant Physiology 22, 357–63.
- Malone M. 1993. Hydraulic signals. *Philosophical Transactions* of the Royal Society of London 341, 33-9.
- Meidner H, Bannister J. 1979. Pressure and solute potential in stomatal cells of *Tradescantia virginiana*. Journal of Experimental Botany **30**, 255–65.
- Monteith JL. 1995. A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment* 18, 357–64.
- Mott KA. 1995. Effects of patchy stomatal closure on gas exchange measurements following abscisic acid treatment. *Plant, Cell and Environment* 18, 1291–1300.
- Mott KA, Cardon ZG, Berry JA. 1993. Asymmetric patchy stomatal closure for the two surfaces of *Xanthium strumarium* L. leaves at low humidity. *Plant, Cell and Environment* 16, 25–34.
- Mott KA, Denne F, Powell J. 1997. Interactions among stomata in response to perturbations in humidity. *Plant, Cell and Environment* 20, 1098–107.
- Osama K, Onoe M. 1984. Measurement of stomatal aperture by digital image processing. *Plant and Cell Physiology* 25, 1379–88.
- **Osama K, Hashimoto Y, Aiga I.** 1983. Observation of stomatal movements of intact plants using an image instrumentation system with a light microscope. *Plant and Cell Physiology* **24**, 281-8.
- Pospíšilová J, Šantrůček J. 1994. Stomatal patchiness. *Biologia Plantarum* 36, 481–510.
- Pospíšilová J, Šantrůček J. 1997. Stomatal patchiness: effects on photosynthesis. In: Pessarakli M, ed. Handbook of photosynthesis. New York: Marcel Dekker, 427–41.
- **Raschke K.** 1977. The stomatal turgor mechanism and its responses to  $CO_2$  and abscisic acid: observations and a hypothesis. In: Marrè E, Ciferri O, eds. *Regulation of cell membrane activities in plants*. Amsterdam: Elsevier/North-Holland Biomedical Press, 173–83.
- Raschke K, Patzke J, Daley PF, Berry JA. 1990. Spacial and temporal heterogeneities of photosynthesis detected through analysis of chlorophyll-fluorescence images of leaves. In: Baltscheffsky M, ed. *Current research in photosynthesis*, Vol. IV. Netherlands: Klower Academic Publishers, 573-8.
- Salisbury FB, Ross CW. 1992. Plant physiology, 4th edn.

Belmont, California: Wadsworth Publishing Company, 369-70.

- Saxe H. 1979. A structural and functional study of the co-ordinated reactions of individual *Commelina communis* L. stomata (Commelinaceae). *American Journal of Botany* 66, 1044–52.
- Shackel KA, Brinckmann E. 1985. In situ measurement of epidermal cell turgor, leaf water potential, and gas exchange in *Tradescantia virginiana* L. Plant Physiology **78**, 66–70.
- Sharkey TD, Seemann JR. 1989. Mild water stress effects on carbon-reduction-cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. *Plant Physiology* **89**, 1060–5.
- Sharpe PJH, Wu H, Spence RD. 1987. Stomatal mechanics. In: Zeiger E, Farquhar GD, Cowan IR, eds. *Stomatal function*. Stanford University Press, 91–114.
- Sheriff DW, Meidner H. 1974. Water pathways in leaves of *Hedera helix* L. and *Tradescantia virginiana* L. Journal of *Experimental Botany* 25, 1147–56.
- Siebke K, Weis E. 1995. Assimilation images of leaves of *Glechoma hederacea*: analysis of non-synchronous stomata related oscillations. *Planta* **196**, 155–65.
- Snaith PJ, Mansfield TA. 1982a. Stomatal sensitivity to abscisic acid: can it be defined? *Plant, Cell and Environment* 5, 309–11.
- Snaith PJ, Mansfield TA. 1982b. Control of the CO<sub>2</sub> responses of stomata by indol-3ylacetic acid and abscisic acid. *Journal* of Experimental Botany 33, 360–5.
- Spence RD. 1987. The problem of variability in stomatal responses, particularly aperture variance, to environmental and experimental conditions. *New Phytologist* 107, 303–15.
- Spence RD, Sharpe PJH, Powell RD, Rogers CA. 1983. Epidermal and guard cell interactions on stomatal aperture in epidermal strips and intact leaves. *Annals of Botany* 52, 1–12.
- Terashima I. 1992. Anatomy of non-uniform leaf photosynthesis. *Photosynthesis Research* **31**, 195–212.
- Terashima I, Wong S-C, Osmond CB, Farquhar GD. 1988. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant and Cell Physiology* 29, 385–94.
- Weyers JDB, Lawson T. 1997. Heterogeneity in stomatal characteristics. *Advances in Botanical Research* 26, 317–51.
- Wilson JA, Ogunkanmi AB, Mansfield TA. 1978. Effects of external potassium supply on stomatal closure induced by abscisic acid. *Plant, Cell and Environment* 1, 199–201.
- Wise RR, Ortiz-Lopez A, Ort DR. 1992. Spacial distribution of photosynthesis during drought in field-grown and acclimated and non-acclimated growth chamber-grown cotton. *Plant Physiology* **100**, 26–32.
- Yang S, Tyree MT. 1996. Hydraulic architecture of Acer saccharum and A. rubrum: comparison of branches to whole trees and the contribution of leaves to hydraulic resistance. Journal of Experimental Botany 45, 179-86.