**Coordination of leaf and stem traits in 25 species of
Fagaceae from three biomes of East Asia**

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Coordination of leaf and stem traits in 25 species of Fagaceae from three biomes of East Asia

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Abstract

It has been debated whether leaf and stem economics spectra are coordinated across species as previous studies provide contradictory results. These studies have been restricted to single biomes, and we hypothesize that climate seasonality may determine the strength of coordination between leaf and stem trait combinations. Herein, using 25 Fagaceae species from East Asia, we investigated the coordination of 15 leaf and four stem traits across and within three biomes (cool temperate, warm temperate, and tropical forests). Traits were chosen to reflect multiple aspects of plant adaptive strategies, such as water, carbon, and nutrient use. The leaf and stem traits of species that reflect resource-use strategies for different resources were functionally coordinated, forming a single axis of trait variation across biomes. This axis represented the trade-off between fast and slow resource-use strategies. We found the trend that the coordination between leaf and stem traits was the strongest in cool temperate forests after removing two *Fagus* species, followed by warm temperate forests but was not observed in tropical forests. Our results support the proposed model that plants vary from slow to fast resource exploitation, using closely related species, and suggest that temperature modulates the coordination of leaf and stem economics spectra.

Key words; functional traits, hydraulic traits, economic spectrum, trait coordination, vein

Introduction

One of the goals in plant ecology is to understand the mechanisms generating trait diversity. Different traits are expected to reflect different costs and benefits that influence fitness in a given environment (McGill et al. 2006; Violle et al. 2007). Thus, examining the relationships among traits would clarify the patterns of cost-benefit relationships in plant evolution and give insights into the mechanisms of trait diversification.

Previous studies of cross-species comparisons have revealed the important axes of traits variations that constrain species' life history strategies and distribution. One example is the leaf economics spectrum (LES) (Wright et al. 2004), in which fast resource user are associated with high photosynthetic rate, short leaf lifespan, low leaf dry mass per area (LMA), and high leaf nitrogen and phosphorus concentrations, whereas slow resource user are associated with the opposite suite of traits. LES also underpins the differences in life history strategies across species: fast species achieve high resource acquisition rates and grow rapidly under high resource conditions, whereas slow species use resources conservatively and can persist under low resource conditions (Díaz et al. 2004; Poorter and Bongers 2006; Wright et al. 2010; Sterck et al. 2011).

This fast–slow spectrum for resource-use strategies has been applied to stems as the stem economics spectrum (SES, Chave et al. 2009). In this spectrum, wood density is proposed as a central trait explaining the differences in the resource-use strategies of stems and life history strategies. Species with low wood density are associated with high xylem hydraulic conductivity, low mechanical strength, and low resistance to cavitation, possibly achieving fast growth rates but with low tolerance to shaded and drought conditions, whereas species with high wood density are associated with low hydraulic conductivity but with high mechanical strength and high resistance to cavitation, possibly leading to high stress tolerance but with low growth rates (e.g., Hacke et al. 2001; Jacobsen et al. 2005; Preston et al. 2006; Chave et al. 2009; Wright et al. 2010; Kitajima et al. 2013).

Because leaf and stem are hydraulically and developmentally coupled, it is expected that resource use strategies in leaf and stem are tightly coordinated across species. So far, several studies reported this coordination in different biomes. In subarctic region, leaf and stem traits related to resource capture were highly coordinated in 26 species (Freschet et al. 2010). In a seasonally-dry Mediterranean

climate, leaf, stem, and root traits were coordinated across 38 species and the strength of the coordination changed among sites with different water availability (de la Riva et al. 2016). In a tropical dry forest, leaf and stem traits related to drought tolerance and construction cost were tightly coordinated across 17 species (Méndez-Alonzo et al. 2012). In the subtropical Bonin Islands, stem wood density was correlated with LMA and leaf-level drought tolerance across 32 angiosperm species (Ishida et al. 2008). By contrast, in a lowland tropical rain forest of French Guiana, LES was decoupled from SES (Baraloto et al. 2010). These results suggest that in harsh or stressful environment with a short growing season leaf and stem traits are coupled whereas in benign environments with a long growing season, they are decoupled. This contrast may be because a longer growing season allows more combinations of leaf and stem traits that achieve similar fitness due to a longer period of time available to compensate for sub-optimal designs. A single analytic framework, however, has not previously been applied in multiple biomes with contrasting temperature-precipitation patterns, limiting our understanding of the extent of the coordination between leaf and stem traits. In this study, using temperature gradient in East Asia, we hypothesized that in the colder region, with a short period favorable for growth, leaf and stem traits would be tightly coordinated across species by strong selection limiting the range of traits (e.g., Weiher and Keddy 1995; Cornwell and Ackerly 2009), whereas warmer regions would allow different combinations of traits in leaf and stem. By exploring the role of climate on the patterns of organ coordination in higher plants, we will gain insights on the ecological and evolutionary forces that determine the diversification of structure and function globally.

Herein, we extended previous research by 1) incorporating leaf and stem traits that reflect water-use strategies, 2) testing the evolutionary robustness of the coordination between leaf and stem by using closely related species, and 3) testing differences in trait coordination among multiple biomes. For the first point, most previous studies examined easily-measurable traits reflecting carbon and nutrient-use strategies (e.g., leaf nitrogen concentration and wood density) to define species' resource use strategies along the economics spectrum (e.g., Wright et al. 2004; Baraloto et al. 2010; Díaz et al. 2015; de la Riva et al. 2016). On the other hand, plant water use strategies (e.g., hydraulic efficiency and drought tolerance) have been less frequently measured, especially at the community scale (Griffin-Nolan et al. 2018) despite their importance in determining plant adaptive strategies. In particular, hydraulic conductance in organs is a determinant of photosynthetic rate (Santiago et al. 2004; Brodribb

et al. 2007; Sack et al. 2013), relative growth rate (Poorter et al., 2010), and tolerance to shade and drought (Markesteijn et al. 2011) across a wide range of plant species. Herein, we considered minor vein density and vessel anatomy as proxies for hydraulic efficiency in leaves and stems, respectively (Tyree and Zimmermann 2002; Kawai and Okada 2016; Scoffoni et al. 2016) and examined their relationships with LES and SES.

For the second point, most previous studies examined the relationships between leaf and stem traits using species with different evolutionary histories (e.g., Freschet et al. 2010; Méndez-Alonzo et al. 2012). Therefore, trait relationships have not been confirmed among closely related species in a phylogenetic context. Herein, we investigated Fagaceae, an ecologically and economically important group in the Northern Hemisphere, which shows diverse leaf and stem traits across species (Cavender-Bares et al. 2004; Kawai and Okada 2016; Aguilar-Romero et al. 2017).

For the third point, to our knowledge, there are few studies to examine the coordination between leaf and stem traits in different biomes. Because Fagaceae dominates in wide range of biomes in Northern Hemisphere, the family is appropriate to test the trait coordination while limiting phylogenetic differences.

Our study aims to examine, using 25 Fagaceae species from East Asia, (1) whether the leaf and stem traits that represent resource-use strategies concerning carbon, water, and nutrient are functionally coordinated and (2) how this coordination changes among three biomes (cool temperate forest, warm temperate forest, and tropical forest). We hypothesized that in the colder region, with a short period favorable for growth, leaf and stem traits would be tightly coordinated across species by strong selection limiting the range of traits, whereas warmer regions would allow different combinations of traits in leaf and stem.

Materials and methods

Plant species and habitats

We studied 25 Fagaceae species varying in leaf and stem traits sampled from seven forest sites in Japan and one in Thailand (Table 1, for detail of the site information, see Supplementary data, Table S1¹). We

¹ Supplementary data are available with the article through the journal Web site

classified each site to three biomes (cool temperate, warm temperate, and tropical forests, including subtropical forest) based on the mean annual temperature (MAT) and dominant forest type. In Japan, deciduous species are highly dominant in a cool temperate forest, and their abundance peaks around 9°C in MAT, whereas evergreen species increase their abundance starting around 10°C in MAT (Aiba et al. 2016). Therefore, we defined cool temperate, and warm temperate, and tropical forests as forests with MAT <12°C, from 12°C to 20°C, and >20°C, respectively (Table S1). We used temperature for classification of biomes because all study sites receive relatively high precipitation (aridity index, defined as the mean annual precipitation divided by the mean annual evapotranspiration, was above 1.4, see Table S1 for detail) and because temperature mainly determines species' distribution and forest types in East Asia (e.g., Kira 1977; Kubota et al. 2014). Our study site covered a latitudinal range from 14.48°N to 43.25°N, mean annual temperature range from 4.6°C to 26.3°C, and mean annual precipitation from 846 to 2843 mm. The number of Fagaceae species sampled was 10, 14, and eight from cool temperate, warm temperate, and tropical forests, respectively. Six species occurred in two biomes (Table 1, Supplementary data, Table S2). Approximate number of Fagaceae in each site is shown in Table S1. Collection of leaves and stem cores were carried out from June to November 2016. A total of 15 leaf and four stem traits were measured in this study (Table 2).

Measurement of leaf traits

We collected at least 10 fully expanded, sun-exposed leaves at a height of 3–11 m from one to six mature trees (mean 4.6 ± 1.4 SD) per species and biome. Most trees sampled were between 10–40 cm in DBH (diameter at breast height) and between 7–18 m in height. Samples were immediately enclosed in plastic bags and transported to the laboratories for further analyses. Some leaves were chemically fixed with c. 3% w/w glutaraldehyde for the observation of the stomata and veins.

Lamina thickness (LT: mm) was measured at the middle point of the leaf lamina for 10 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 600dpi. Leaves were subsequently oven-dried (65°C, >72 h) and weighed. Individual leaf area (LA: cm²) was calculated using the scanned images, using Fiji (Schindelin et al. 2012). Leaf dry mass per area (LMA: g m⁻²) was calculated as total leaf dry mass divided by leaf area. Lamina tissue density (TD:

g cm^{-3}) was calculated as LMA divided by lamina thickness. We included the petiole in the calculation of LA and LMA.

To determine lamina mechanical strength, we carried out a punch test. We tested at the middle point of the leaf lamina for at least 10 leaves per tree, avoiding the primary and secondary veins, using a flat-ended cylindrical steel punch (2 mm in diameter) attached with a digital force gauge (DS2-50N, IMADA, Toyohashi, Japan) and a steel die with a small hole (2.1 mm in diameter). We standardized the force to punch (unit: N) in three ways. Firstly, we calculated force to punch per unit perimeter of fracture (F_p : kN m^{-1}) and obtained force to punch per unit fracture surface area (or specific force to punch, F_{ps} : MN m^{-2}) by dividing F_p by lamina thickness (LT). Secondly, density-corrected specific force to punch (F_{TD} : $\text{N g}^{-1} \text{m}$) was obtained by dividing F_{ps} by lamina tissue density (TD). The latter two are classified as material strength, which do not depend on the size or morphology of the leaf, whereas F_p is classified as structural strength, which reflects the size or morphology of the leaf as well as its material strength (Westbrook et al. 2011). We used F_{TD} as a correlate of leaf cellulose per unit leaf mass (Kitajima et al. 2012).

To evaluate leaf gas exchange capacity, we measured stomatal density (SD: mm^{-2}) and calculated potential maximum stomatal conductance to water vapor (g_{smax} : $\text{mol m}^{-2} \text{s}^{-1}$). A replica of the abaxial surface of one leaf per tree was obtained by Suzuki's Universal Micro-Printing method (KENIS LIMITED, Osaka, Japan). In pubescent leaves, trichomes were gently removed by duct tape and a razor blade. The images were captured with a digital camera (EOS kiss X3, Canon, Tokyo, Japan) coupled to a light microscope (BX50, Olympus, Tokyo, Japan). Stomatal images were captured at two different resolutions; 1919 and 7679 pixels per mm with a full extent of $1.3 \text{ mm} \times 0.8 \text{ mm}$ and $0.3 \text{ mm} \times 0.2 \text{ mm}$ for the calculation of SD and g_{smax} , respectively. The number of stomata was manually counted in at least three different views (c. 0.24 mm^2 each) using Fiji. The g_{smax} was estimated by equation 1 (Franks and Farquhar 2001), which is as follows:

$$\text{(Eqn1)} \quad g_{smax} = \frac{d}{v} \text{SD} \frac{a}{l + \frac{\pi}{2} \sqrt{a/\pi}}$$

where d is the diffusivity of water in air at 20°C ($2.42 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$); v is the molar volume of air at 20°C ($0.024 \text{ m}^3 \text{ mol}^{-1}$); SD is the stomatal density (m^{-2}); a is the maximum pore area (m^2); and l is the pore depth (m). The mean guard cell width was substituted for pore depth (l) based on the assumption that guard cells are approximately circular in cross section. Maximum pore area (a) was calculated from the guard cell length, assuming that a stomatal pore is a circle (Brodribb et al. 2013). We measured at least 20 stomata for the calculation of a and l and averaged values were used.

To evaluate leaf hydraulic efficiency, we measured minor vein density (VLA_{min} : mm mm^{-2}). For one leaf per tree, we cut a small section (c. $1.5 \times 1.5 \text{ cm}^2$) 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) coupled to a light microscope (BX50, Olympus). The resolution of the images was 763 pixels per mm with a full extent of $3 \text{ mm} \times 2 \text{ mm}$. The length of the minor vein (>3rd order vein) was calculated by manually tracing the veins in a c. 2 mm^2 region, using Fiji. The minor vein density was calculated as the minor vein length divided by the area of the analyzed region. The minor vein thickness (VT: mm) was calculated by averaging the thickness of each order vein measured at one point. The minor vein volume per unit area (VVA: $\text{mm}^3 \text{ cm}^{-2}$), an index of the construction cost of minor veins, was calculated as $\pi (\text{VT}/2)^2 \times \text{VLA}_{\text{min}}$.

We also measured lamina $\delta^{13}\text{C}$ (unit: ‰), 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. $\delta^{13}\text{C}$ 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 nitrogen concentration (N_{mass} : %) was measured using a coupled elemental analyzer (Flash 2000, Thermo Fisher Scientific). Area-based leaf nitrogen concentration (N_{area} : g m^{-2}) was obtained by multiplying N_{mass} by LMA. We used both area- and mass- based expressions which represent investment of nitrogen per unit area and per unit leaf dry mass investment, respectively, to examine how these variables are related to resource use strategies in leaf and stem.

Measurement of stem traits

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 five to eight trees per species and biome, including tress 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: g cm⁻³) was calculated as the dry weight divided by the fresh volume.

We quantified vessel anatomy with the same sample for three trees per species and biome. After being rehydrated in tap water for overnight, transverse sections approximately 20–30 µm thick were prepared with a sliding microtome. Samples were double-stained with 1.0% w/v safranin (in 50% ethanol) and 1.0 % w/v fast green (in 95% ethanol) and dehydrated through an ethanol series (50%, 95%, and 100%). Finally, samples were dehydrated in xylene and mounted on a glass slide with Canada balsam. Images were captured using a digital camera (EOS kiss X3, Canon) coupled to a stereoscopic microscope (SZX-12, Olympus). The resolution of images was 789 pixels per mm with a full extent of c. 6 mm × 4 mm. Images were binarized using GIMP ver. 2.8.18 (GNU Image Manipulation Program; available on www.gimp.org) and a rectangle-shaped region (10–23 mm²) was set on each image for analysis of xylem anatomy. Vessel density (VD: mm⁻²) was defined as the number of vessels divided by the area of the analyzed region. Mean hydraulically weighted diameter (D_h : mm) was calculated using equation 2 (Tyree and Zimmermann 2002), which is as follows:

$$(Eqn2) \quad D_h = \sqrt[4]{\frac{1}{n} \sum_{i=1}^n d^4}$$

where d is the diameter of each vessel. Since a vessel cross section is not exactly a circle, d was calculated as the average of the mean lengths of the major and minor axes of ellipses fitted to each vessel. Using VD and D_h , we calculated the potential specific hydraulic conductivity (K_h : kg m⁻¹ MPa⁻¹ s⁻¹) using equation 3 (Poorter et al., 2010), which is as follows:

$$(Eqn3) \quad K_h = \frac{\pi \rho_w}{128 \eta} VD D_h^4$$

where ρ_w is the density of water at 20°C (998.2 kg m⁻³); η is the viscosity of water at 20°C (1.002×10^{-3} Pa s). Note that K_h is higher than the measured conductivity since K_h does not consider the flow resistance through intervessel pits, which typically accounts for >50 % of the total resistance of the vessel network (Wheeler et al. 2005; Choat et al. 2008) and embolized vessels. Since pore zones (earlywood containing relatively large vessels) accounted for >90% of K_h in ring-porous species (deciduous *Quercus* and *Castanopsis sieboldii*, Table S2), we reported VD, D_h , and K_h on pore zones for these species. Olson et al. (2014) showed that intraspecific variation in vessel traits is largely driven by tree size, but we considered that, at least for our studied species, variation in vessel traits is driven by other factors (e.g., environment), because neither DBH nor height were correlated to vessel traits across species ($r = 0.03\text{--}0.27$, $P > 0.20$).

Statistical analyses

All analyses were carried out in R (ver. 3.3.2; R core team, 2016). We calculated the mean trait values for each species in each biome and some trait values were log₁₀-transformed before analyses to improve normality and heteroscedasticity (Table 2; Table S2). Firstly, we examined the relationships among traits by generating the correlation matrix for all of the variables using the Pearson correlation coefficient (r). Then, to summarize the relationships, we performed two principal component analyses (PCA); the first PCA included leaf and stem traits (global PCA) and the second PCA included either leaf or stem traits (individual PCAs). We conducted the above PCA analyses for both across and within biomes. In the global PCA across biomes, we averaged the trait values of species, which occurred in multiple biomes. Finally, to assess the degree of coordination between leaf and stem traits, we used the Pearson correlation coefficient between the species' scores of the first and second component of individual PCAs which included either leaf and stem traits. In above PCAs, we also included species' evergreenness (deciduous = 0, evergreen = 1, Table S2) and vessel porosity in stem (ring-porous = 1, diffuse-porous = 0, Table S2) to examine whether these characteristics are linked to species' resource use strategies. We did not conduct phylogenetic analyses because at present a well-resolved phylogenetic tree is not available for our species.

Results

Overall patterns of relationships between leaf and stem traits

The 25 species of Fagaceae of this study exhibited a broad range of variation in leaf and stem traits (Table 2; Table S2). Many leaf and stem traits were significantly correlated (Fig. 1; Table 3). Overall, F_{ps} , F_{TD} , g_{smax} , SD, N_{mass} , and LA were the leaf traits that strongly correlated with stem traits. Hydraulic traits were positively correlated between leaves and stems (e.g., g_{smax} and K_h ; Fig. 1a) and among leaves (e.g., SD and VLA_{min}), whereas several traits could be automatically related (e.g., SD and g_{smax} because g_{smax} was estimated from SD).

The first axis in the global PCA across biomes accounted for 36.77% of the variance of 21 traits with positive loadings of leaf and stem structural properties (evergreenness, F_p , F_{ps} , F_{TD} , VT, VVA, LT, LMA, and WD; Fig. 2a, Table 2) and area-based leaf nitrogen content (N_{area}) and with negative loadings of leaf and stem hydraulic traits (g_{smax} , SD, VLA_{min} , vessel porosity, D_h , and K_h), mass-based leaf nitrogen content (N_{mass}), and LA. The second axis explained 18.34% of the variance with positive loadings of VD and negative loading of hydraulic and structural traits in leaf and stem (g_{smax} , $\delta^{13}C$, N_{area} , LT, LMA, vessel porosity, D_h , and K_h).

The global PCA indicated that most leaf and stem traits vary along a single axis (Fig 2a; Table 2), along which one end was characterized by species with an efficient leaf and stem transport systems, high gas exchange capacity, and low leaf and stem construction cost associated with low mechanical strength (fast resource use). The opposite end was characterized by species with mechanically tough leaves and stems with high construction cost and by low photosynthetic capacity and low hydraulic efficiency of leaves and stems (slow resource use). Species' scores in PC1 of the global PCA (Fig. S1) did not differ among genera (one-way ANOVA, $P = 0.20$) but those in PC2 was greater for *Fagus* than other four genera (one-way ANOVA, $P < 0.001$; Tukey's test, $P < 0.05$) due to their high VD (Table S2). Consistently with loading of evergreenness, we found that deciduous species had lower values in PC1 than evergreen species (t-test, $P < 0.001$) but that values of PC2 was not significantly different between evergreen and deciduous species.

Individual PCAs including either leaf or stem traits explained 55.16% and 87.82% of the variances in the first two axes, respectively (Supplementary data, Figs. S2a, b). They showed qualitatively similar patterns to the global PCA. The PC1 scores in the stem PCA and leaf PCA were positively correlated across 25 Fagaceae species ($r = 0.55$, $P = 0.004$, Fig. 2b). Excluding two species

of *Fagus*, which showed slightly higher PC1 scores than others in the stem PCA (Fig. 2b), increased the strength of the correlation ($r = 0.71$, $P < 0.001$).

Patterns of relationships between leaf and stem traits in the three biomes

The PCAs for each of the three biomes (cool temperate, warm temperate, and tropical forests) showed generally similar patterns to the PCA across the three biomes (Figs. 2a, c, e, and g) although the loadings of traits for PC1 were the opposite in the warm temperate forest. This similarity was also observed for the PCAs including either leaf or stem traits (Fig. S2). The species' PC1 scores in leaf and stem PCA were significantly correlated in the warm temperate forest but not in the cool temperate and tropical forests (Figs. 2d, f, and h). Removing two outliers (*Fagus crenata* and *Fagus japonica*) with high scores of the stem PC1, however, made the relationships highly significant at the cool temperate forest (Fig. 2d).

Discussion

Using 25 Fagaceae species with strong differentiations in leaf and stem traits, we observed tight coordination in the resource-use strategies between leaf and stem. The principle novel findings were that, 1) in resource-use strategies of species for carbon, water, and nutrient, leaves and stems were functionally coordinated, forming a single axis of trait variation and that 2) the strength of this coordination changed among biomes.

Coordination between leaf and stem traits

We demonstrated that leaf and stem traits that reflect resource-use strategies in carbon, water, and nutrient were functionally integrated into a single axis of trait variation in 25 Fagaceae (Table 2; Figs. 1, 2a, b). These results support the life history theory that leaf and stem traits are coordinated irrespective of resources such that a single axis of trait variation explains most woody plant strategies along the trade-off between fast and slow resource-use strategies (Grime et al. 1997; Méndez-Alonzo et al. 2012; Reich 2014). Along this spectrum, one extreme is characterized by cheap leaves and stems, high water supply capacity, and high photosynthetic rate, possibly associated with high growth rates (e.g., Poorter and Bongers 2006; Fan et al. 2012), whereas the opposite extreme is characterized by tough leaves and

stems, low photosynthetic capacity, possibly associated with low mortality (e.g., Wright et al. 2010; Kitajima et al. 2013). In the present study, deciduous species were generally fast resource user, whereas evergreen species were slow resource user. Our results also provided evidence of evolutionary robustness in frequently-reported relationships in water use strategies, including the positive relationships between stem hydraulic conductance and gas exchange capacity (Brodribb and Feild 2000; Santiago et al. 2004; Meinzer et al. 2008; Zhang and Cao 2009) and between liquid and gaseous phase conductance in leaves (Sack et al. 2003; Boyce et al. 2009), both of which have been reported in species with different evolutionary histories.

As novel relationships, we found that minor vein thickness (VT) and minor vein volume per area (VAA) were positively correlated with LT and LMA (Table 3). There are two possible explanations for this. Firstly, since veins are made of cellulose-rich material with high tissue density (Villar et al. 2009), their abundance may positively influence leaf construction cost (LMA) via an increase in VAA. Secondly, the thickness of minor veins is mainly accounted for by the thickness of bundle sheath cells, which scales with lamina thickness across species (John et al. 2013) due to developmental constraints (Pantin et al. 2012). Thus, developmental coordination between leaf tissues and organs could cause the positive relationship observed between VT and LT. This relationship may further cause the positive relationship of VT with LMA since LMA was determined by LT and not by TD in the present study ($LMA = LT \times TD$; Table 3). The weaker association of LMA with VAA than with VT (Table 3), however, seems to support the latter explanation. On the other hand, VT was positively correlated with F_{ps} and F_{TD} which were independent from LT or LMA (Table 3). This suggests that diameter of minor veins directly affects lamina mechanical strength and may affect leaf lifespan (e.g., Kitajima et al. 2012) irrespective of leaf structural properties. Future studies should evaluate the contribution of VT to leaf strength in Fagaceae by examining the degree of reinforcement of minor veins by sclerenchyma or collenchyma and by considering possibly co-varying anatomical traits outside minor veins (e.g., sclereids).

We also found that leaf area was positively correlated with g_{smax} , D_h , and K_h (Table 3) and was involved in the trade-off between fast–slow resource use strategies (Fig. 2a, Table 2). Because larger leaf area enhances light interception per leaf, leaf area would be functionally coordinated with traits related to other resource use (e.g., water). The positive relationship between LA and K_h indicates

the balance between water-transpiring surface area and capacity of water conduction, given the limited variation of leaf area: sapwood area at shoot level among species (K. Kawai & N. Okada unpublished data).

Influence of climate on stem and leaf relationships

Consistent with our hypothesis, the strongest relationship between leaf and stem was observed in the cool temperate forest (except two *Fagus* species) with short growth-favorable period, followed by the warm temperate forest, and tropical forests (Fig. 2). This trend is generally consistent with the compilation of previous studies (Ishida et al. 2008; Baraloto et al. 2010; Freschet et al. 2010) conducted in different regions and with different species. However, because of the lower number of species examined at each biome, this result should be seen as a trend. Moreover, even in cool and warm temperate forests, the coordination between leaf and stem PCA was largely brought by the contrasting resource-use strategies between deciduous and evergreen species (Figs. 2d, f). Therefore, at least in Fagaceae, the change in the composition of deciduous and evergreen species along climatic gradient may partially influence the strength of coordination between leaf and stem traits.

Decoupling of leaf and stem traits in tropical species (Fig. 2h) may be related to low variation in stem traits, especially in vessel traits but to comparable variation in leaf traits (Supplementary data Table S3). In addition, the lowest strength of coordination in the tropical forest mirrored the low variance explained by the PC1 in both the global and individual PCAs (Figs. 2g and S2g). This suggests the presence of other axes of trait variation in tropical forests apart from the trade-off between slow and fast resource-use strategies, probably due to more niches available in tropical forests than in temperate ones. The removal of two *Fagus* species in the cool temperate forest (Fig. 2d) may be justified to some degree because *Fagus* is located in a distant clade in the evolution of Fagaceae (Manos and Stanford 2001; Kamiya et al. 2003) and because they exhibit distinct vessel anatomy (denser vessels with smaller diameters than others, Table S2). Nevertheless, this result highlights that the relationship among leaf and stem traits are to some degree dependent on the evolutionary histories of the species concerned, as shown in LES (Heberling and Fridley 2012).

Since our study sites generally receive high precipitation (Table S1), difference in temperature or associated length of growth-favorable period (e.g., Kikuzawa et al. 2013) may be related

to the observed change in the coordination between leaf and stem traits among biomes, as in LES (e.g., Wright et al. 2005; Funk and Cornwell 2013). Our observation suggests that warm and humid regions with long growth-favorable period can accommodate larger number of combinations of leaf and stem traits apart from the trade-off between slow and fast resource-use strategies. This is consistent with the observation of greater ranges in trait variation in tropical forests than in temperate forests (e.g., Swenson and Enquist 2007; Lamanna et al. 2014).

We conclude that suites of leaf and stem traits that reflect resource-use strategies concerning carbon, water, and nutrient are functionally coordinated, forming a single axis of trait variation in closely related species. This result supports the theory of a plant economics spectrum in an evolutionary context. We also suggest that temperature or associated growth-favorable period affect the strength of the coordination between leaf and stem traits under humid conditions. Future research is required to examine the observed relationships in different plant lineages with different evolutionary histories. Such research will help to clarify how evolution constrains the trait correlation networks in leaves and stems.

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Table 1. The 25 Fagaceae species studied, species code, and biomes

Species	Code	Biome
<i>Castanea crenata</i> Siebold et Zucc.	Ccr	CTM-WTM
<i>Castanopsis piriformis</i> Hickel & A.Camus	Cpi	TROP
<i>Castanopsis sieboldii</i> (Makino) Hatus. ex T.Yamaz. et Mashiba	Csi	WTM-TROP
<i>Fagus crenata</i> Blume	Fcr	CTM
<i>Fagus japonica</i> Maxim.	Fja	CTM
<i>Lithocarpus edulis</i> (Makino) Nakai	Led	WTM-TROP
<i>Lithocarpus glaber</i> (Thunb.) Nakai	Lgl	WTM
<i>Lithocarpus</i> sp1	Ls1	TROP
<i>Lithocarpus</i> sp2	Ls2	TROP
<i>Lithocarpus</i> sp3	Ls3	TROP
<i>Quercus acuta</i> Thunb.	Qac	CTM-WTM
<i>Quercus aliena</i> Blume	Qal	WTM
<i>Quercus crispula</i> Blume	Qcr	CTM
<i>Quercus dentata</i> Thunb.	Qde	CTM
<i>Quercus gilva</i> Blume	Qgi	WTM
<i>Quercus glauca</i> Thunb.	Qgl	WTM
<i>Quercus hondae</i> Makino	Qho	WTM
<i>Quercus kerrii</i> Craib	Qke	TROP
<i>Quercus miyagii</i> Koidz.	Qmi	TROP
<i>Quercus myrsinifolia</i> Blume	Qmy	WTM
<i>Quercus phillyreoides</i> A.Gray	Qph	WTM
<i>Quercus salicina</i> Blume	Qsa	CTM-WTM
<i>Quercus serrata</i> Murray	Qse	CTM-WTM
<i>Quercus sessilifolia</i> Blume	Qss	CTM-WTM
<i>Quercus variabilis</i> Blume	Qva	CTM

CTM = cool temperate forest, CTM-WTM = cool and warm temperate forest, WTM = warm temperate forest, WTM-TROP = warm temperate and tropical forest, TROP = tropical forest.

Table 2. Leaf and stem traits studied in this study, including minimum species mean, the mean species of means, the maximum species mean of 25 Fagaceae, groups of traits, correlation (r) with the global PCA Axes 1 and 2 and their ecological significance

Trait	Symbol	Unit	Mean	Min	Max	Group	r with PC1	r with PC2	Ecological significance
Force to punch per unit perimeter of fracture	F_p	kN m ⁻¹	1.39	0.61	2.48	Leaf	0.86 ***	ns	Defense from abiotic and biotic stress
Specific force to punch	F_{ps}	MN m ⁻²	5.88	3.42	8.03	Leaf	0.75 ***	ns	Defense from abiotic and biotic stress
Density-corrected specific force to punch ^a	F_{TD}	N g ⁻¹ m ¹	12.28	7.88	20.72	Leaf	0.65 ***	ns	Defense from abiotic and biotic stress
Potential maximum stomatal conductance to water vapor	g_{smax}	mol m ⁻² s ⁻¹	1.74	0.81	3.23	Leaf	-0.65 ***	-0.57 **	Gas exchange capacity
Stomatal density	SD	mm ⁻²	544	195	828	Leaf	-0.60 **	ns	Gas exchange capacity
Minor vein density	VLA _{min}	mm mm ⁻²	10.8	7.7	15.20	Leaf	-0.52 **	ns	Water transport capacity in leaf
Minor vein thickness	VT	mm	0.03	0.02	0.04	Leaf	0.71 ***	ns	Vein construction cost
Minor vein volume per unit area	VVA	mm ³ cm ⁻²	0.92	0.56	1.39	Leaf	0.41 *	ns	Vein construction cost
Stable carbon isotope ratio	$\delta^{13}C$	‰	-30.0	-32.1	-27.4	Leaf	ns	-0.47 *	Long term water use efficiency
Dry-mass based leaf nitrogen concentration	N_{mass}	%	1.60	1.20	2.11	Leaf	-0.52 **	ns	Photosynthetic capacity
Area-based leaf nitrogen concentration	N_{area}	g m ⁻²	1.78	0.96	2.65	Leaf	0.44 *	-0.69 ***	Photosynthetic capacity
Lamina thickness	LT	mm	0.23	0.11	0.38	Leaf	0.68 ***	-0.61 **	Photosynthetic capacity
Lamina tissue density	TD	g cm ⁻³	0.49	0.39	0.57	Leaf	ns	ns	Photosynthetic capacity, drought tolerance, Defense from abiotic and biotic stress
Leaf area ^a	LA	cm ²	34.8	5.05	145.6	Leaf	-0.56 **	ns	Light capture
Leaf dry mass per area	LMA	g m ⁻²	114	58	181	Leaf	0.68 ***	-0.58 **	Photosynthetic capacity, leaf longevity
Wood density	WD	g cm ⁻³	0.69	0.50	0.85	Stem	0.45 *	ns	Defense from abiotic and biotic stress, resistance to cavitation
Vessel density	VD	mm ⁻²	16.4	2.1	158.5	Stem	ns	0.81 ***	Water transport capacity
Mean hydraulically weighted vessel diameter	D_h	mm	0.16	0.04	0.30	Stem	-0.68 ***	-0.67 ***	Water transport capacity
Potential hydraulic conductivity	K_h	kg m ⁻¹ MPa ⁻¹ s ⁻¹	216	15.2	1191	Stem	-0.81 ***	-0.40 *	Water transport capacity

^a Log₁₀-transformed before analysis. For each species' trait data, see Table S2.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns not significant

Table 3. Pairwise relationships among 19 leaf and stem traits in 25 Fagaceae

	Leaf traits															Stem traits		
	F_p	F_{ps}	F_{TD}^a	g_{max}	SD	VLA _{min}	VT	VVA	$\delta^{13}C$	N_{mass}	N_{area}	LT	TD	LA ^a	LMA	WD	VD	D_h
Leaf traits	F_{ps}	0.69 ***																
	F_{TD}^a	0.64 ***	0.93 ***															
	g_{max}	-0.29	-0.40	-0.26														
	SD	-0.44 *	-0.47 *	-0.47 *	0.68 ***													
	VLA _{min}	-0.50 *	-0.46 *	-0.44 *	0.41 *	0.47 *												
	VT	0.65 ***	0.50 *	0.51 **	-0.32	-0.42 *	-0.44 *											
	VVA	0.37	0.22	0.25	-0.07	-0.14	0.15	0.82 ***										
	$\delta^{13}C$	-0.04	-0.22	-0.25	0.41 *	0.39	0.17	-0.02	0.15									
	N_{mass}	-0.37	-0.03	0.05	0.42 *	0.10	0.14	-0.36	-0.30	0.07								
	N_{area}	0.71 ***	0.24	0.15	0.04	-0.16	-0.17	0.35	0.26	0.21	0.05							
	LT	0.88 ***	0.28	0.27	-0.14	-0.28	-0.32	0.58 **	0.41 *	0.05	-0.46 *	0.79 ***						
	TD	-0.07	-0.09	-0.44 *	-0.27	0.13	0.15	-0.13	-0.03	0.13	-0.29	0.15	-0.04					
	LA ^a	-0.28	-0.29	-0.12	0.60 **	0.3	0.07	-0.23	-0.17	0.26	0.39	-0.16	-0.25	-0.46 *				
	LMA	0.83 ***	0.24	0.12	-0.2	-0.24	-0.27	0.54 **	0.40 *	0.11	-0.52 **	0.81 ***	0.95 ***	0.26	-0.37			
	Stem traits	WD	0.37	0.41 *	0.30	-0.01	0.14	-0.15	0.26	0.19	0.33	-0.27	0.25	0.26	0.22	-0.10	0.33	
VD		-0.40 *	-0.01	-0.03	-0.33	-0.30	0.29	-0.29	-0.17	-0.22	0.13	-0.51 **	-0.52 **	0.10	-0.16	-0.48 *	-0.41 *	
D_h		-0.30	-0.53 **	-0.43 *	0.82 ***	0.54 **	0.23	-0.28	-0.12	0.34	0.48 *	0.18	-0.09	-0.19	0.64 ***	-0.13	-0.16	-0.47 *
K_h		-0.47 *	-0.60 **	-0.51 **	0.76 ***	0.52 **	0.28	-0.43 *	-0.28	0.14	0.45 *	-0.06	-0.27	-0.13	0.61 **	-0.30	-0.37	-0.16

The numbers shown are Pearson correlation coefficients. See trait abbreviations for Table 2. Significant correlations were shown in bold face.

^a Log₁₀-transformed before analysis.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure captions

Fig. 1 Relationships between (a) potential maximum stomatal conductance to water vapor (g_{max}) and potential hydraulic conductivity at stem (K_h) and (b) between specific force to punch (F_{ps}) and stem wood density for 25 Fagaceae species in East Asia. The curve in (a) is a positive exponential function fitted to the data ($y = 43.6 + 1.90\exp(1.97x)$). The line in (b) was fitted by standard major axis (SMA; $y = 0.16 + 0.09x$). *** $P < 0.001$; * $P < 0.05$.

Fig. 2 Principle component analysis of 16 leaf traits (black) and five stem traits (gray) traits for (a) three biomes, (c) cool temperate forest, (e) warm temperate forest, and (g) tropical forests. Right column shows the relationships between species scores of PC1 in the individual PCAs which included either leaf or stem traits for (b) three biomes, (d) cool temperate forest, (f) warm temperate forest, and (h) tropical forests. Numbers in parentheses represent the percentage of variance explained by each axis. Please note that x-axis in (e) and y-axis in (c) were reversed to locate the same traits to a similar position. The number of Fagaceae examined was 25, 10, 14, and 8 for three biomes, cool temperate forest, warm temperate forest, and tropical forests, respectively. Symbols in (b) represent species' climatic occurrence; CTM = cool temperate forest, CTM-WTM = cool and warm temperate forest, WTM = warm temperate forest, WTM-TROP = warm temperate and tropical forest, TROP = tropical forest. The lines in (b), (d), and (f) were fitted by standard major axis (SMA). *** $P < 0.001$; ** $P < 0.01$; ns not significant. For (d), exclusion of two outliers (two *Fagus* species), which showed high PC1 scores in the stem PCA increased in the significance of the relationship. See Table 2 and Table S2 for abbreviations of species and traits, respectively. See Figure S2 for the results of individual PCAs which included either leaf or stem trait.

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