Variations of leaf and stem traits in relation to altitudinal distributions of 12 Fagaceae species of Mount Kinabalu, Borneo

Kiyosada Kawai1,2*, Berhaman Ahmad1, Ismawati Palle3 and Naoki Okada4

1 Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan
2 Department of Plant Ecology, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan
3 Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
4 Graduate School of Global Environmental Studies, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501, Japan
* Corresponding author: k.kawaikx@gmail.com

ABSTRACT

Fagaceae in Southeast Asia shows diverse distributions along altitude, but the underlying mechanisms remain unclear. Since species' traits (morphological and physiological characteristics) can dictate their resource use strategies (e.g., the way of resource acquisition and allocation), they potentially influence their distributions along altitude. Here, to examine the linkage between species' traits and altitude, we quantified the variation of 10 leaf and stem traits of 12 Fagaceae species growing in Mount Kinabalu, Borneo and related traits to species' lowest and highest limits and altitudinal range. Species with high leaf dry mass per area (LMA) and lamina thickness showed higher upper limits of altitudinal distribution whereas no traits were correlated with the lower limits. LMA and leaf nitrogen content positively covaried with species' altitudinal range. These results demonstrate that species with conservative resource use had higher altitudinal limits and wider altitudinal range, highlighting the role of leaf traits in the diversification of altitudinal distributions among closely-related species. We further suggest that diversifications in leaf traits potentially lead to the coexistence of Fagaceae species in tropical montane forests.

Key words: altitudinal distribution, Borneo, Fagaceae, leaf vein, tropical montane forest

INTRODUCTION

Tropical trees show species-specific distributions along the environmental gradients at local (e.g., Davies et al. 1998; Itoh et al. 2003; Russo et al. 2005) and regional scales (e.g., John et al. 2007; Toledo et al. 2012; Condit et al. 2013). Such species' distributions, in turn greatly influence spatial patterns of forest structure and functions in the tropics (e.g., Culmsee et al. 2010). However, the underlying mechanisms of species' distributions are not fully understood.

Recently, studies of plant traits (morphological, phenological, and physiological characteristics) have shed light on this mechanism. In this trait-based approach, species' traits determine their resource use strategies (e.g., acquisition and allocation) of vital resources (water, nutrient, and carbon) and thus demography (e.g., rates of growth and survival), finally influencing fitness in a given environment (McGill et al. 2006; Viole et al. 2007). So far, several traits have been identified to explain species' distributions in the tropical regions. For example, the water potential at which 50% of stem conductivity is lost was negatively correlated with the mean annual precipitation of its habitat in Callitris species (Larter et al. 2017). Similar linkages between species' drought tolerance and water availability were observed in various angiosperms (Blackman et al. 2012; Trueba et al. 2017).

Tropical mountains generally contain many environmental gradients along altitude such as air temperature, precipitation, solar radiation, frequency of low clouds, and soil fertility (Grubb 1977; Bruijnzeel and Veneklaas 1998; Tanner et al. 1998; Körner 2007). Therefore, it is expected that species' resource use of different resources may underpin their altitudinal distributions. Here, using Fagaceae in Southeast Asia, we related species' leaf and stem traits which reflect carbon, water, and nutrient use to their altitudinal limits. This taxon-specific approach would result in reduced interspecific trait variability because evolutionary processes at lower taxonomic level usually do not produce extreme phenotypic variations observed at higher taxonomic level. Such tests, however, have the potential to establish the truly evolutionary coordination between traits and distribution which may not emerge at a broad phylogenetic scale (Cavender-Bares 2019).

Fagaceae is an important component of tropical
montane forests of Southeast Asia in terms of abundance and diversity (Aiba and Kitayama 1999; Culmsee et al. 2010). This family also shows diverse species-specific distributions both horizontally (Cavender-Bares et al. 2004; Aguilar-Romero et al. 2016) and altitudinally (Beam et al. 2001; Fujii et al. 2006; Har 2019). In subtropical forests of Florida, it is shown that leaf and stem traits of *Quercus* species correspond to their distribution along the gradients of soil moisture, nutrient availability, and fire regime and that trade-offs among traits promote species’ niche partitioning and coexistence at a local scale (Cavender-Bares et al. 2004). Even at a broader scale, Koehler et al. (2012) found that the trade-off between freezing tolerance and growth rate limits species’ northern and southern limits for four *Quercus* species occurring in temperate-tropical transitions. Therefore, Fagaceae is an ideal lineage to investigate the mechanisms of diversification in altitudinal distributions based on plant traits.

In order to identify traits responsible for altitudinal distributions, we quantified 10 leaf and stem traits that reflect resource use strategies of water, nutrient, and carbon (Table 1) of 12 Fagaceae species in Mount Kinabalu, Borneo. Then, we examined the relationships between these traits and species’ elevational limits (lowest and highest) and range. In Mount Kinabalu, environmental factors such as temperature, vapor pressure deficit (VPD), annual water budgets (mean annual rainfall – mean annual evaporation), and soil nutrients vary greatly along altitude (Kitayama 1992; Kitayama and Aiba 2002). Among these factors, the mean annual temperature declines linearly with elevation, potentially acting as strong selection pressure on organisms. Indeed, boundaries among four altitudinal vegetation zones in Mt. Kinabalu coincide with temperature thresholds of latitudinal vegetation zones, indicating that temperature is a primary factor controlling species’ distributions along altitude in a similar way to the latitudinal gradient (Kitayama 1992). Other important environmental variables such as atmospheric aridity and soil nutrients do not show such consistent change along altitude (Kitayama et al. 2000; Kitayama and Aiba 2002): for example, mean VPD and water budgets dramatically decreased and increased, respectively, at around 2000 m a.s.l. coinciding with the lower limit of the cloud belt. On the other hand, wind velocity increases at higher altitudes (Kitayama et al. 1999).

Then, based on the responses of traits to temperature along latitude (e.g., Wright et al. 2005; Kikuzawa et al. 2013), we predicted that species with resource conservation strategies, associated with high leaf dry mass per area (LMA), low leaf nitrogen concentration per dry mass (N\(\text{max}\)), low minor vein density (VLA\(\text{min}\)), and high wood

### Table 1. Leaf and stem traits as well as altitudinal distributions, including the minimum species mean, the mean of species mean, and standard deviation (SD) of species mean of 12 Fagaceae species on Mount Kinabalu, Borneo.

| Trait                              | Symbol | Unit  | Mean (SD)       | Min  | Max  | Group | Ecological significance                                      |
|------------------------------------|--------|-------|-----------------|------|------|-------|-------------------------------------------------------------|
| Leaf dry mass per area             | LMA    | g m\(^{-2}\) | 161.0 (45.8) | 98.7 | 237.3 | Leaf  | Leaf longevity, photosynthetic rate                         |
| Leaf dry matter content            | LDNC   | g g\(^{-1}\) | 0.52 (0.04)   | 0.46 | 0.56  | Leaf  | Drought tolerance                                           |
| Lamina thickness                   | LT     | mm    | 0.30 (0.08)    | 0.17 | 0.45  | Leaf  | Mechanical resistance, leaf longevity                       |
| Leaf area\(^{1}\)                  | LA     | cm\(^2\) | 61.9 (65.7)    | 16.7 | 260.8 | Leaf  | Efficiency of light capture and heat exchange, drought tolerance |
| Leaf carbon isotope ratio          | \(\delta^{13}\)C | %       | -31.6 (0.68) | -32.9 | -30.8 | Leaf  | Long term water use efficiency                              |
| Dry-mass based leaf carbon concentration | C\(_{\text{max}}\) | %       | 53.3 (1.40) | 50.3 | 55.3  | Leaf  | Cell wall investment, shade tolerance                       |
| Dry-mass based leaf nitrogen concentration | N\(_{\text{max}}\) | %       | 1.54 (0.21) | 1.20 | 1.83  | Leaf  | Photosynthetic rate, respiration rate                       |
| Area-based leaf nitrogen concentration | N\(_{\text{max}}\) | g m\(^{-2}\) | 2.42 (0.50) | 1.51 | 3.20  | Leaf  | Photosynthetic rate, respiration rate                       |
| Minor vein density\(^{1}\)        | VLA\(_{\text{min}}\) | mm m\(^{-2}\) | 8.46 (2.23) | 5.37 | 14.04 | Leaf  | Water transport capacity                                    |
| Wood density                       | WD     | g cm\(^{-2}\) | 0.71 (0.07) | 0.54 | 0.82  | Stem  | Drought tolerance, shade tolerance, defence from abiotic and biotic stresses |

**Altitudinal distribution**

|                      |                      |                      |                      |                      |                      |                      |                      |
|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Lowest altitude      | ALT\(_{\text{min}}\) | m                    | 1142 (348)           | 500                  | 1800                 | –                    | –                    |
| Highest altitude\(^{1}\) | ALT\(_{\text{min}}\) | m                    | 2217 (524)           | 1700                 | 3400                 | –                    | –                    |
| Range of altitudinal distribution\(^{1}\) | ALT\(_{\text{min}}\) | m                    | 1050 (565)           | 500                  | 2100                 | –                    | –                    |

\(^{1}\) Log\(_{\text{er}}\)-transformed before analysis. For each species’ trait data, see Appendix 1.
Variation in traits and altitudinal distribution of tropical Fagaceae

Density (WD) can extend their distribution to high elevation but not to low elevation due to limited competitive abilities. On the other hand, species with the opposite suite of traits representing the acquisitive resource use would be distributed to low elevation but not to high elevation due to limited stress tolerance. We first tested this prediction and then examined the coordination among traits to find the physiological constraints underlying the diversification in species’ distribution.

MATERIALS AND METHODS

Plant species, habitat and altitudinal distributions

This study was conducted in Mount Kinabalu, Borneo (6°05’N, 116°33’E, 4095 m a.s.l.) where forest structure and species composition vary greatly among altitudes and soil substrates (Kitayama 1992; Aiba and Kitayama 1999; Kitayama and Aiba 2002). Mean annual temperature and precipitation, as measured at 1560 m a.s.l., is ca. 18°C and ca. 2800 mm year⁻¹, respectively (Kitayama 1992). We chose 12 common Fagaceae species varying in leaf and stem traits, and altitudinal distributions (Appendix 1). In Mount Kinabalu, approximately 61 Fagaceae species (11 Castanopsis, 37 Lithocarpus, 11 Quercus, and one Trigonobalanus species) can be found between 300 to 3400 m a.s.l. (Beaman et al. 2001). In the present study, we targeted species that generally occur on sedimentary rocks and did not include species whose distribution is mostly restricted to ultrabasic rocks. We used three indices to characterize species’ altitudinal distributions (lowest and highest altitude and their range [difference between highest and lowest altitude]). This information was subtracted from the flora of Mount Kinabalu (Beaman et al. 2001). Plant nomenclature followed The Plant List (http://www.theplantlist.org/), accessed on 23rd September 2019.

In February and March of 2019, we collected leaves and stem core samples from trees growing from 1490 m to 1860 m a.s.l. Because of their tall stature, we mostly targeted trees growing on the ridges. Subsequently, a total of 12 leaf and stem traits were measured (Table 1 and Appendix 1). All studied species had the same life forms (evergreen trees).

Measurements of leaf traits

We collected at least five fully expanded, sun-exposed leaves at a height of 3 to 11 m from one to five mature trees (mean 4.3 ± 1.2 SD) per species, using a sickle. Samples were immediately enclosed in plastic bags and transported to the laboratories for further analyses. Some leaves were chemically fixed with c. 3 % v/v glutaraldehyde for the observation of the veins.

Lamina thickness (LT) was measured at the middle point of the leaf lamina for five leaves per tree, avoiding the primary and secondary veins, using a micrometer (MDC-25MX, Mitutoyo, Kanagawa, Japan). We then scanned the same leaves using a flat-bed scanner (Epson, Nagano, Japan) at 300 dpi. Subsequently, leaf fresh weight was measured and then leaves were oven-dried (65°C, >72 h) and weighed again. Individual leaf area (LA) was calculated using the scanned images, using Fiji (Schindelin et al. 2012). Leaf dry mass per area (LMA) was calculated as total leaf dry weight divided by leaf area. Leaf dry matter content (LDMC) was calculated as dry weight divided by fresh weight. We included the petiole in the calculations of LA, LMA, and LDMC.

As an index of leaf hydraulic conductance (Kawai and Okada 2016; Scoffoni et al. 2016), we measured minor vein density (VLA_m). For one leaf per tree, we cut a small section (c. 1.5 × 1.5 cm²) from the middle of the lamina and immersed it in 5 % w/v NaOH(aq) until it became transparent. Then, the section was rinsed with tap water and stained with 1.0 % w/v safranin (in 50 % ethanol). After being rinsed with ethanol, images were captured with a digital camera (EOS kiss X3, Canon, Tokyo, Japan) coupled to a light microscope (BX50, Olympus, Tokyo, Japan). The resolution of the images was 763 pixels per mm with a full extent of 3 mm × 2 mm. The length of the minor vein (>3rd order vein) was calculated by manually tracing the veins in a c. 2 mm² region, using Fiji. The minor vein density was calculated as the minor vein length divided by the area of the analyzed region.

We also measured lamina δ¹³C, an indicator of long term water use efficiency (Farquhar et al. 1989). The same leaves used for the calculation of LMA were ground with a mill, avoiding petiole and midrib. δ¹³C was measured using an isotope-ratio mass spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Massachusetts, USA) at the Center for Ecological Research (CER), Kyoto University. At the same time, dry-mass based carbon (C_mm) and nitrogen concentration (N_mm) as an index of maximum photosynthetic rate (Wright et al. 2004) were measured using a Flash 2000 Elemental Analyzer (Thermo Fisher Scientific). Area-based leaf nitrogen concentration (N_mm) was obtained by multiplying N_mm by LMA.
Measurements of stem trait

We collected one stem wood core (3–4 cm in length) with an increment borer (5.15 mm in diameter, Haglöf, Långsele, Sweden) at breast height of one to five trees (mean 3.8 ± 1.1 SD) per species, including trees from which we sampled leaves. The fresh volume was determined by measuring the diameter and length of a core, assuming a cylindrical shape. The samples were then oven-dried (65°C, >72 h) and weighed. Wood density (WD) was calculated as the dry weight divided by the fresh volume.

Statistical analysis

All analyses were carried out in R (ver 3.5.0; R core team 2018). We calculated the mean trait values for each species and some trait values were log₁₀-transformed before analyses to improve normality and heteroscedasticity (Table 1). We examined the relationships between traits and altitudinal distributions and the relationships among traits using the Pearson correlation coefficient (r). Lines were fitted by standard major axis (SMA), using R package ‘smatr’ (Warton et al. 2012). We did not conduct phylogenetic analyses because at present a well-resolved phylogenetic tree was not available for our species.

RESULTS

Altitudinal distribution, 10 leaf and stem traits showed large interspecific variation in 12 Fagaceae of Mount Kinabalu, Borneo (Table 1, Appendix 1). L. havilandii showed the highest upper limit of the altitudinal distribution (ALTₘₐₓ) of 3400 m a.s.l. whereas L. luteus showed the lowest of 1800 m a.s.l. L. ruminatus showed the highest lower limit of the altitudinal distribution (ALTₘᵋₜ) of 1800 m a.s.l. whereas Q. subsericea showed the lowest of 500 m a.s.l. Leaf area varied 15-fold across species from 16.7 cm² in L. havilandii to 261 cm² in L. lampadarius. The LMA, LT, and VLAₘᵋₜ also showed large differences among species, varying 2.4-fold, 2.6-fold, and 2.6-fold, respectively. Exclusion of L. lampadarius which showed exceptionally large leaf area among species did not change the overall results except that the relationship between LA and ALTₘᵋₜ became significant (r = -0.73, P = 0.01, n = 11).

Relationships between traits and altitudinal distributions

Species with higher altitudinal limits showed wider altitudinal distribution (r = 0.63, P < 0.05, Table 2). Consistent with our prediction, we found that the species with greater LMA and LT have higher altitudinal limits (Fig. 1a, 1b, Table 2). We also found the positive relationship between Nₖₑₑᵋₑ and highest altitude in trend (r = 0.56, P = 0.06). On the other hand, no traits were correlated with species’ lowest limits (Table 2). The LMA and Nₖₑₑᵋₑ positively co-varied with the altitudinal range across species (Table 2), showing that species with high LMA and Nₖₑₑᵋₑ could occur at various elevations including high altitudes.

Table 2. Pearson’s correlation coefficients among traits and altitudinal distribution for 12 Fagaceae species in Mount Kinabalu, Borneo.

|          | LMA | LDMC | LT | LA¹ | δ¹³C | C_max | N_max | N_min | VLA_max¹ | WD | ALT_min | ALT_max¹ |
|----------|-----|------|----|-----|------|-------|-------|-------|----------|----|---------|----------|
| LDMC     | 0.05| –     | –  | –   | –    | –     | –     | –     | –        | –  | –       | –        |
| LT       | 0.90| – 0.27| –  | –   | –    | –     | –     | –     | –        | –  | –       | –        |
| LA¹      | 0.14| – 0.43| 0.16| –   | –    | –     | –     | –     | –        | –  | –       | –        |
| δ¹³C     | 0.62| – 0.13| 0.72| 0.02| –    | –     | –     | –     | –        | –  | –       | –        |
| C_max    | 0.65| – 0.03| 0.58| – 0.07| 0.72| –     | –     | –     | –        | –  | –       | –        |
| N_max    | – 0.67| – 0.31| – 0.46| – 0.13| – 0.40| – 0.30| –     | –     | –        | –  | –       | –        |
| N_min    | 0.90| – 0.16| 0.92| 0.07| 0.57| 0.64| – 0.29| –     | –        | –  | –       | –        |
| VLA_max¹ | – 0.29| – 0.50| – 0.45| – 0.53| – 0.24| 0.13| 0.37| – 0.20| –        | –  | –       | –        |
| WD       | – 0.13| – 0.07| – 0.05| 0.26| 0.52| 0.28| – 0.15| – 0.28| – 0.15| – 0.15| –     | –        |
| ALT_min  | – 0.38| – 0.28| – 0.26| – 0.21| – 0.31| – 0.24| 0.48| – 0.20| 0.37| 0.38| –     | –        |
| ALT_max¹ | 0.61| 0.17| 0.59| – 0.12| 0.36| 0.46| – 0.35| 0.56| 0.22| – 0.15| 0.20| –        |
| ALT_max²| 0.64| 0.38| 0.54| – 0.14| 0.13| 0.32| – 0.27| 0.66| 0.16| – 0.35| 0.63| –        |

Significance of correlation is indicated by bold (P < 0.05) and underlined bold (P < 0.01).
¹Log₁₀-transformed prior to analysis.
Variation in traits and altitudinal distribution of tropical Fagaceae

Coordination of traits

LMA was most frequently correlated with other traits across species (Table 2). Consistently with global patterns (e.g., Wright et al. 2004), we found the negative correlation between LMA and N\textsubscript{mass} (Fig. 2a) which indicates the trade-off between structural investment and maximum photosynthetic capacity. The leaves with high LMA, LT, and C\textsubscript{mass} showed less negative δ\textsubscript{13}C (Fig. 2b). LT was strongly positively correlated with LMA and N\textsubscript{area} (Table 2), suggesting the relatively constant investment of dry mass and N per unit leaf volume across studied species. While several leaf traits were correlated with each other, wood density was not correlated with any leaf trait (Table 2).

DISCUSSION

Using 12 Fagaceae species of Mount Kinabalu, Borneo, we demonstrated that leaf traits reflecting carbon and nutrient use strategies (LMA and N\textsubscript{area}) are associated with species’ altitudinal distributions. Although these traits are found to change along altitude at both species and community levels in Mount Kinabalu (Hikosaka et al. 2002; Kitayama and Aiba 2002) and in other mountains worldwide (e.g., Grubb 1977; Moser et al. 2007; Bresson et al. 2011; Hernández-Calderón et al. 2014; Read et al. 2014), our study is the first to demonstrate this trend in closely-related species. This, in turn, suggests that evolutionary changes of leaf traits cause niche differentiation in altitude in Fagaceae of Southeast Asia.

Consistent with our prediction, we found that species with higher LMA and LT have higher upper limits of altitudinal distribution (Fig. 1a, 1b, Table 2). Both high LMA and LT lead to a longer leaf lifespan yet with low photosynthetic capacity (Fig. 2a, Wright et al. 2004; Onoda et al. 2011) and contribute to longer residence time of nutrient and carbon in the leaf, potentially enhancing whole-plant carbon and nutrient use efficiency (Berendse and Aerts 1987). Such resource conservation strategies would be adaptive in surviving and growing on high elevations of tropical forests where low temperature...
constrains the capacities of photosynthesis and nutrient absorption. In addition, although the effects of wind on plants vary depending on the characteristics of plants and microhabitats, thus hard to be generalized, thick leaves may be important to enhance mechanical resistance (Onoda et al. 2011) and thus prolong leaf lifespan at windy sites of high altitudes (Anten et al. 2010). Higher LMA and LT for high altitude species than low altitude species are also found in other mountains and could be attributable to larger cell size, thicker cell wall, particularly in the epidermis, and more palisade cell layers (Tanner and Kapos 1982; Körner et al. 1989; Moser et al. 2007). Although available soil water dramatically increases above 2000 m a.s.l. due to frequent clouds (Kitayama 1992), this unlikely explains high LMA and LT for high altitude species because soil saturation hardly affect leaf traits (Lopez and Kursar 2003).

We also found the marginally positive relationship between N_{max} and species’ upper limits of altitudinal distribution. Because the increase of N_{max} does not contribute to the photosynthetic rate at a higher elevation, the role of N_{max} on photosynthesis could be limited (Hikosaka et al. 2002). Rather, because cell wall inevitably contains a certain amount of N (Onoda et al. 2017), the larger amount of N would be a prerequisite to maintain high cell wall content (high LMA) and thus to enhance mechanical resistance and leaf longevity at high elevations.

Both LMA and N_{max} were also correlated with species’ altitudinal range (Table 2). This suggests that species with resource conservation strategies can survive and grow in various elevations, including high elevations with harsh environments. This may be because evergreen species with high LMA are highly shade-tolerant at seeding and juvenile stage (Kitajima 1994; Poorter and Bongers 2006; Lusk and Warton 2007; Poorter 2009) which confer a competitive advantage in forest floor at each elevation. After the exclusion of *L. lampadarius* which showed exceptionally large leaf area among species, LA and species’ altitudinal range were negatively correlated, indicating that species with small leaf could occur at the wide range of altitude. These results may reflect high shade tolerance for small leaved-species (Poorter 2009).

We expected that species with acquisitive traits such as low LMA, high N_{max} and high VLA_{min} which can achieve high growth rate at resource-rich conditions are distributed to low elevations. However, there are no significant relationships between traits and species’ lowest limits (Table 2). This suggests a weak environmental filtering that allows a wide range of trait values at benign low elevations (Weiher and Keddy 1995). Rather, other processes such as competition which is difficult to be identified by traits may determine species’ lowest limits.

Traits related to leaf-level water relations, such as δ_{13}C, VLA_{min}, LA, and LDMC were not related to species’ altitudinal distributions (Table 2). This is probably because rainfall is generally ample (>2000 mm) and varies little with altitude in Mount Kinabalu (Aiba and Kitayama 1999; Kitayama and Aiba 2002), suggesting that drought stress generally does not influence species’ distributions here except during occasional droughts (Aiba and Kitayama 2002).

We confirmed several previously-reported relationships and independences in evergreen tropical Fagaceae, providing the evidence for the correlated evolutions of traits in closely-related species. For example, the negative relationship between LMA and N_{max} and the positive relationship between LMA and N_{max} (Fig. 2a, Table 2) were found globally (Wright et al. 2004) and also in temperate and tropical Fagaceae in East Asia (Kawai and Okada 2019). The independence of VLA_{min} from LMA is consistent with previous studies using diverse angiosperms (Sack et al. 2013; Li et al. 2015; Kawai and Okada 2018).

The positive relationships of δ_{13}C with LMA, LT, and C_{max} (Fig. 2b, Table 2) have been reported in previous studies (Vitousek et al. 1990; Schulze et al. 1998; Cordell et al. 1999; Hanba et al. 1999). There are at least two possible explanations: firstly, variation in photosynthetic capacity among species may determine δ_{13}C. High N_{max} associated with high LMA, LT, and C_{max} (Table 2) would lead to high assimilation rate per unit area (Hanba et al. 1999; Hikosaka et al. 2002) and thus decrease the ratio of the concentration of CO_{2} at chloroplast (c_{i}) to that in the air (c_{a}), resulting in less negative δ_{13}C. Indeed, δ_{13}C was weakly positively correlated with N_{max}, an indicator of area-based photosynthetic rate \(r = 0.57, P = 0.05\). Secondly, stomatal conductance may be lower for species with high LMA (Jacobsen et al. 2008; Blackman et al. 2016), thus decreasing c_{i}/c_{a} and resulting in low discrimination of δ_{13}C (less negative δ_{13}C).

Similar to our previous study, we found that LT was strongly positively correlated with LMA \(r = 0.90\) for this study; \(r = 0.95\) for 25 Fagaceae species of Kawai and Okada 2019) and N_{max} \(r = 0.92\), this study; \(r = 0.79\) for 25 Fagaceae species. These results collectively suggest that investment of dry mass and nitrogen per unit lamina volume are evolutionarily conservative among Fagaceae species. Therefore, lamina thickness may be a key driver of variation in carbon and nutrient use strategies and thus leaf functioning among Fagaceae species. Indeed, for temperate and tropical Fagaceae, leaf lamina thickness was well correlated with leaf hydraulic conductance (Kawai and
Variation in traits and altitudinal distribution of tropical Fagaceae

Okada 2016) and leaf mechanical resistance (Kawai and Okada 2019), both of which greatly influence species' demography (e.g., Kitajima and Poorter 2010; Iida et al. 2016).

There are two limitations to this study. The first limitation is the assumption that genetic controls on traits are greater than phenotypic plasticity. However, Bresson et al. (2011) showed that in temperate mountains, intraspecific trait variation along altitude is largely driven by phenotypic plasticity, not by genetic controls. In such a case, trait values expressed at species' altitudinal limits may not reflect the trait values expressed in a given altitude. Therefore, future studies should examine how much degree the trait plasticity influences species’ distributions. Secondly, the relationships among species-level traits could be different at different altitudes (e.g., the relationship between nitrogen concentration and photosynthetic rate, Hikosaka et al. 2002), thus the interpretation made at a given elevation might not be applied at other elevations.

In conclusion, using 12 Fagaceae species of Mount Kinabalu, Borneo, we demonstrated that species with leaf-level conservative resource use showed higher altitudinal limits and wider altitudinal range, highlighting the role of leaf traits in the diversification of species’ altitudinal distributions. This observation further suggests that even within lineage, diversification in leaf traits that reflect species' biogeographic and phylogenetic history (Cavender-Bares 2019) leads to species coexistence in tropical montane forests. Future studies should examine 1) the generality of the results in other tropical mountains in Southeast Asia because species' altitudinal distributions could vary among mountains which reflect the differences in climatic and edaphic gradients (e.g., precipitation, Hara 2019), 2) confirm observed patterns in other important tropical lineages such as Dipterocarpaceae and Myrtaceae which also show diverse altitudinal distributions among species (e.g., Ghazoul 2016), and 3) clarify the mechanisms underlying interspecific variation in species’ lowest limits.

ACKNOWLEDGMENTS We thank the staff members of Sabah Parks for their kind assistance with our study. Dr. Kitayama Kancheiro provided logistic support. Sabah Biodiversity Council permitted us to conduct this research (Access license no. JKM/MBS.1000-2/2 JLD.7 (124); Licence for transfer of biological resources no. JKM/MBS. 1000-2/3 JLD.3 (94)). Dr. Aiba Shin-ichiro and two anonymous reviewers provided valuable suggestions which improved the manuscript greatly. Carbon isotope analysis was conducted using Cooperative Research Facilities (Isotope Ratio Mass Spectrometer) of the Center for Ecological Research, Kyoto University. This research was funded by Grant-in-Aid for JSPS Research Fellows to K.K. (17J05458).

REFERENCE

Aguilar-Romero R, Garcia-Oliva F, Pineda-Garcia F, Torres I, Peña-Vega E, Ghilardi A, Oyama K. 2016. Patterns of distribution of nine Quercus species along an environmental gradient in a fragmented landscape in central Mexico. Botanical Sciences 94: 471–482.

Aiba S, Kitayama K. 1999. Structure, composition and species diversity in an altitude-substrate matrix of rainforest tree communities on Mount Kinabalu, Borneo. Plant Ecology 140: 139–157.

Aiba S, Kitayama K. 2002. Effects of the 1997 & 98 El Niño drought on rain forests of Mount Kinabalu, Borneo. Journal of Tropical Ecology 18: 215–230.

Anten NPR, Alcalá-Herrera R, Schieving F, Onoda Y. 2010. Wind and mechanical stimuli differentially affect leaf traits in Plantago major. New Phytologist 188: 554–564.

Beaman JH, Anderson C, Beaman RS. 2001. The Plants of Mount Kinabalu. 4. dicotyledon families Acanthaceae to Lythraceae. 570 pp. Natural History Publications (Borneo) Sdn. Bhd., Kota Kinabalu in association with The Royal Botanic Gardens, Kew Richmond

Berendse F, Aerts R. 1987. Nitrogen-use-efficiency: a biologically meaningful definition? Functional Ecology 1: 293–296.

Blackman CJ, Aspinwall MJ, Resco de Dios V, Smith RA, Tissue DT. 2016. Leaf photosynthetic, economics and hydraulic traits are decoupled among genotypes of a widespread species of eucalypt grown under ambient and elevated CO₂. Functional Ecology 30: 1491–1500.

Blackman CJ, Brodribb TJ, Jordan GJ. 2012. Leaf hydraulic vulnerability influences species’ bioclimatic limits in a diverse group of woody angiosperms. Oecologia 168: 1–10.

Bresson CC, Vitasse Y, Kremer A, Delzon S. 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? Tree Physiology 31: 1164–1174.

Brujinzeel LA, Veneklaas EJ. 1998. Climatic conditions and tropical montane forest productivity: the fog has not lifted yet. Ecology 79: 3–9.

Cavender-Bares J. 2019. Diversification, adaptation, and community assembly of the American oaks (Quercus), a model clade for integrating ecology and evolution. New Phytologist 221: 669–692.

Cavender-Bares J, Kitajima K, Bazzaz FA. 2004. Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. Ecological Monographs 74: 635–662.

Condit R, Engelbrecht BMJ, Pino D et al. 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. Proceedings of the National Academy of Sciences 110: 5064–5068.
Kawai K. Okada N. 2019. Coordination of leaf and stem traits in 25 species of Fagaceae from three biomes of East Asia. *Botany* 97: 391–403.

Kawai K, Okada N. 2018. Roles of major and minor vein in leaf water deficit tolerance and structural properties in 11 temperate deciduous woody species. *Trees - Structure and Function* 32: 1573–1582.

Kikuzawa K, Onoda Y, Wright IJ, Reich PB. 2013. Mechanisms underlying global temperature-related patterns in leaf longevity. *Global Ecology and Biogeography* 22: 982–993.

Kitajima K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98: 419–428.

Kitajima K, Poorter L. 2010. Tissue-level leaf toughness, but not lamina thickness, predicts sapling leaf lifespan and shade tolerance of tropical tree species. *New Phytologist* 186: 708–721.

Kitayama K. 1992. An altitudinal transect study of the vegetation on Mount Kinabalu, Borneo. *Vegetatio* 102: 149–171.

Kitayama K, Aiba S. 2002. Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus on Mount Kinabalu, Borneo. *Journal of Ecology* 90: 37–51.

Kitayama K, Lakim M, Wahab MZ. 1999. Climate profile of Mount Kinabalu during late 1995-early 1998 with special reference to the 1998 drought. *Sabah Parks Nature Journal* 2: 85–100.

Kitayama K, Majalap-Lee N, Aiba S. 2000. Soil phosphorus fractionation and phosphorus-use efficiencies of tropical rainforests along altitudinal gradients of Mount Kinabalu, Borneo. *Oecologia* 123: 342–349.

Koehler K, Center A, Cavender-Bares J. 2012. Evidence for a freezing tolerance-growth rate trade-off in the live oaks (Quercus series Virentes) across the tropical-temperate divide. *New Phytologist* 193: 730–744.

Körner C. 2007. The use of ‘altitude’ in ecological research. *Trends in Ecology and Evolution* 22: 569–574.

Körner C, Neumayer M, Menendez-Riedl SP, Smeets-Scheel A. 1989. Functional morphology of mountain plants. *Flora* 182: 353–383.

Larter M, Pfäutsch S, Domec JC et al. 2017. Aridity drove the evolution of extreme embolism resistance and the radiation of conifer genus *Callitris*. *New Phytologist* 215: 97–112.

Li L, McCormack ML, Ma C et al. 2015. Leaf economics and hydraulic traits are decoupled in five species-rich tropical-subtropical forests. *Ecology Letters* 18: 899–906.

Lopez OR, Kursar TA. 2003. Does flood tolerance explain tree species distribution in tropical seasonally flooded habitats? *Oecologia* 136: 193–204.

Lusk CH, Warton DJ. 2007. Global meta-analysis shows that relationships of leaf mass per area with species shade tolerance depend on leaf habit and ontogeny. *New Phytologist* 176: 764–774.

McGill BJ, Enquist BJ, Weiher E, Westoby M. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21: 178–185.
Moser G, Hertel D, Leuschner C. 2007. Altitudinal change in LAI and stand leaf biomass in tropical montane forests: a transect study in ecuador and a pan-tropical meta-analysis. Ecosystems 10: 924–935.

Onoda Y, Westoby M, Adler PB et al. 2011. Global patterns of leaf mechanical properties. Ecology Letters 14: 301–312.

Onoda Y, Wright IJ, Evans JR et al. 2017. Physiological and structural tradeoffs underlying the leaf economics spectrum. New Phytologist 214: 1447–1463.

Poorter L. 2009. Leaf traits show different relationships with shade tolerance in moist versus dry tropical forests. New Phytologist 181: 890–900.

Poorter L, Bongers F. 2006. Leaf traits are good predictors of plant performance across 53 rain forest species. Ecology 87: 1733–43.

R core team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing

Read QD, Moorhead LC, Swenson NG et al. 2014. Convergent effects of elevation on functional leaf traits within and among species. Functional Ecology 28: 37–45.

Russo SE, Davies SJ, King DA, Tan S. 2005. Soil-related performance variation and distributions of tree species in a Bornean rain forest. Journal of Ecology 93: 879–889.

Sack L, Scoffoni C, John GP et al. 2013. How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. Journal of Experimental Botany 64: 4053–4080.

Schindelin J, Arganda-Carreras I, Frise E et al. 2012. Fiji: an open-source platform for biological-image analysis. Nature methods 9: 676–82.

Schulze ED, Williams RJ, Farquhar GD et al. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Australian Journal of Plant Physiology 25: 413–425.

Scoffoni C, Chatelut DS, Pasquet-Kok J et al. 2016. Hydraulic basis for the evolution of photosynthetic productivity. Nature Plants 2: 16072.

Tanner EVJ, Kapos V. 1982. Leaf structure of Jamaican upper montane rain-forest trees. Biotropica 14: 16–24.

Tanner EVJ, Vitousek PM, Cuevas E. 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. Ecology 79: 10–23.

Toledo M, Peña-Claros M, Bongers F et al. 2012. Distribution patterns of tropical woody species in response to climatic and edaphic gradients. Journal of Ecology 100: 253–263.

Trueba S, Pouteau R, Lens F et al. 2017. Vulnerability to xylem embolism as a major correlate of the environmental distribution of rain forest species on a tropical island. Plant Cell and Environment 40: 277–289.

Violle C, Navas M-L, Vile D et al. 2007. Let the concept of trait be functional! Oikos 116: 882–892.

Vitousek PM, Field CB, Matson PA. 1990. Variation in foliar δ 13C in Hawaiian Metrosideros polymorpha: a case of internal resistance? Oecologia 84: 362–370.

Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. smatr 3-an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3: 257–259.

Weiher E, Keddy PA. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74: 159–164.

Wright IJ, Reich PB, Cornelissen JHC et al. 2005. Modulation of leaf economic traits and trait relationships by climate. Global Ecology and Biogeography 14: 411–421.

Wright IJ, Reich PB, Westoby M et al. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.
### APPENDIX

Appendix 1. Mean trait values and altitudinal distribution of 12 Fagaceae species in Mount Kinabalu, Borneo.

| Species                                      | LMA (g m$^{-2}$) | LDMC (g g$^{-1}$) | LT (mm) | LA (cm$^2$) | δ$^{13}$C (%) | $C_{\text{max}}$ (%) | $N_{\text{max}}$ (%) |
|----------------------------------------------|------------------|-------------------|---------|-------------|--------------|----------------------|---------------------|
| *Lithocarpus bullatus* Hatus. ex Soepadmo     | 226              | 0.55              | 0.35    | 25.7        | −31.7        | 55.3                 | 1.32                |
| *Lithocarpus clementianus* (King ex Hook.f.) A. Camus | 178              | 0.54              | 0.32    | 66.6        | −30.8        | 53.7                 | 1.20                |
| *Lithocarpus confertus* Soepadmo              | 133              | 0.54              | 0.24    | 26.7        | −32.9        | 50.3                 | 1.68                |
| *Lithocarpus hatusimae* Soepadmo              | 199              | 0.55              | 0.33    | 43.0        | −30.9        | 54.3                 | 1.38                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 197              | 0.54              | 0.40    | 16.7        | −30.9        | 54.1                 | 1.49                |
| *Lithocarpus luteus* Soepadmo                 | 140              | 0.50              | 0.28    | 69.5        | −31.7        | 54.9                 | 1.80                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 197              | 0.54              | 0.40    | 16.7        | −30.9        | 54.1                 | 1.49                |
| *Lithocarpus luteus* Soepadmo                 | 140              | 0.50              | 0.28    | 69.5        | −31.7        | 54.9                 | 1.80                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 197              | 0.54              | 0.40    | 16.7        | −30.9        | 54.1                 | 1.49                |
| *Lithocarpus luteus* Soepadmo                 | 140              | 0.50              | 0.28    | 69.5        | −31.7        | 54.9                 | 1.80                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 197              | 0.54              | 0.40    | 16.7        | −30.9        | 54.1                 | 1.49                |

| Species                                      | Nleaf (g m$^{-2}$) | VLA (mm mm$^{-2}$) | WD (g cm$^{-2}$) | ALTmin (m) | ALTmax (m) | ALTrun (m) | $n_{\text{leaf}}$ | $n_{\text{stem}}$ |
|----------------------------------------------|-------------------|-------------------|-----------------|-------------|------------|------------|-------------------|------------------|
| *Lithocarpus bullatus* Hatus. ex Soepadmo     | 2.98              | 10.8              | 0.62            | 1200        | 3000       | 1800       | 4                 | 4                |
| *Lithocarpus clementianus* (King ex Hook.f.) A. Camus | 2.13              | 6.0               | 0.82            | 900         | 2200       | 500        | 4                 | 4                |
| *Lithocarpus confertus* Soepadmo              | 2.24              | 7.2               | 0.54            | 1300        | 1800       | 1000       | 1                 | 1                |
| *Lithocarpus hatusimae* Soepadmo              | 2.75              | 9.3               | 0.70            | 1100        | 2100       | 1000       | 5                 | 4                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 2.90              | 9.3               | 0.69            | 1300        | 3400       | 2100       | 5                 | 5                |
| *Lithocarpus lampadarius* (Gamble) A. Camus    | 3.23              | 5.4               | 0.73            | 600         | 2400       | 1800       | 5                 | 5                |
| *Lithocarpus luteus* Soepadmo                 | 2.50              | 8.3               | 0.73            | 1200        | 1900       | 700        | 5                 | 6                |
| *Lithocarpus havilandii* (Stapf) Barnett      | 2.34              | 7.9               | 0.72            | 1800        | 2400       | 600        | 5                 | 5                |
| *Quercus pseudoverticillata* Soepadmo         | 1.51              | 8.1               | 0.72            | 1400        | 1900       | 500        | 4                 | 4                |
| *Quercus subsericea* A. Camus                 | 2.07              | 7.7               | 0.77            | 500         | 1700       | 1200       | 5                 | 4                |
| *Quercus valdinervosa* Soepadmo               | 1.80              | 14.0              | 0.76            | 1200        | 2100       | 900        | 5                 | 4                |
| *Trigonobalanus verticillata* Forman          | 2.55              | 7.5               | 0.71            | 1200        | 1700       | 500        | 4                 | 4                |

See Table 1 for trait abbreviation. $n_{\text{leaf}}$: sample size for leaf traits. $n_{\text{stem}}$: sample size for stem trait.