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VARIATION IN LEAF STOMATAL TRAITS OF 28 TREE SPECIES IN RELATION TO GAS EXCHANGE ALONG AN EDAPHIC GRADIENT IN A BORNEAN RAIN FOREST¹

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- *Premise of the study:* Quantifying variation in functional traits associated with shifts in the species composition of plant communities along resource gradients helps identify environmental attributes important for community assembly. Stomates regulate the balance between carbon assimilation and water status in plants. If environmental attributes affecting photosynthetic water-use efficiency govern species distribution along an edaphic gradient, then adaptive variation in stomatal traits of plant species specializing on different soils should reflect belowground resource availability.
- *Methods:* We tested this hypothesis by quantifying stomatal trait variation in understory saplings of 28 Bornean tree species in relation to gas exchange and water-use efficiency (WUE).
- *Key results:* Comparisons between congeneric specialists of the more fertile, moister clay and the less fertile, well-drained sandy loam revealed little evidence of similar shifts in stomatal traits across genera, nor was stomatal pore index correlated with g_{\max} , A_{\max} , or WUE (A_{\max}/g_{\max} or $\Delta^{13}\text{C}$), suggesting that stomates may be overbuilt in these shaded juveniles. A_{\max} was higher on sandy loam, likely due to higher understory irradiance there, but there were no other significant differences in gas exchange or WUE.
- *Conclusions:* Despite substantial diversity in stomatal anatomy, there were few strong relationships between stomatal, photosynthetic, and WUE traits in relation to soil resources. Routine differences in water availability therefore may not exert a dominant control on the distributions of these Bornean tree species. Furthermore, the clades represented by these 12 genera may possess alternative functional designs enabling photosynthetic WUE that is sufficient to these humid, understory environments, due to whole plant-functional integration of stomatal traits with other, unmeasured traits influencing gas exchange.

Key words: gas exchange; leaf functional traits; Malaysia; mixed dipterocarp forest; phenotypic plasticity; photosynthetic rate; phylogenetic comparisons; stable isotope ^{13}C ; stomatal conductance; water-use efficiency.

The ecological roles of plant species can be described by their functional traits, which are physiological, morphological, or anatomical characteristics facilitating the acquisition of light, water, carbon, and mineral nutrients, the essential resources for plant growth, survival, and reproduction. Functional traits often covary consistently along environmental gradients that shift in

resource availability (Chapin, 1980; Grime, 2001; Reich et al., 2003). Such suites of covarying functional traits are considered evidence of coordinated ecological strategies that reflect correlated evolution and that underlie floristic variation at many scales (Westoby et al., 2002; Ackerly, 2003; Reich et al., 2003). Analyses of functional trait variation on resource gradients therefore provide a means to link patterns in species composition with the environmental attributes that are important for community assembly (Weiher and Keddy, 1999; Ackerly, 2003).

Natural selection should favor plants with suites of functional traits that maximize their net rate of carbon gain, contingent on survival (Givnish, 1988). Traits that maximize carbon gain, such as the ability of the leaf to support high maximum stomatal conductance (g_{\max}) and fast rates of transpiration and photosynthesis (A_{\max}), should therefore experience positive selection in environments favoring fast growth, such as more fertile, mesic habitats (Chapin, 1980). Conversely, the capacity to avoid desiccation and increase water-use efficiency should reflect selection to minimize water loss in environments with reduced water availability, while simultaneously optimizing carbon gain (Chapin et al., 1993; Kozłowski and Pallardy, 2002). Because of their dual role in mediating the balance between carbon assimilation and water status, adaptive variation in stomatal traits of plant species specializing on different habitats should mirror the availability of resources affecting these functions. Here, we

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test this idea using data on stomatal and gas-exchange traits of tree species specializing on contrasting soil types in ever-wet Bornean rain forest.

As the terminal element of the hydraulic system in higher plants, stomates mediate gas exchange between the leaf and atmosphere. Stomatal apertures change as a result of the direct and indirect responses of guard cells to environmental factors that affect carbon assimilation and evaporation of water, such as light, ambient humidity, temperature, CO₂ concentration, and soil nutrient and water availability. The stomatal complex is therefore a pivotal structure that simultaneously acts to prevent desiccation of plant tissue and to vary the conductance to diffusion so as to optimize the balance between water vapor efflux and CO₂ influx to the leaf in a varying environment (Cowan, 1978; Cowan and Farquhar, 1977). Indeed, stomatal and vascular traits controlling gas- and liquid-phase transport are often found to be functionally coordinated with the capacity of the photosynthetic system to fix CO₂ (Meinzer, 2002; Sack et al., 2003; Maherali et al., 2008; Brodribb et al., 2009; Nobel, 2009).

Many environmental attributes affect carbon gain. Principal among these is light. In the understory of closed-canopy, tropical forest, which is the focus of our study, light is often extremely limiting (Chazdon and Pearcy, 1986). Identifying functional traits that determine carbon gain in low light is key to understanding the growth and survival of juvenile trees as they reach maturity. Understories of wet, tropical forest are also often humid and still, which reduces the leaf boundary layer conductance, which combined with low light, can limit the total leaf conductance to CO₂ and, hence, the delivery of CO₂ into the leaf (Hanba et al., 2003; Seibt et al., 2008). The availability of belowground resources also has important consequences for carbon gain. Soil moisture affects whole-plant hydraulic conductivity, which influences stomatal function by inducing stomatal movements that preserve a dynamic equilibrium between transpiration and hydraulic conductance while avoiding xylem cavitation (Meinzer et al., 1997; Brodribb et al., 2003). In addition, soil mineral nutrients are required to support photosynthetic catalytic capacity (e.g., chlorophyll and RuBisCO) (Chapin, 1980).

In northwest Borneo, the floristic composition of mixed dipterocarp rain forest varies along gradients of elevation and of soil texture, moisture, and fertility (Ashton, 1964; Baillie et al., 1987; Potts et al., 2002). Within a forest dynamics plot at Lambir Hills National Park (Lambir) in this region, most tree species have distributions restricted to particular soil types, causing high turnover in species composition along the gradient (Davies et al., 2005). Two soils, clay and sandy loam, are at the extremes of the moisture–fertility gradient: clay is more fertile and has greater water-holding capacity than sandy loam (Baillie et al., 2006) and is more productive in that it supports faster tree diameter growth rates (Russo et al., 2005).

We used this gradient to test hypotheses concerning the relationships between soil resource availability, anatomy of the stomatal complex, and gas-exchange and water-use efficiency parameters for 28 angiosperm tree species. If environmental attributes related to photosynthetic water-use efficiency govern species distribution on contrasting soil types, then variation in stomatal traits between clay vs. sandy loam specialists should reflect both the greater soil moisture and the larger gas exchange capacity required to support the faster growth rates observed on clay relative to sandy loam. We therefore expected tree species specializing on clay to have a higher density of larger stomates, resulting in larger stomatal pore area per unit lamina area on a leaf, relative to species specializing on sandy loam. We also

expected larger stomatal pore area to be associated with faster maximum stomatal conductance and carbon assimilation rates and less efficient water use among species.

We evaluated these predictions using interspecific comparisons of stomatal density and guard cell length for 24 Bornean tree species representing congeneric species pairs (12 genera), in which one was a specialist of sandy loam and the other was a specialist of clay, and intraspecific comparisons within four generalists (similarly abundant on both clay and sandy loam). Comparisons between congeneric specialists allow a phylogenetically controlled test of whether there are consistent soil-related shifts in stomatal, gas-exchange, and water-use efficiency traits, whereas comparisons between populations of generalist species growing on sandy loam and clay allow an assessment of phenotypic plasticity in stomatal traits. We predicted that the detection of within-species differences would parallel those predicted between specialists.

MATERIALS AND METHODS

Study site—This research was conducted in Lambir Hills National Park, Sarawak, Malaysia (4°11'N, 114°01'E). The Park encompasses 6800 ha of low-land, mixed dipterocarp forest with the highest tree species richness ever recorded in the palaeotropics (Ashton and Hall, 1992; Lee et al., 2002). Rainfall is ~3000 mm·y⁻¹, with all months averaging >100 mm (Watson, 1985). In 1991, a 52-ha plot (hereafter, Lambir) was established in the Park using methods from similar studies by the Center for Tropical Forest Science (Condit, 1998). All trees ≥1 cm in diameter, excluding palms (~1200 woody species), were tagged, mapped, identified, and their diameters measured to the nearest 1 mm, with recensuses every ~5 yr.

Floristic composition, stand structure, soils, and geomorphology of Lambir are described in Lee et al. (2002), Baillie et al. (2006), and Tan et al. (2009). Soils range from coarse loams that are sandstone-derived, leached, nutrient-depleted and well drained, with substantial raw humus (named humult in previous studies), to clays that are shale-derived, less nutrient-depleted, and not as well drained, with little raw humus (previously named udult). Davies et al. (2005) identified four soil types in Lambir based on variation in nutrients (total C, N, and P and exchangeable K, Ca, and Mg) and elevation at a 20 × 20 m scale. Ranked in ascending order of fertility and moisture, they are: sandy loam, loam, fine loam, and clay. Means of total nitrogen, total phosphorus, pH, exchangeable magnesium and calcium, and elevation for each of the four soil types are reported in Davies et al. (2005). Means for exchangeable and reserve nutrients, total exchangeable bases, cation exchange capacity, and base saturation the sandy loam and clay habitats are reported in Baillie et al. (2006).

The distributions of most tree species in Lambir are strongly biased with respect to these four soil types (Davies et al., 2005). Among the 764 species tested, 73% had distributions significantly aggregated on one or two soil types (specialists), and 13% had a neutral distribution with respect to soil fertility and topography (generalists). Here, we focused on 24 tree species specializing on the extremes of this edaphic gradient, sandy loam and clay, and four generalist species that are found on all soil types. From Davies et al. (2005), the means and standard errors (with their respective units) of selected mineral nutrient concentrations in surface soil of sandy loam and clay, respectively, are total N (%): 0.093 (0.001) and 0.107 (0.003); total P (mg/kg): 43.7 (0.7) and 133.6 (4.1); exchangeable Mg (cmol_c/kg): 0.12 (<0.01) and 0.70 (0.04).

Soil volumetric water content, vapor pressure deficit, and understory light availability (see below) were measured to characterize the abiotic environment on sandy loam and clay soil. Three replicate measurements of soil volumetric water content were made ~25 cm from each other at a location using a hand-held time-domain reflectometer (HydroSense, Campbell Scientific, Logan, Utah) on a single, rain-free day approximately every 2–8 wk from June 2004 to May 2005 (13 measurement dates); 17 locations were sampled on sandy loam and clay soil (34 locations in total). Differences between soil types were tested with a repeated-measures analysis of variance with date of measurement treated as a random effect and soil type as a fixed effect.

Ambient vapor pressure deficit (VPD; mmol/mol) was calculated based on air temperature and relative humidity (Murray, 1967; Campbell, 1977) measured at 1 m above the soil at seven locations on each soil type from October to December 2007 (21 day–location combinations on each soil). Sensors

(CS-215L, Campbell Scientific, Logan, Utah) were calibrated to each other in the laboratory and were rotated between locations every 3 d, and maximum and minimum relative humidity and air temperature ($^{\circ}\text{C}$) were recorded every 15 min.

Study species and individuals—The study species comprised 28 angiosperm tree species in 12 genera and 10 families (Table 1). All study species are shade-tolerant, C_3 plants, but *Macaranga lamellata* and *M. umbrosa* are the most light-demanding. Study species were selected to represent phylogenetically controlled comparisons and thus were congeneric species pairs of sandy loam and clay specialists. For four of the 12 genera, a generalist species was also included. Interspecific comparisons of sandy loam and clay specialists within genera allow a test of the hypothesis that, across different genera, there have been consistent shifts in stomatal traits linked to shifts in soil-type specialization by these tree species. Generalists were sampled from individuals growing on both sandy loam and clay and allow assessment of the phenotypic plasticity of stomatal traits in response to variation in edaphic resources.

All individual trees (1–3 cm in diameter at 1.3 m height) used in this study were selected randomly within the Lambir plot, conditional on appearing healthy with no major breaks in the stem or crown. To minimize variation in the light environment of study individuals, only trees growing in shade under fully closed canopy were selected. To account for the possibility that light availability affected estimates of gas exchange parameters and stomatal traits, hemispherical photographs were taken at 1-m above the soil, centered under the crown of most study individuals ($N = 98$ trees) with a Nikon CoolPix 4500 using a FC-E8 fisheye lens converter. The study individual's crown was moved out of the image when the photograph was taken. Photographs were taken under uniform sky light conditions: early in the morning, on overcast days, and after sunset. Images were analyzed with Winscanopy software (Regent Instruments, Canada); canopy openness (%) and total average daily photosynthetically active photon flux density under the canopy (PPFD; $\text{mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) were estimated for each tree. The mean canopy openness across all study individuals was 6.2% (standard deviation 1.3%). In analyses in which the individual was the unit of replication, PPFD was included as a covariate (see below). Student's t test was used to test for differences between the clay and sandy loam soils in canopy openness and PPFD.

Stomatal measurements—Leaves used for measurement of stomatal traits were collected from the Lambir plot in November 2005 from 3–8 trees per species ($N = 165$ trees; Table 1) growing under closed-canopy forest on their home soil type (specialists) or on both sandy loam and clay soils (generalists). Recent, mature, fully expanded leaves that were generally undamaged and largely free of epiphylls, lichens, or fungi were preferred and were immediately preserved in 100% ethanol. The terminal leaflet was used for measurements of compound leaves. Although impressions of the abaxial surface of the lamina of fresh leaves were initially made using fingernail varnish, we found the cell walls of guard cells to be poorly defined in the impressions, most likely because of the thick cuticles and layers of epicuticular wax typical of many tropical tree species. Instead, confocal microscopy (Olympus FluoView 500 on an Olympus BX60) of the preserved leaf tissue was used to make images of the abaxial and adaxial surfaces of laminae. Excitation was with a 405 nm diode laser, and emission spectra were detected in three channels: 430–460, 505–525, and 560–600 nm. The autofluorescence of the walls of the leaf epidermal cells and of the extracellular components is highly visible on micrographs and clearly outlines the walls of guard cells.

For each specimen, three micrographs of the abaxial surface of the lamina were taken at 200 \times or 400 \times magnification (Appendix S1, see Supplemental Data at <http://www.amjbot.org/cgi/content/full/ajb.0900344/DC1>). Images were taken at haphazardly selected, nonoverlapping locations on $\sim 2\text{-cm}^2$ sections of the leaf, taken from between the midrib of the lamina and its edge and in the middle of the length of the lamina. This area is likely to be the most photosynthetically active portion of the lamina. The density (number of stomates mm^{-2}), guard cell length (μm), and inner pore length (μm) were measured on micrographs using Image-J software (Abramoff et al., 2004). For density, only the stomates for which the entire pore was visible in the field of view were counted. Guard cell length and inner pore length were measured on three haphazardly selected stomates per image. The stomatal pore index (SPI; a dimensionless index of stomatal pore area per lamina area measuring the stomatal conducting capacity of the leaf), was calculated as (stomatal density) \times (guard cell length) 2 on the abaxial surface of the lamina (Sack et al., 2003).

A more limited data set on stomatal traits was collected using scanning electron microscopy (online Appendix S1). Scanning electron micrographs (400 \times

and 1200 \times magnification) were made at haphazardly selected, nonoverlapping locations on leaf punches ~ 5 mm in diameter; the punch was made immediately adjacent to the section of the leaf used for confocal micrographs. The guard cell lengths for 2–19 stomates were measured using Image-J for 1–2 trees per species. Mean guard cell length measured using images from confocal and SEM micrographs were correlated among species ($r = 0.81$, $P < 0.001$; Appendix S2, see online Supplemental Data), and all analyses were consistent between the two types of measurements. Therefore, the results presented are based on the more extensive data from the confocal micrographs.

Gas-exchange and water-use efficiency parameters—Recent, mature, fully expanded leaves that were largely free of epiphylls, lichens, and damage were selected for estimation of gas exchange parameters and water-use efficiency. Maximum stomatal conductance (g_{max} ; $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and carbon assimilation (A_{max} ; $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) rates were measured on naturally growing trees (1–3 cm in diameter) at Lambir on sunny days from June to October 2005 using a portable infra-red gas analyzer (LI-6400, LiCor Environmental Sciences, Lincoln, Nebraska, USA) on 2–4 individuals per species–soil-type combination ($N = 102$ trees). Leaves were first acclimated with light supplied by red-light-emitting diodes (LI-6400-02) having a photon flux density of $500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Once gas exchange rates had stabilized, light was increased progressively up to $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which was considered saturating. After gas exchange rates had again stabilized, several gas exchange measurements were recorded, and A_{max} and g_{max} were taken to be the averages of these measurements at steady state and saturating light. The CO_2 concentration of the reference air entering the leaf chamber was adjusted with a CO_2 mixer control unit to be 400 ppm CO_2 . All gas exchange data were collected in the mornings between 0800 and 1200 hours. The chamber temperature was controlled by maintaining the Peltier block temperature at 30°C . The relative humidity of the reference air was kept as close to ambient (usually 70–85%) as possible. Leaf vapor pressure deficits within the leaf chamber were < 1.8 kPa for all measurements. The air flow rate was 650 mL/min. Close examination of the data during and after measurements were complete showed no evidence of photoinhibition. Instantaneous water-use efficiency (WUE) was calculated as $A_{\text{max}}/g_{\text{max}}$.

The content of the stable isotope ^{13}C in leaves was used as an estimate of the WUE integrated over the period of leaf construction (integrated WUE; Farquhar et al., 1982). $\delta^{13}\text{C}$ was quantified on 1–2 leaves of 10–12 trees per species–soil-type combination. Depending on leaf size, 1–3 leaves per individual were dried at 60°C for a minimum of 72 h, finely ground together so as to pass through a 40-mesh screen, and analyzed for ^{13}C content on a Finnigan Delta $^+$ mass spectrometer (Finnigan MAT, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (NA1500 CHN combustion analyzer; Carlo Erba Strumazione, Milan, Italy) via a Finnigan ConFlo II Interface at the University of Arkansas Stable Isotope Analysis Laboratory, USA.

To account for the possibility that differences in the ^{13}C content of the ambient source CO_2 available for assimilation on each soil type might bias inferences concerning integrated WUE of species on sandy loam vs. clay, we quantified $\delta^{13}\text{C}$ in ambient air. Air samples at 1-m height in the understorey of 10 locations on each soil were collected in 100 mL glass flasks from 0800 to 1100 hours on 2 d using an air pump using methods similar to those in Lai et al. (2005). Air that had been dried with magnesium perchlorate was pumped through the flask for ~ 2 min, with the collection point ~ 10 m from the observer to prevent exhaled CO_2 from entering the flask. Flasks were shipped to the Stable Isotope Ratio Facility for Environmental Research at the University of Utah, where they were analyzed on a GC-IRMS system as in Schauer et al. (2003). Differences in $\delta^{13}\text{C}$ of the source CO_2 were tested using a linear, mixed-effect model with measurement date as a random effect and soil type as a fixed effect. $\delta^{13}\text{C}$ was significantly higher on the sandy loam soil (-9.39‰), relative to clay (-10.39‰ ; $t = 2.59$, $\text{df} = 17$, $p = 0.019$). $\Delta 13\text{C}$ of leaf tissue was calculated as $(\delta_a - \delta_p)/(1 + \delta_p)$ where δ_a is the $\delta^{13}\text{C}$ of the source CO_2 for the appropriate soil and δ_p is the $\delta^{13}\text{C}$ of the leaf tissue (Farquhar and Richards, 1984).

Statistical analysis—Differences in stomatal traits between clay and sandy loam specialists in each genus and between populations of generalists growing on clay or sandy loam in each species were analyzed using separate linear models for specialists and generalists. For specialists, differences between genera were modeled as fixed effects. The soil specialization (sandy loam or clay) of congeneric species was treated as a fixed effect nested within genus, because specialists were represented by different species in each case and were only sampled on their home soil type. For generalists, the model structure was the same, except the grouping factor was species. We chose a nested-effect design because we were interested in whether there was a significant effect of soil

TABLE 1. Taxonomy, abbreviation codes, growth form, leaf arrangement, and soil specialization pattern of the 28 study species in Bornean rain forest. Sample sizes (*N*) on sandy loam (SL) and clay (C) are the number of trees sampled for stomatal traits.

Code	Species	Family	Growth form	Leaf arrangement	Soil specialization	<i>N</i>
APOR	<i>Aporosa hosei</i> Merr.	Phyllanthaceae	subcanopy tree	simple, alternate	sandy loam	5
	<i>Aporosa sarawakensis</i> A. Schott	Phyllanthaceae	subcanopy tree	simple, alternate	clay	5
CALO	<i>Calophyllum ferrugineum</i> Merr.	Clusiaceae	tree	simple, opposite	sandy loam	6
	<i>Calophyllum gracilipes</i> Merr.	Clusiaceae	tree	simple, opposite	clay	6
DACR	<i>Dacryodes rostrata</i> (Bl.) Lam. f. <i>cuspidata</i> (Bl.) Lam.	Burseraceae	tree	compound, alternate	clay	3
	<i>Dacryodes expansa</i> (Ridl.) H.J. Lam	Burseraceae	tree	compound, alternate	sandy loam	5
DIOS	<i>Diospyros decipiens</i> C.B. Clarke	Ebenaceae	tree	simple, alternate	clay	5
	<i>Diospyros ferruginescens</i> Bakh.	Ebenaceae	tree	simple, alternate	sandy loam	5
DIOSPE	<i>Diospyros pendula</i> Hasselt ex Hassk.	Ebenaceae	tree	simple, alternate	generalist	4 C, 5 SL
DIPTCT	<i>Dipterocarpus confertus</i> Sloot.	Dipterocarpaceae	canopy tree	simple, alternate	generalist	5 C, 6 SL
DIPT	<i>Dipterocarpus globosus</i> Vesque	Dipterocarpaceae	canopy tree	simple, alternate	sandy loam	5
	<i>Dipterocarpus kunstleri</i> King	Dipterocarpaceae	tree	simple, alternate	clay	8
DRYO	<i>Dryobalanops aromatica</i> C.F. Gaertn.	Dipterocarpaceae	canopy tree	simple, alternate	sandy loam	5
	<i>Dryobalanops lanceolata</i> Burck	Dipterocarpaceae	canopy tree	simple, alternate	clay	7
MACA	<i>Macaranga lamellata</i> Whitmore	Euphorbiaceae	subcanopy tree	simple, whorled	sandy loam	6
	<i>Macaranga umbrosa</i> S.J. Davies	Euphorbiaceae	subcanopy tree	simple, whorled	clay	4
PALA	<i>Palaquium cryptocarpifolium</i> P. Royen	Sapotaceae	tree	simple, whorled	sandy loam	5
	<i>Palaquium dasyphyllum</i> Pierre ex Dubard	Sapotaceae	tree	simple, alternate	clay	5
POLY	<i>Polyalthia clavigera</i> King	Annonaceae	tree	simple, alternate	sandy loam	6
	<i>Polyalthia sarawakensis</i> Diels	Annonaceae	subcanopy tree	simple, alternate	clay	7
POLYRU	<i>Polyalthia rumphii</i> (Bl.) Merrill	Annonaceae	subcanopy tree	simple, alternate	generalist	4 C, 4 SL
RINO	<i>Rinorea bengalensis</i> (Wall.) Kuntze	Violaceae	subcanopy tree	simple, alternate	clay	4
	<i>Rinorea lanceolata</i> Kuntze	Violaceae	subcanopy tree	simple, alternate	sandy loam	5
SHOR	<i>Shorea inappendiculata</i> Burck	Dipterocarpaceae	canopy tree	simple, alternate	clay	5
	<i>Shorea laxa</i> Slooten	Dipterocarpaceae	canopy tree	simple, alternate	sandy loam	4
SHORPT	<i>Shorea patiensis</i> Ashton	Dipterocarpaceae	canopy tree	simple, alternate	generalist	5 C, 5 SL
EUGE	<i>Syzygium</i> cf. <i>grande</i> (Wight) Walp.	Myrtaceae	tree	simple, opposite	sandy loam	6
	<i>Syzygium kingii</i> (Merr.) Merr. & L.M. Perry	Myrtaceae	tree	simple, opposite	clay	5

specialization within each genus (or species, for generalists). For all models, PPFD was included as a covariate. We chose not to model genus (or species, for generalists) as a random effect because visual inspection of mean trait values showed a nearly uniform distribution, indicating that the assumption that they came from a common distribution was not appropriate. Consistent with this, model comparison based on the Akaike information criterion (Bolker, 2007) showed greater support for the fixed-effects, relative to the mixed-effects, models (online Appendix S3), indicating in each case that variation in the dependent variable was better explained using independent intercept parameters for each group. Analyses with and without PPFD as a covariate (the latter included a larger number of trees) were consistent with each other, and only analyses with PPFD are presented. We also tested whether there was a consistent soil-type effect on stomatal traits across all genera (for specialists) and species (for generalists). Means of stomatal traits were calculated for each taxonomic group, and linear models with soil type and genus (or species, for generalists) as main effects were fitted.

Comparisons between congeneric species pairs allow a test of whether there are consistent trait shifts between specialists of each soil type, but do not account for the deeper evolutionary history that our study species share. To test for correlated evolution between stomatal gas exchange and water-use efficiency traits, we used analyses of phylogenetically independent contrasts (PICs). We assembled a hypothesis of the phylogenetic relationships among the 28 species in our data based on the most recent, maximally resolved Angiosperm Phylogeny Group hypothesis (Stevens, 2001 onward) as represented in the software Phylomatic (available at <http://www.phylodiversity.net/phylomatic/>, part of the phylodiversity network, sponsored by Arnold Arboretum of Harvard University), using a speciation model of evolution for branch lengths (Grafen, 1989). The phylogeny was not fully bifurcating. We therefore generated 5000 topologies comprising random resolutions of all nodes containing polytomies, then calculated independent contrasts, and performed a Pearson correlation for each. If the 95th percentile of the distribution of probabilities from the Pearson correlation tests was ≤ 0.05 , then that correlation was considered statistically significant, and the correlation coefficient (*r*) was taken to be the 95th (for $r < 0$) or 5th (for $r > 0$) percentile of the distribution of coefficients. We used the R package ape (R Development Core Team, 2006) for all phylogenetic comparative analyses.

We examined whether gas exchange and water-use efficiency parameters differed between soil types, accounting for variation in the light environments of individual trees (PPFD). Replication of gas exchange data were insufficient to model the effect of soil type within genera, so data for soil specialists were pooled across all species and individuals. The 2–4 data points per species are, however, likely to be more similar to each other than to heterospecifics, potentially generating correlated errors. Therefore, models using individual-level data, but with a random effect on the individual tree, were used to account for the correlated error structure (Bolker, 2007). Relationships between species' means and PICs in stomatal, gas-exchange, and water-use traits were modeled using Pearson correlation (Zar, 1996). Log-transformation was used when required to improve normality of model residuals. The statistical package R (R Development Core Team, 2006) was used for all analyses.

RESULTS

Soil-related environmental variation—Soil volumetric water content was significantly higher on average throughout the year on the clay soil, relative to the sandy loam soil (no significant interaction between soil type and measurement date: $df = 406$, $t = 0.57$, $P = 0.569$; main effect of soil type: $df = 32$, $t = -6.70$, $P < 0.001$; Fig. 1). Vapor pressure deficit at a given air temperature tended to be higher on sandy loam than on clay, although air temperatures also tended to be higher on clay than on sandy loam (Appendix S4, see online Supplemental Data). Understory light availability was significantly greater on the sandy loam, relative to the clay soil (Welch's *t* test: $df = 91.2$, $t = -4.49$, $P < 0.001$), based on hemispherical photographs of the study trees. The mean and standard deviation (in parentheses) of average daily PPFD was 6.22 (1.24) and 5.15 (1.11) $\text{mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ on the sandy loam and clay soils, respectively, corresponding to a 1.6% difference in canopy openness.

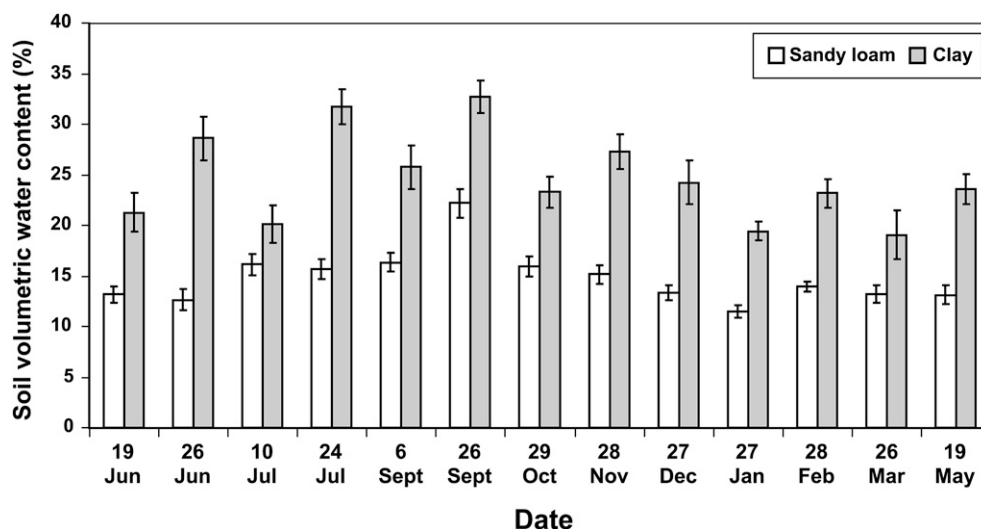


Fig. 1. Variation in soil volumetric water content between sandy loam and clay soils underlying mixed-dipterocarp forest in Borneo.

Stomatal traits—The 28 study species differed substantially in stomatal form (online Appendix S1) and varied 15-fold in mean stomatal density, 3-fold in guard cell length, and 6-fold in stomatal pore length (online Appendix S5). Mean guard cell length and stomatal pore length were highly correlated among species ($r = 0.90$, $P < 0.001$). There was a significant negative correlation between species' mean stomatal density and guard cell length (species' values: $r = -0.72$, $P < 0.001$; PICs: $r = -0.72$; $P = 0.003$; Fig. 2). Based on species' values, this relationship was fit by a power-law with a scaling exponent of -1.78 (SE = 0.34; $\log(\text{density}) = -1.78 \times \log(\text{length}) + 10.47$; $F_{1,26} = 27.91$, $P < 0.001$ $R^2 = 0.50$).

Most species had few or no stomates on the adaxial surface of the lamina, with the exception of three clay specialists, *Aporosa sarawakensis*, *Rinorea bengalensis*, and *Syzygium kingii*. Adaxial densities of stomates were appreciably lower than the abaxial densities for *A. sarawakensis* and *S. kingii*. They were more comparable, but still significantly lower, for *R. bengalensis* (Student's t test: $t = -5.90$, $df = 3.3$, $P = 0.008$). Sizes of stomates for *R. bengalensis* were similar on the abaxial and adaxial surfaces (Student's t test: $t = -1.24$, $df = 5.54$, $P = 0.263$). *Macaranga lamellata*, a sandy loam specialist, was observed to have papillate structures on the leaf, with stomates embedded within them (online Appendix S1).

Soil-related inter- and intraspecific variation in stomatal traits—Differences between soil specialists within each genus explained more of the total variation in stomatal traits than did variation in light availability for all three stomatal traits (online Appendix S6). Stomatal traits varied greatly between soil specialists within each genus (Fig. 3), but not always in the predicted direction. We predicted that clay specialists would have significantly greater stomatal densities than sandy loam specialists, and this was true of the comparisons for three of 12 genera (*Aporosa*, *Diospyros*, and *Syzygium*; Fig. 3A). Two genera, *Dipterocarpus* and *Polyalthia*, had significantly greater stomatal densities on the leaves of sandy loam specialists, a pattern opposite to that predicted. For the remaining seven genera, there was no significant variation between specialists.

Clay specialists were expected to have leaves with significantly larger stomates (guard cell length) than sandy loam spe-

cialists, and this was true of the comparisons for four of 12 genera (*Dacryodes*, *Dipterocarpus*, *Palaquium*, and *Polyalthia*; Fig. 3B). Three genera (*Aporosa*, *Diospyros*, and *Syzygium*) exhibited significantly larger stomates on the leaves of sandy loam specialists, a pattern opposite to that predicted. For the other five genera, there was no significant variation between specialists. Largely due to the negative correlation between stomatal density and size (Fig. 2), the genera that were consistent or inconsistent with our predictions shifted in the analyses of stomatal density and size.

We predicted that clay specialists would have significantly greater stomatal conducting capacity, as measured by the

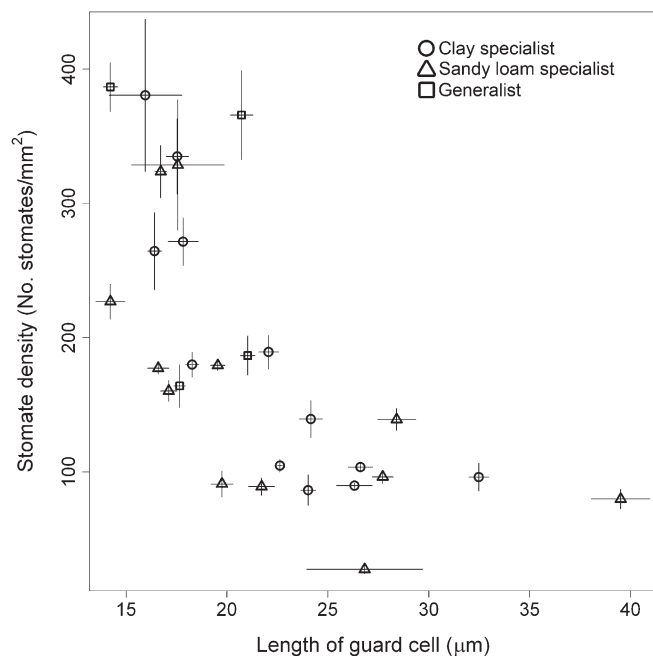


Fig. 2. The relationship between mean stomatal density and guard cell length among 28 Bornean tree species. Triangles and circles represent species specializing on sandy loam and clay soils, respectively; squares represent generalists. Error bars are ± 1 standard error.

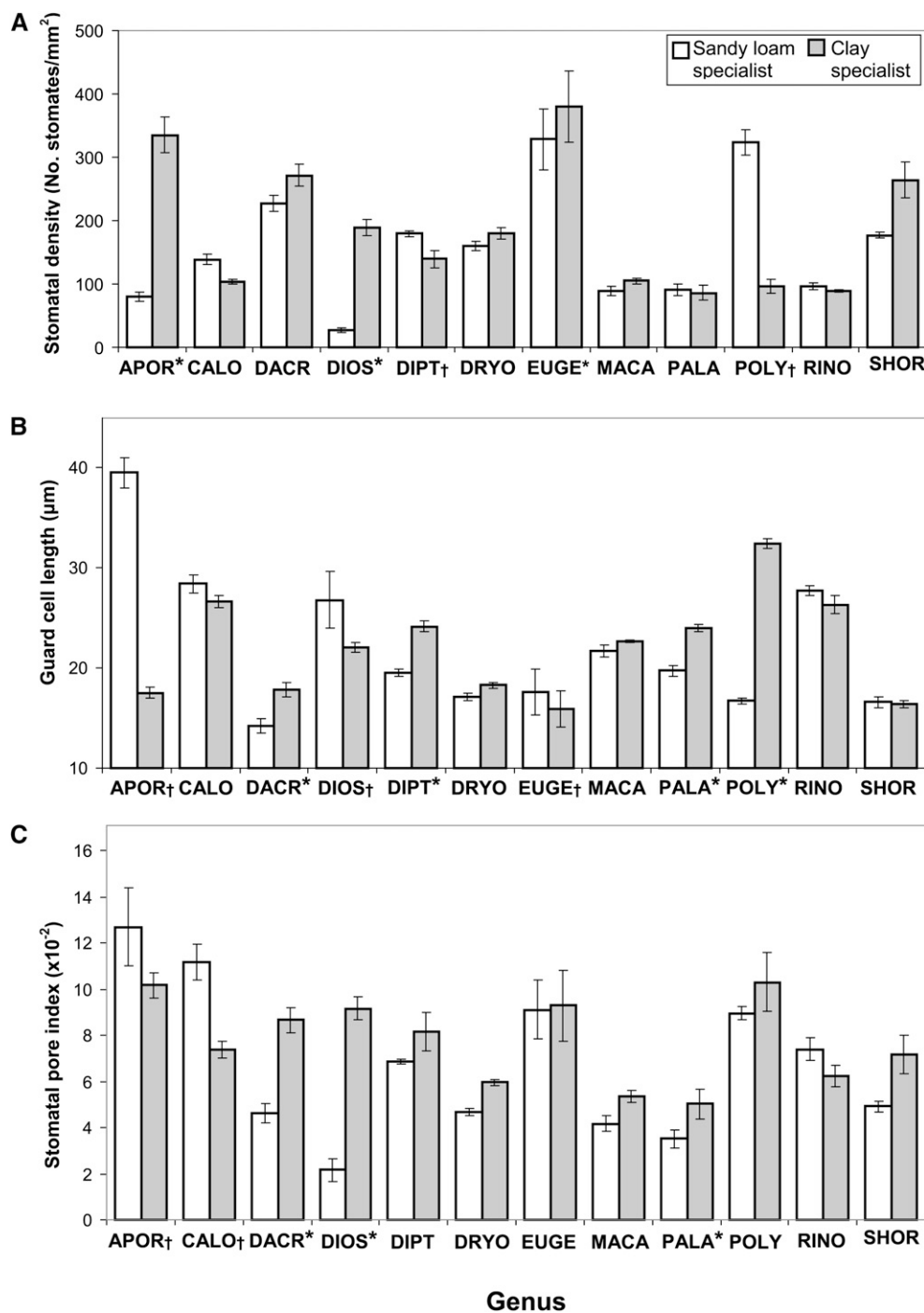


Fig. 3. Interspecific variation in stomatal density, guard cell length, and stomatal pore index between tree species specializing on sandy loam or clay soils in Bornean rain forest. Abbreviations for genera are given in Table 1. Means \pm 1 standard error are shown. Asterisks and daggers on the x-axis indicate genera in which there were significant differences ($P < 0.05$) between soil specialists in the predicted, or opposite to the predicted, direction, respectively.

stomatal pore index (SPI), than would the sandy loam specialists, and for three of 12 genera, this prediction was borne out (*Dacryodes*, *Diospyros*, and *Palaquium*; Fig. 3C). For two genera (*Aporosa* and *Calophyllum*), the SPI of sandy loam specialists significantly exceeded that of clay specialists, contrary to our prediction. For the remaining six genera, there were no significant differences between soil specialists in SPI.

There was little soil-related phenotypic plasticity in stomatal traits among soil generalists (Fig. 4). None of the four generalist species exhibited statistically significant variation between populations growing on sandy loam vs. clay soils in any stomatal trait (online Appendix S7). Based on mean trait values for soil specialists and for populations of generalists on each soil type across all taxonomic groups, there were no significant differences between soils for any stomatal trait, which is likely due

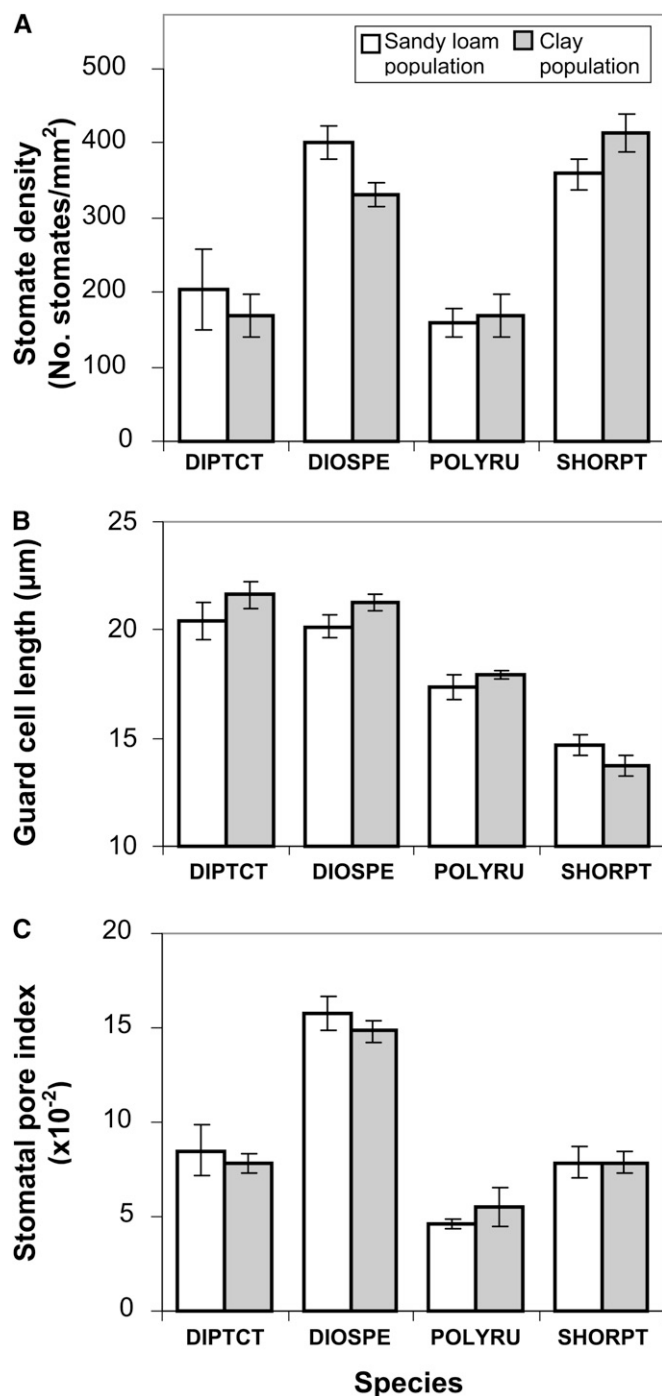


Fig. 4. Intraspecific variation in stomatal density, guard cell length, and stomatal pore index between populations of four generalist tree species growing on sandy loam or clay soils in Bornean rain forest. Abbreviations for genera are given in Table 1. Means ± 1 standard error are shown. There were no significant differences between populations on each soil ($p > 0.05$).

to the fact the direction of the significant effects of soil specialization differed among genera.

Covariation of gas exchange and water-use efficiency traits with light availability—Across all individuals, there was only

marginally statistically significant covariation of daily average PPFD with A_{\max} ($P = 0.049$) and g_{\max} ($P = 0.054$). There was no significant covariation of PPFD with instantaneous WUE ($P = 0.434$). Based on species mean values, there were no differences in g_{\max} or instantaneous or integrated WUE between soil specialists ($F_{1,11} = 1.29$, $P = 0.280$; $F_{1,11} = 0.05$, $P = 0.834$; $F_{1,11} = 3.87$, $P = 0.075$; respectively). However, A_{\max} was higher for sandy loam than for clay specialists (5.65 vs. 4.67 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively; $F_{1,11} = 7.02$, $P = 0.023$). Among soil specialists, the higher A_{\max} on sandy loam remained significant after accounting for the light environment of individual trees (soil main effect, $P = 0.018$; online Appendix S8), and individuals of sandy loam specialists displayed a slightly stronger relationship between A_{\max} and light (sandy loam: slope = 0.04 , standard error of slope = 0.03 , $P = 0.137$, $\text{df} = 25$; clay: slope = 0.01 , standard error of slope = 0.04 , $P = 0.716$, $\text{df} = 18$). This is likely explained by acclimation to the overall higher understory irradiance on sandy loam than clay. A similar but weaker trend was observed in mean A_{\max} of generalist individuals (5.18 vs. 4.93 $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for sandy loam vs. clay), but the effect of soil type was not significant, although this test had lower replication than that for specialists ($F_{1,14} = 0.15$, $P = 0.705$).

Relationships between stomatal and gas exchange or water-use efficiency traits—There were relatively few significant interspecific relationships between stomatal traits and either gas exchange or water-use efficiency variables, based on species' values (Fig. 5) and PICs. Based on species' values and PICs, A_{\max} was not significantly related to any stomatal trait. Similarly, contrary to expectation, g_{\max} was not related to mean SPI (Fig. 5J), nor to either of the components of SPI, stomatal size or density (Fig. 5B and 5F).

Although there was no significant relationship between the time-integrated estimate of WUE ($\Delta^{13}\text{C}$ of leaves) and any stomatal trait based on species' values and PICs, there was a strong negative relationship between instantaneous WUE (A_{\max}/g_{\max}) and stomatal size (Fig. 5C) and, more weakly, with stomatal density (Fig. 5G) based on species' values. For the relationship between instantaneous WUE and stomatal size, the shape of the scatterplot suggests a boundary relationship in that, for a given guard cell length, there appears to be a maximum attainable instantaneous WUE. Thus, variation in instantaneous WUE appears to be driven more by variation in stomatal size than density or total conducting capacity (as measured by SPI), and increases in instantaneous WUE appear to require reduction in the size of stomates. However, this relationship was not statistically significant after accounting for phylogeny using PICs.

DISCUSSION

Despite high rainfall, interspecific variation in nutrient- and water-use efficiency and drought-tolerance have been shown to be important determinants of the distributions of tropical tree species (Brunig, 1974; Baltzer et al., 2005, 2008; Engelbrecht et al., 2007), and periodic drought at Lambir and elsewhere in Southeast Asia can increase tree mortality rates (Becker and Wong, 1993; Burslem et al., 1996; Delissio and Primack, 2003; Potts, 2003; Newbery and Lingenfelder, 2004). In addition, studies based on theory and observation generally have found coordination among leaf hydraulic architecture, resistance to water vapor diffusion, and stomatal traits (Parlange and Waggoner, 1970; Sack et al., 2003; Franks, 2004). We therefore

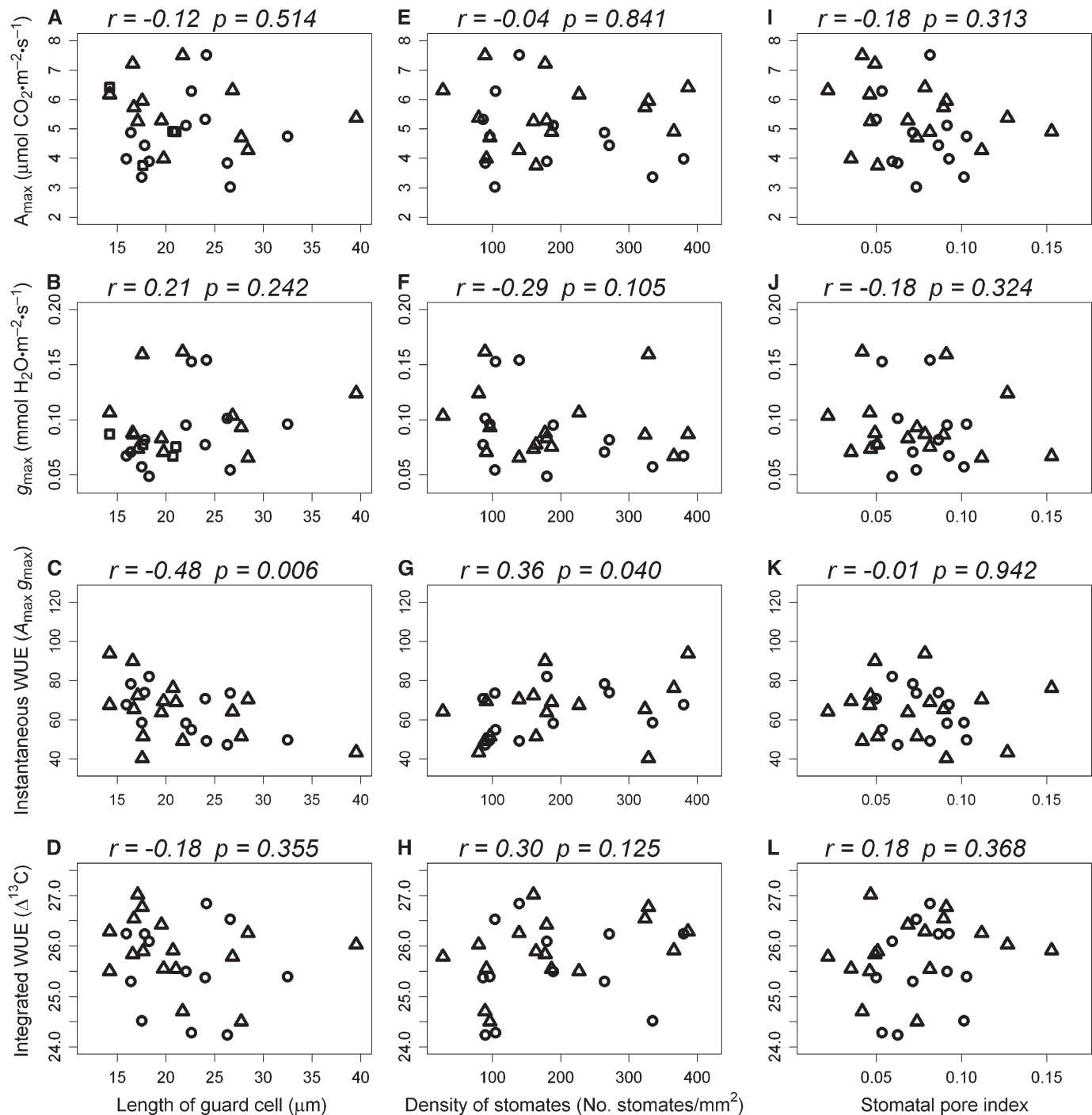


Fig. 5. The correlation of mean stomatal density, mean guard cell length, and mean stomatal pore index with gas exchange and water-use efficiency parameters for 28 Bornean tree species. Pearson correlation coefficients (r) and probabilities (p) are listed above each plot. Triangles and circles represent species specializing on sandy loam and clay soils, respectively; squares represent generalists.

expected stomatal traits of tree species specializing on different soil types to reflect variation in the availability of soil moisture and nutrients and for variation between stomatal, gas exchange, and WUE traits to be correlated. Our results, however, provide only weak support for these ideas: SPI, a measure of the maximum stomatal conducting capacity of a leaf, was larger for tree species specializing on the more fertile, moister clay soil in

only three of 12 congeneric comparisons. Furthermore, the substantial diversity in stomatal anatomy observed among these Bornean tree species did not strongly correlate with function. Of the gas exchange and water-use efficiency parameters that we measured, the only significant relationship with a stomatal trait was between species' mean instantaneous water-use efficiency and stomatal size.

Implications for understanding species' distributions—Our findings have a number of important implications for understanding the distributions of tree species along resource gradients. First, gradients vary simultaneously in multiple resources, and at least for some taxa at Lambir, soil moisture may not be the key factor directly determining species' distributions along this edaphic gradient. Although this inference is in contrast to some findings in other tropical forests (e.g., Baltzer et al., 2005, 2008; Engelbrecht et al., 2007), there is no reason to expect that water should be the most important limiting resource in all tropical forests. Furthermore, tolerance of catastrophic drought, rather than responses to the routine differences in water availability that are measured by water-use efficiency parameters, may be more important in determining species' distributions in this ever-wet forest (Becker and Wong, 1993; Burslem et al., 1996; Delissio and Primack, 2003; Potts, 2003; Newbery and Lingenfelder, 2004; Blackman et al., 2009). Thus, although many studies have demonstrated that tree species are associated with and exhibit varying performance in particular soil-defined habitat types (e.g., Clark et al., 1998; Davies et al., 2005; Russo et al., 2005; Paoli et al., 2006; Comita and Engelbrecht, 2009), fewer studies have evaluated which of the several possible resources that vary along gradients, and the nature of their restriction, are the most important in explaining those distributions.

Second, the fact that the availability of different types of resources can covary inversely along soil-defined resource gradients (Coomes and Grubb, 2000; present study) has consequences for natural selection on and the resulting diversity of functional traits. At Lambir, the clay soil is more productive in that it supports significantly faster diameter growth than does the less-fertile, better-drained sandy loam soil (Russo et al., 2005). In such productive habitats, species with higher A_{\max} would presumably have a competitive advantage (Poorter and Garnier, 1999), which could indirectly select for coordinated increases in g_{\max} and SPI (Geber and Dawson, 1997; Arntz and Delph, 2001). In contrast to the availability of soil resources at Lambir, understory light is more limited on clay than on sandy loam due to denser canopy cover, and this would presumably result in opposing selection on these traits. A_{\max} of saplings appeared to respond more to variation in the availability of understory light than directly to availability of soil resources, since A_{\max} was higher and displayed a slightly stronger relationship with light on the less fertile, well-drained sandy loam soil, compared to clay. Functional traits are thus likely to experience conflicting environmental selection pressures, even on the same habitat type, which has the potential to maintain genetic variation and result in the large within-species variance in trait values that we observed (Levins, 1968).

Third, the fitness consequences of variation in a particular trait, such as SPI, depend on its integration with other traits in the whole plant (Reich et al., 2003; Marks and Lechowicz, 2006). In other words, traits can be integrated functionally in quantitatively different ways that result in similar whole-plant performance: such higher-order functional integration has been shown in a simulation model of tree seedling growth and survival to contribute to functional trait diversity in forests via selection for alternative functional designs (Marks and Lechowicz, 2006). Stomatal, gas exchange, and water-use efficiency traits should therefore be considered as only part of the overall functional program influencing the performance of these Bornean tree species, and they are expected to be integrated with other traits, such as those related to hydraulic conductance and vascular anatomy (Meinzer et al., 1997; Brodribb et al., 2003; Sack,

et al., 2003). For example, all else being equal, similar rates of CO_2 diffusion to the sites of carboxylation could be achieved by different combinations of SPI, stomatal pore depth, and thickness of the mesophyll (Nobel, 2009). Our results suggest that in the clades represented at Lambir by our 12 study genera, alternative functional designs for gas exchange may contribute both to the diversity in stomatal traits and to the lack of correlation between stomatal traits and photosynthetic and WUE responses observed along this edaphic gradient.

Stomatal traits in relation to WUE and gas exchange—The weak relationships between stomatal, gas exchange, and WUE traits may also be explained because stomatal anatomy (SPI) is not the only determinant of conductance. In humid, shaded understories, where our study was conducted, carbon fixation is strongly light-limited. Our results suggest that carbon assimilation may have been more constrained by light limitation than by any direct, soil-related effects that stomatal traits may have on water loss from or CO_2 diffusion to the sites of carboxylation in the leaf. Even in the saturating light of gas exchange measurements, it is possible that stomates may not have been fully open, due to acclimation to their extremely shaded environments (Chazdon and Pearcy, 1986). If so, our g_{\max} estimates would not reflect the maximal conducting capacity of stomates of these dimensions and densities. Instead, our data suggest that the saplings of these Bornean tree species have higher SPI than is necessary to achieve the g_{\max} and A_{\max} that are realizable in the shaded understory. Cornelissen et al. (2003) also found the leaves of shaded, laboratory-grown seedlings to be over-built in terms of SPI, relative to leaves on adults in higher irradiance. Furthermore, conductance inversely scales with stomatal pore depth [$g_{\max} \propto \text{SPI}/(\text{stomatal depth} + \text{stomatal pore radius})$; (Ting and Loomis, 1963; Parlange and Waggoner, 1970; Ajiri et al., 1973; Sack et al., 2003)]. We lack information on stomatal pore depths of these Bornean tree species, but given the variability in stomatal form (Appendix S1), they may also vary in pore depth, which could account for the lack of correlation between g_{\max} and SPI. Interference between neighboring stomates and wax occlusions over the pore would also increase resistance to diffusion through stomates (Ting and Loomis, 1965; Parlange and Waggoner, 1970; Ajiri et al., 1973). The dispersion of stomates on the epidermis varied from appearing clustered (e.g., *Dryobalanops* spp.) to more regular (e.g., *Aporosa* spp.), and stomatal pores of some species appeared more occluded than those of others (Appendix S1). Variation among species in these features would also act to decouple the inter-specific relationship between g_{\max} and SPI.

Stomatal pore index is a function of stomatal density and guard cell length, and we found these latter traits to be negatively related, in parallel to other studies (Salisbury, 1928; Grubb et al., 1975; Bongers and Popma, 1990; Sack et al., 2003, 2005). The scaling exponent fit to our data were within two standard errors of that found for six temperate tree species (Sack et al., 2003). Increases in SPI can therefore be achieved by increases in stomatal density or size, but apparently not both simultaneously. Such a scaling constraint on SPI might be expected if either the size or density is developmentally fixed based on the rate of leaf expansion (Salisbury, 1928) or if the maximal capacity for stomatal conductance is coordinated with the maximal capacity for carbon fixation so as not to exceed it (Cowan, 1977; Farquhar et al., 1980). If the stomatal size–density relationship were driven purely by constraints associated with leaf expansion, then we would not necessarily expect to

see an evolutionary correlation between these traits. The fact that the analyses based on PICs showed a significant relationship provides more support for the hypothesis that evolutionary coordination between the maximal capacities for stomatal conductance and C-fixation (Geber and Dawson, 1997; Arntz and Delph, 2001) may underlie the scaling relationship.

Published findings relating variation in stomatal traits to water stress and mineral nutrient availability are inconsistent. Studies in Southeast Asian heath and Brazilian caatinga forests, which can experience extreme, periodic drought stress, also failed to find a relation between leaf anatomy, including stomatal characters, and traits related to drought tolerance (Ferri, 1960; Peace and McDonald, 1981; Medina et al., 1990; Juhrendt et al., 2004). Moreover, another study of Bornean tree species also observed no significant covariation between stomatal and gas exchange traits (Cao and Booth, 2001). Nevertheless, our results contrast with studies finding SPI and stomatal density to be associated with maximal stomatal or whole-leaf epidermal conductance (Koerner et al., 1979; Sack et al., 2003, 2004; Juhrendt et al., 2004).

Phenotypic plasticity in stomatal traits—We found no evidence of phenotypic plasticity in stomatal density or guard cell length among conspecific individuals that could be explained by edaphic variation. Applying this finding to the soil specialists suggests that significant differences between specialists in stomatal traits, when they were found, were likely to be better explained by genotypic differences between species than by phenotypic plasticity in response to soil-related environmental variation. Our result is consistent with an experiment in which there was no change in the stomatal density or guard cell length of individuals fertilized with nitrogen, relative to unfertilized controls, for six temperate deciduous tree species (Aasamaa et al., 2001). That study did, however, find that experimentally water-stressed individuals exhibited reduced guard cell length, but no change in stomatal density, relative to unstressed controls.

Stomatal traits are generally found to vary between sun and shade leaves of the same species (Abrams and Kubiske, 1990; Poole et al., 1996; Herrick et al., 2004; Sack et al., 2006). The fact that we did not observe within-species differences in stomatal traits of generalists growing on sandy loam vs. clay suggests that the magnitude of variation between soil types in understory light was not strong enough to induce them. The lack of plasticity that we observed may partly result from the fact that all generalists examined for plasticity are very shade tolerant. Extremely shade-tolerant species are considered to be less flexible in growth rate, structure, and physiology, compared to more light-demanding species (Bazzaz, 1979; Abrams and Kubiske, 1990; Chazdon, 1992).

Conclusions—Identifying the functional basis of performance differences of juvenile trees as they compete for a spot in the canopy is critical to understanding forest community dynamics. In tropical forests, it is still a matter of debate whether mineral nutrient limitation, water deficit, or herbivory most strongly controls functional trait variation and the distribution of tree species along edaphic gradients (Janzen, 1974; Peace and McDonald, 1981; Medina et al., 1990; Palmiotto et al., 2004; Baltzer et al., 2005, 2008; Fine et al., 2006). Denser canopies, however, form over more fertile, compared with less fertile, soils (Ashton, 1964; Coomes and Grubb, 2000; present study). As such, light availability is also likely to play a significant role in determining both the performance differences of

trees in the understory and the functional trait variation that underlies those differences, even on edaphically defined resource gradients. Thus, multiple environmental factors and their interactions may make it difficult for low-dimensional functional trait trade-offs to be the principal determinants of performance (Ricklefs, 1977; Grubb, 1992; Clark et al., 2007). Indeed, we found strong covariation between stomatal density and size, but not between anatomical and functional traits of stomates among shaded juveniles of Bornean tree species. Instead, higher-order functional integration of traits (sensu Marks and Lechowicz, 2006) is likely to be more closely linked to the performance differences between tree species at the sapling stage that ultimately determine forest community structure.

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