FIELD NOTE

Temporal fluctuation of patchy stomatal closure in leaves of *Dipterocarpus sublamellatus* at upper canopy in Peninsular Malaysia over the last decade

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ABSTRACT

In the southeast Asian lowland dipterocarp forest of Peninsular Malaysia (Pasoh Forest Reserve), upper canopy tree species with heterobaric leaves show severe midday depression of net assimilation rate (*A*) accelerated by patchy stomatal closure, although it is still unclear whether it always happens or not. We added the *in situ* observations at various meteorological conditions over the last decade to evaluate the frequency and environmental conditions of patchy stomatal closure in *Dipterocarpus sublamellatus* Foxw., an emergent tree with heterobaric leaves through leaf–gas exchange measurements combined with numerical simulations and chlorophyll fluorescence imaging. Our observation revealed that on days with moderate leaf temperature (≤35°C) and vapor pressure deficit (VPD), which consist four of nine measurement days during 2003–2015, uniform stomatal behavior could explain observed *A* during the day. The patterns of stomatal behavior shifted from ‘uniform’ to ‘patchy’ even within a day depending on increases in leaf temperature and VPD according to irradiation of sunlight. However, it did not return from ‘patchy’ to ‘uniform’ once patchy stomatal closure happened in a day.

Key words: chlorophyll fluorescence imaging, leaf temperature, midday depression of photosynthesis, patchy stomatal closure, vapor pressure deficit

INTRODUCTION

In tropical rainforests, the proportion of tree species with heterobaric leaves was higher under high light intensity and drought conditions such as in the emergent and canopy layers; by contrast, homobaric leaf species were distributed in areas with shady and moist conditions, such as the understory (Kenzo et al. 2007). Previous studies reported that heterogeneous (‘non-uniform’) stomatal closure occurs in trees with heterobaric leaves in which bundle sheath extensions delimit the mesophyll vertically, but not in trees with homobaric leaves. In heterobaric leaves, the diffusion of CO₂ and H₂O is restricted, causing stomatal patchiness coupled with depression of net assimilation rate (*A*) under drought conditions, such as low humidity (Loreto and Sharkey 1990, Beyschlag et al. 1992, Mott et al. 1993), short-term water stress (Sharkey and Seeman 1989, Gunasekara and Berkowitz 1992) or application of abscisic acid (ABA) (Downton et al. 1988, Tearashima et al. 1988). However, in homobaric leaves, there are no bundle-sheath extensions, and uniform stomatal behavior occurs. A physiological study suggests that bundle-sheath extensions may respond quickly to drought signals, such as ABA, by reducing mesophyll water potential or increasing the concentration of ABA (Terashima 1992). Several studies have revealed the hydraulic mechanism of stomatal patchiness (Mott and Buckley 2000, Peak et al. 2004, Buckley 2005, West et al. 2005, Mott and Peak 2007). Heterobaric leaves of canopy species in a tropical rainforest have a larger vessel diameter and stomatal pore index than the homobaric leaves, which allows them to achieve a high carbon gain with large water use under strong light condition (Inoue et al. 2015). It has also been shown that the density of bundle-sheath extensions is more related to the gas exchange properties and drought tolerance of the leaves, such as stomatal conductance and water potential, than to their mechanical and structural properties (Kawai et al. 2017). Since depression in *A* at leaf level may affect the photosynthetic CO₂ fixation of the entire ecosystem (Gross primary
production, GPP), it is important to clarify when and under what conditions patchy stomatal closure occurs and to what extent it reduces $A$ in natural environments, but few studies have demonstrated this.

Patchy stomatal closure coupled with depression of $A$ has been observed in several dipterocarp species with heterobaric leaves distributed in lowland tropical rainforests using numerical simulations of leaf–gas exchange rates and/or observation of stomatal aperture distribution (Hiromi et al. 1999, Takanashi et al. 2006, Kosugi et al. 2009, Kamakura et al. 2011). Kosugi et al. (2009) reported that patchy stomatal closure occurred in wet periods (higher volumetric soil water content, VSWC) as well as mild-dry periods (lower VSWC), although the magnitude of inhibition of $A$ was different. Although stomatal patchiness in leaves of dipterocarp trees subjected to artificial soil dry conditions was significant than that of untreated trees (Inoue et al. 2017), VSWC may not be the main factor in whether patchy stomatal closure occurs or not. On sunny days, patchy stomatal closure occurred only in upper canopy trees due to the high light intensity, leaf temperature ($T_{leaf}$) and vapor pressure deficit (VPD), whereas subcanopy or understory trees (including seedlings of canopy tree species) have shown uniform stomatal closure with a moderate decrease in $A$, even though they have heterobaric leaves (Kamakura et al. 2015). Thus, the vertical micro-meteorological gradient in the forest determines the occurrence of stomatal patchiness. Moreover, continued observation of leaf–gas exchange rates of canopy sunlit leaves of *Dipterocarpus sublamellatus* Foxw., one of the dominant canopy tree species in lowland tropical rainforests showed that even canopy tree species, which had previously shown patchy stomatal closure to varying degrees, did not show patchy stomatal closure on some days. Then, the crucial question was how frequently and under what environmental conditions patchy stomatal closure occurs in trees occupying the top canopy. To answer it, we added more leaf–gas exchange data of *D. sublamellatus* to Kosugi et al. (2009). Moreover, to detect the occurrence of heterogeneity of the photosynthetic electron transport rate ($J$) in leaves induced by patchy stomatal closure, as reported in previous studies (Guàrdia et al. 2012, Kamakura et al. 2012a, Kamakura et al. 2015), we also observed diurnal changes in chlorophyll fluorescence imaging.

**MATERIALS AND METHODS**

**Study site**

The study site was Pasoh Forest Reserve of the Forest Research Institute Malaysia (FRIM), near Simpang Pertang in Negri Sembilan, Peninsular Malaysia (2°58’ N, 102°18’ E, 75–150 m a.s.l.). This site is one of the Asia flux, Japan flux, FLUXNET, and LTER (Long-Term Ecological Research Network) sites (http://www.bluemoon.kais.kyoto-u.ac.jp/pasoh/). The core area (600 ha) of the reserve (2,450 ha) was covered by a primary lowland mixed dipterocarp forest (a tropical evergreen broad-leaved forest), which consisted of various species of *Shorea* and *Dipterocarpus*. The continuous canopy height was $\sim$35 m, although some emergent trees exceeded 45 m. Soil type around the tower was Haplic Acrisol, based on the FAO classification. The A horizon was thin (0–5 cm) and the lateritic gravels were abundant below a depth of 30 cm (Yamashita et al. 2003). The mean annual rainfall was 1,857 mm (2003–2015). In Peninsular Malaysia, there are two wet and mild-dry periods, but the seasonality and amount of rainfall fluctuate year-to-year. To represent soil moisture conditions, an antecedent precipitation index (API$_{60}$) from 2003 to 2015 was shown in Fig. 1 because and positively correlated with VSWC at Pasoh site (Kosugi et
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al. 2007, Noguchi et al. 2016). API was defined as follows:

\[ API = \sum_{i=1}^{n} P_i / i. \]  

(1)

Materials

*In situ* leaf gas exchange measurements and stomatal observations were conducted on one emergent tree (*Dipterocarpus sublamellatus* Foxw.) accessible from a 30-m-high triangular canopy walkway built between three aluminum towers (Fig. 2a). We confirmed that this species had heterobaric leaves from observations of transverse slices of its leaves under a light microscope (Eclipse E200; Nikon, Tokyo, Japan; Fig. 2b). We could access two individuals of *D. sublamellatus* from the tower, but one tree has been shaded by other tree species. Although we conducted gas exchange measurements in shaded leaves of the *D. sublamellatus* tree, at times, no stomatal patchiness was observed (data not shown). At this site, Takanashi et al. (2006) and Kamakura et al. (2015) reported stomatal patchiness of another emergent species with heterobaric leaves (i.e., *Neobalanocarpus heimi* (King) Ashton), but this tree can no longer be accessed from the canopy walkway. The studied tree is the only one emergent tree with heterobaric sunlit leaves that we can access from the canopy walkway.

Gas exchange measurements

We measured diurnal changes in \( A \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), stomatal conductance for the water vapor behavior (\( g_s \), mol m\(^{-2} \text{s}^{-1} \)), and \( J \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) of intact leaves of one emergent tree of *D. sublamellatus* under saturated (1,000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) photosynthetic photon flux density (PPFD) using an LI-6400 gas-exchange measurement system (LI-Cor Inc., Lincoln, NE) with a 6400-40 circular (2 cm diameter) leaf chamber fluorometer. Gas exchange measurements were carried out under saturated light to evaluate causal factors of patchy stomatal closure because light conditions at top of the canopy vary moment by moment. Leaves were set in the cuvette for approximately 1 min, and then measurements were conducted under ambient conditions, except for the light level. Observation of stomatal patchiness under natural light conditions has been reported at this site (Kamakura et al. 2011). We calculated \( J \) in the light using a Photosystem II quantum yield (\( \varPhi_{II} \)) measured with LI6400-40.

Measurements of \( A, g_s \), and \( J \) were conducted nine days during the period from March 2003 to January 2015. On each observation day, we sampled 3–5 fully expanded sunlit leaves at the top of the canopy and the measurements were repeated 5–17 times during the day using the same leaves.

Gas exchange analysis

To detect environmental factors affecting patchy stomatal closure, we calculated the degree of stomatal patchiness \( (P) \) as follows:

\[ P = \frac{A_{obs} - A_{sim\_uni}}{A_{sim\_patchy} - A_{sim\_uni}} \]  

(2)

where \( A_{obs} \) is the observed \( A \), and \( A_{sim\_uni} \) and \( A_{sim\_patchy} \) are the simulated \( A \) at \( g_s \) for either uniform or patchy bimodal

![Fig. 2. (a) Canopy corridor and the observed *Dipterocarpus sublamellatus* tree and (b) a transverse slice of *Dipterocarpus sublamellatus* leaves observed under a light microscope. Arrows in (b) indicate bundle-sheath extensions.](image-url)
stomatal behavior, respectively. When the absolute difference between \( A_{\text{sim,uni}} \) and \( A_{\text{sim,patchy}} \) was small (\( \leq 0.5 \)), we distinguished these \( P \) values from the other \( P \) values because there is no significance for calculating \( P \).

To explore either uniform or patchy stomatal behavior of the leaf, we simulated values of net assimilation rate \( (A_{\text{sim,uni}} \text{ or } A_{\text{sim,patchy}}) \) and the partial pressure of CO\(_2\) in the chloroplasts \( (p(C_i)_{\text{sim,uni}} \text{ or } p(C_i)_{\text{sim,patchy}}) \) for an entire leaf at observed \( g_s \) based on the Farquhar–von Caemmerer–Berry model (Farquhar et al. 1980). Uniform stomatal behavior indicates that individual stomatal apertures distributed in the leaf are synchronous and equal to the whole-leaf conductance. For uniform stomatal behaviour, we calculated \( A_{\text{sim,uni}} \) as follows:

\[
A_{\text{sim,uni}} = \min \{ W_s, W_p \}
\]

\[
W_s = V_{c,max} \times \exp \left( \frac{\Delta H_{c,R}(T_{\text{leaf}} - 25)}{298 R (T_{\text{leaf}} + 273)} \right) \times \frac{p(C_o) - p(O)}{p(C_o) + K_v \left( 1 + \frac{p(O)}{K_o} \right)}
\]

\[
W_p = 4 \times \frac{1}{\Delta} \exp \left( \frac{\Delta S(T_{\text{leaf}} + 273) - \Delta H_{c,R}}{R (T_{\text{leaf}} + 273)} \right) \times \frac{p(C_o) - p(O)}{p(C_o) + \Delta S}
\]

where \( V_{c,max} \) is the maximum rate of carboxylation at 25 \(^\circ\)C (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), \( R_0 \) is the non-photorespiratory respiration rate (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), \( \tau \) is the specifcity factor of Rubisco, \( p(O) \) (21,000 Pa) is the partial pressure of O\(_2\) at the sites of oxygenation, \( K_c \) and \( K_v \) are the Michaelis–Menten constants of Rubisco for CO\(_2\) and O\(_2\), respectively, \( T_{\text{leaf}} \) is leaf temperature (\(^\circ\)C), \( R \) is the gas constant (8.31 J K\(^{-1}\) mol\(^{-1}\)), \( \Delta H_{c,R} \) is the activation energy for \( V_{c,max} \) (J mol\(^{-1}\)), \( \Delta H_{d,R} \) is the deactivation energy for \( V_{c,max} \) (J mol\(^{-1}\)), and \( \Delta S \) is an entropy term (J mol\(^{-1}\)). To estimate \( V_{c,max} \) at 25 \(^\circ\)C \( (V_{c,max}^{25}) \mu \text{mol m}^{-2} \text{s}^{-1} \), we used an inverse method called the “one-point method”. This method has been widely used to determine \( V_{c,max} \) from the actual responses of leaves in the field (Grassi et al. 2005, De Kauwe et al. 2016). The Arrhenius function was used to estimate the temperature dependence of parameters \( K_c, K_v, \tau, V_{c,max} \) and \( R_0 \) (detailed in Kosugi et al. 2003, Kosugi and Matsuo 2006, Takano et al. 2006).

Based on the average line or the median value listed by Kosugi and Matsuo (2006), we used 55,200 J mol\(^{-1}\) for \( \Delta H_{c,R} \), 220,000 J mol\(^{-1}\) for \( \Delta H_{d,R} \) and 650 J mol\(^{-1}\) for \( \Delta S \).

For patchy stomatal behavior, we assumed a patchy "bimodal" distribution of stomata within the leaf, which indicates that whole-leaf conductance reflects total amount of \( g_s \) for open and closed patches in single leaves. Stomatal conductance for open and closed patches was given as maximum \( g_s \) (\( g_{\text{max}} \)) and minimum \( g_s \) (\( g_{\text{min}} \)), respectively, and the open/closed patch ratio \( (r_a) \) was determined from the observed \( g_s \) for the whole leaf. We assumed uniform photosynthetic parameters for each patch, and the average net assimilation rate \( (A_{\text{sim,patchy}}) \) and CO\(_2\) concentration in the chloroplasts \( (p(C_i)_{\text{sim,patchy}}) \) for the whole leaf was calculated by multiplying the open and closed patch ratio in a leaf by \( A \) or \( p(C_i) \) of open and closed patches. The equations are as follows:

\[
A_{\text{sim,patchy}} = r_a A_{\text{op}} + (1 - r_a) A_{\text{cl}}
\]

\[
p(C_i)_{\text{sim,patchy}} = r_a p(C_i)_{\text{op}} + (1 - r_a) p(C_i)_{\text{cl}}
\]

As parameter setting for simulation, we used the optimal values of \( V_{c,max}^{25} \) (19.7 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), \( R_0^{25} \) (1.0 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), and \( g_{\text{max}} \) (0.15 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) obtained from the observed leaf–gas exchange data throughout the whole measurement period. The maximum \( g_s \) was determined from the top 15% of all the data for observed \( g_s \). Although \( V_{c,max} \) shows seasonal change, we used a single value of \( V_{c,max} \) in this study because \( g_s \) in some leaves decreased immediately in the morning due to drought stress and it is difficult to calculate \( V_{c,max} \) for each day (e.g., on Aug 24, 2003, shown in Fig. 3b, high values of \( A \) and \( g_s \) were obtained only once in a day, and it was difficult to determine \( V_{c,max} \) only from measurements taken on only this day). We also used 0.0005 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) for \( g_{\text{min}} \) following Takano et al. (2006). Detailed explanation of the model is described in Kamakura et al. 2011, Kamakura et al. 2012b, and Kamakura et al. 2015.

### Mapping of J using imaging-PAM

On 15 Jan and 29 Jun 2014, we performed imaging of \( \Phi_B \) using the MINI-version of the Imaging-PAM (Walz, Effeltrich, Germany) before the gas exchange measurements, in which leaf areas up to 2.4 cm\(^2\) \times 3.2 cm (50 \( \mu \text{m} \) per pixel) could be assessed. Actinic illumination was 1,000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), which provided the same intensity as used for the leaf–gas exchange measurements. Actinic light was used for \( \sim 1 \) min, after which a saturation pulse was used. The images of \( \Phi_B \) were calculated using Imaging-Win software (Walz). Values of \( \Phi_B \) were corrected for each area of investigation with the instrument-dependent value.
Fig. 3. The observed and simulated plots (simulated by numerical analysis based on the Farquhar–von Caemmerer–Berry model) of diurnal changes in leaf–gas exchange rates with saturated photosynthetic photon flux density (PPFD) (1,000 μmol m⁻² s⁻¹) on nine observation days from 2003 to 2015 (mean ± SD, n = 3–5). The figure shows the net assimilation rate (A), stomatal conductance (gₛ), photosynthetic electron transport rate (J), the degree of stomatal patchiness (P), leaf temperature (T_leaf), vapor pressure deficit (VPD), and PPFD under natural conditions obtained with an LI6400-40. By comparing observed A values and simulations, observation days when (a) uniform or (b) patchy bimodal stomatal behavior explained A are shown. When the difference (A_sim_uni - A_sim_patchy) was ≤0.5, the data were removed from the calculation of the averaged P. The top panel shows an antecedent precipitation index (API₆₀, mm) on each observation day.
the $F_v$-factor (1.378), which was determined by Kamakura et al. (2012a). The photosynthetic electron transport rate ($J$, $\mu$ mol m$^{-2}$ s$^{-1}$) of the illuminated leaves was analyzed from $\Phi_{psii}$. The mapping of $J$ in the leaves was assessed using R-software (ver. 2.13.0; R Development Core Team, Vienna, Austria).

RESULTS

Diurnal patterns of leaf–gas exchange rates with saturated PPFD

On each measurement day, average $A$ and $g_s$ in leaves under saturated PPFD reached their maxima in the morning and then decreased thereafter (Fig. 3). The photosynthetic electron transport rate ($J$) showed a moderate decrease during the day. By comparing observed and simulated $A$ values, the plot of observed $A$ values throughout the day fell on the simulated $A$ line calculated under the assumption of uniform stomatal behavior on 14 Dec 2007, 15 Sep 2009, 15 Jan 2014, and 14 Jan 2015 (Fig. 3a). On 9 Mar 2003, 24 Aug 2003, 23 Nov 2004, 14 Jan 2013, and 29 Jun 2014, however, uniform stomatal behavior could only explain higher values of observed $A$ in the morning, but patchy bimodal stomatal behavior was able to explain depressed values of $A$ during the day (Fig. 3b). On these days, rapid and severe decreases of $A$ and $g_s$ from the beginning of the day were detected. On days when patchy bimodal stomatal behavior explained $A$, $P$ approached close to 1.0 (this indicates the occurrence of patchy bimodal stomatal behavior) with increasing $T_{leaf}$ and VPD according to irradiation of sunlight. The depressed values of $A$ and $g_s$ did not recover and $P$ did not approach close to zero (this indicates the occurrence of uniform stomatal behavior) even though $T_{leaf}$ and VPD decreased thereafter. On days when uniform stomatal closure explained $A$, the average $P$ was smaller than 0.6 with moderate changes in $T_{leaf}$ and VPD. However, whether stomatal behavior pattern was uniform or patchy did not vary according to variation in API. Thus, patchy stomatal closure was related to daily fluctuation of atmospheric conditions, but not related to soil moisture condition.

Relationships between gas exchange parameters and environmental factors

On days when patchy stomatal behavior explained $A$ ($P$ was smaller than 0.6), $A$ at a given $g_s$ tended to be lower than on days when uniform stomatal behavior explained $A$ (Fig. 4). This suggests that $A$ was depressed by patchy bimodal stomatal behavior. Concerning the values of $P$, stomatal patchiness did not occur on days when uniform stomatal behavior explained $A$ because of moderate $T_{leaf}$ (< 35°C) and VPD values (Fig. 5a), whereas it occurred with higher values of $T_{leaf}$ and VPD on days when patchy bimodal stomatal behavior explained $A$ (Fig. 5b). On days

![Fig. 4. Relationship between $A$ and $g_s$ on days when uniform or patchy bimodal stomatal behavior explained $A$. Open circles show data on days when uniform stomatal behavior explained $A$ and closed circles show data on days when patchy bimodal stomatal behavior explained $A$.](image-url)
When patchy bimodal stomatal behavior explained \( A \) (\( P \) approached close to 1.0), the pattern of stomatal behavior did not shift from patchy to uniform because \( P \) values in the afternoon remained high even when \( T_{\text{leaf}} \) and VPD values became moderate (Fig. 5b).

**Diurnal patterns of \( J \) images with saturated PPFD**

Fig. 6 shows the diurnal changes in the spatial distribution of \( J \) under saturated PPFD on the day when uniform stomatal closure was detected based on gas exchange measurements (15 Jan 2014, Fig. 6a), and on the day when patchy bimodal stomatal closure was detected (29 Jun 2014, Fig. 6b). The time course of a transactional profile of \( J \) made from \( J \) images is also shown in this figure. A uniform distribution of \( J \) was observed during the morning of 29 June 2014 and throughout the day of 15 Jan 2014. However, a heterogeneous distribution of \( J \) was observed during the afternoon of 29 Jun 2014, although it was not bimodal. The timing of the occurrence of \( J \) patchiness coincided with the timing of the occurrence of patchy stomatal closure detected from gas exchange observations and simulations. When \( J \) patchiness was observed, \( J \) values decreased from about 100 to 80 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and fluctuated in an almost–sinusoidal pattern (Fig. 6b (4)–(6)).

**DISCUSSION**

How frequently does patchy stomatal closure occur?

Maximum values of \( A \) (around 10 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) were frequently observed under saturated PPFD during the measurement periods (Fig. 3), and this value represents the leaf photosynthetic ability of \( D. \) sublamellatus, as reported by Kosugi et al. (2009). However, by adding leaf-gas exchange data and comparing observed and simulated \( A \) values at a given \( g \), for both uniform and patchy bimodal stomatal behaviors, we demonstrated that patchy bimodal
Fig. 6. Images of the spatial distribution of $J$ and its transected profile under saturated PPFD ($1,000 \mu$mol m$^{-2}$s$^{-1}$) taken with the Imaging-PAM. The images measured $2.4 \times 3.2$ cm$^2$ (50 $\mu$m per pixel). Data on the day when uniform stomatal closure was expected (15 Jan 2014; a) and when patchy bimodal stomatal closure was expected (29 Jun 2014; b) are shown. Maximum and minimum values of $J$ are shown at a relative scale. The leaves were identical to those used for gas exchange measurements in Fig. 3. The number in each image corresponds to the measurement time for the leaf–gas exchange shown on the left side. The black line in each image shows the trajectory on which the transected profile of $J$ was measured.
stomatal behavior sometimes occurred and sometimes not even in the same individuals. Stomatal patchiness occurred in five of the nine measurement days during 2003–2015, but this was not determined by the magnitude of API.<br>

These results indicate that stomatal behavior would change with daily fluctuations of atmospheric conditions and not with soil moisture conditions. On 29 Jun 2014, uniform stomatal behavior explained the higher values of A in the morning, and patchy bimodal stomatal behavior explained the depressed values of A afterward (Fig. 3b). This indicates that patterns of stomatal behavior in leaves of *D. sublamellatus* shifted from uniform to patchy even within a day depending on increases in $T_{\text{leaf}}$ and VPD according to irradiation of sunlight. Such shift of stomatal behavior has also been reported for leaves of several canopy tree species of tropical rainforests (Hiromi et al. 1999) and cool temperate deciduous forest ecosystems (Kamakura et al. 2012b). The degree of stomatal patchiness ($P$) also showed that stomatal patchiness occurred with increasing $T_{\text{leaf}}$ and VPD during the day, and that stomatal behavior did not shift from patchy to uniform even though $T_{\text{leaf}}$ and VPD decreased thereafter (Fig. 5b). A heterogeneous distribution of $J$ was also observed on the day when patchy bimodal stomatal behavior explained $A$ (Fig. 6). A patchy distribution of $J$, coupled with patchy stomatal closure, suggests heterogeneous distribution of internal CO$_2$ concentration ($C_i$) within a leaf, but distribution of $C_i$ in a leaf could not be estimated at high light intensities as discussed in Kamakura et al. (2012a). Although no direct relationship between stomatal patchiness and photosynthesis was detected in this study, changes in the distribution of $J$ within a day, and simulated results of leaf–gas exchange suggest that patchy stomatal closure affects photosynthetic activity. Thus, $J$ patchiness in leaves of *D. sublamellatus* provides more evidence of stomatal patchiness. At the Pasoh site, heterogeneous distribution of $J$ and stomatal patchiness detected from numerical simulations of leaf–gas exchange rates were also observed in the leaves of *Neobalanocarpus heimii*, another higher canopy tree species, whereas subcanopy and understory tree species with heterobasic leaves showed uniform stomatal behavior throughout the day (Kamakura et al. 2015). These results suggest that detection of stomatal behavior from numerical simulations of leaf–gas exchange rates is reasonable and that increased $T_{\text{leaf}}$ and VPD on top of the canopy affect patterns of stomatal behavior.

**What environmental conditions should be met to cause patchy stomatal closure?**

Our study showed that $A$ at a given $g_s$ decreased on days when patchy bimodal stomatal behavior occurred rather than on days when uniform stomatal behavior occurred (Fig. 4), and that patchy bimodal stomatal behavior was the main factor causing severe depression of $A$ in leaves of *D. sublamellatus* on hot, sunny days. By calculating the degree of stomatal patchiness ($P$) based on leaf–gas exchange data, stomatal patchiness did not occur at least when $T_{\text{leaf}}$ was below 35°C, but above that. In future studies, it is necessary to separate the impacts of $T_{\text{leaf}}$ and VPD by varying one but holding the other constant, although it is difficult to distinguish these two parameters under natural conditions. It is clear that stomata in many species close in response to increased VPD (Franks and Farquhar 1999), although it is still unclear how stomata sense VPD directly (Leuning 1995). Alternatively, it has been suggested that increased VPD increases the rate of transpiration (water loss not passing through the stomatal pore), which would lead to lowered guard-cell turgor and thus stomatal closure, if the hydraulic flow resistance in the pathway to the evaporating sites on the outer surface of the guard cells is great enough (Buckley 2005). Although the mechanism of how stomata in each patch senses increased VPD is still uncertain, our study indicates that the pattern of stomatal behavior, which determines the magnitude of midday depression of $A$, varies with $T_{\text{leaf}}$ and VPD. Another important point to note is that when patchy bimodal stomatal behavior was detected during the day, the pattern of stomatal behavior did not shift from patchy to uniform even though $T_{\text{leaf}}$ and VPD values became moderate (Fig. 5b). This explains why depressed values of $A$ and $g_s$ did not recover under lower $T_{\text{leaf}}$ and VPD values (Fig. 3b). Thus, it is possible that not only $T_{\text{leaf}}$ and VPD affects diurnal changes of stomatal response, but also circadian rhythms are involved (Hetherington and Woodward 2003, Doughty et al. 2006). A study of circadian regulation of stomata revealed that the accumulation of potassium ions was associated with stomatal opening, whereas later in the day sucrose was the dominant solute for stomatal closing (Talbott and Zeiger 1998). However, it is unclear whether such rhythms relate to patchy stomatal closure. Hiromi et al. (1999, 2012) showed that *Dryobalanops aromatica*, a dipterocarp species, maintained a higher leaf water potential during the day but stomatal patchiness occurred as much or more than in trees with lower leaf water potential, such as *Shorea* species. Because *D. sublamellatus* at the Pasoh site also showed higher leaf water potential (higher than $-1.1$ MPa) during the day (Fuku et al. 2007), this species has a more conservative strategy to reduce water loss and maintain a stable water balance.
Although further studies are necessary to clarify the underlying mechanisms of stomatal patchiness, it has been suggested that an inhibition of canopy photosynthesis in the afternoon throughout a year and a decrease in daily GPP in dry periods at the Pasoh site may be explained by the depressed values of leaf photosynthesis coupled with patchy stomatal closure (Kosugi et al. 2012). Thus, severe depression in A caused by patchy stomatal closure should be considered in analyzing canopy CO2 exchange. Because dramatic decreases in daily GPP coupled with patchy stomatal closure may be seen in the future associated with unusually severe drought events (e.g., the El Nino Southern Oscillation), long-term observations of both leaf- and canopy-scale gas exchange are critical.

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