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Full Length Research Paper

The leaf size-twig size spectrum in evergreen broad-leaved forest of subtropical China

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Plant twig characters for 82 woody species including individual leaf and total leaf mass, stem mass, individual leaf area, total leaf area and stem cross-sectional area were investigated at the twig level in different altitudes and life forms on Meihuashan Mountain in Southeastern China. The results showed that twig cross-sectional area of plant twigs were found to allometrically scale to individual leaf area and total leaf area that the twig supported, all with the common SMA (standardized major axis) slope being significantly larger than 1.0. However, the spectrum of twig leaf mass–stem mass was found to be mostly isometric and the slope had no significant deviation from 1.0, the same as the spectrum of twig total leaf area–twig leaf mass. The allometric constants (y-intercepts) of the relationships between the stem cross-sectional area and leaf area (both total leaf area and individual leaf area) were found to decrease significantly along the altitudinal gradient. Compared to deciduous broad-leaved species, the evergreen broad-leaved species were smaller in total leaf area for a given cross-sectional area or stem mass. This suggests that the species would support less leaf area at a given twig cross-sectional area with increasing environmental stress. And the life form can modify leaf-stem scaling relationship because of the difference of function.

Key words*:* Twig, leaf size, allometric scaling, altitudinal gradient, subtropical evergreen broad forest.

INTRODUCTION

Allometry, the study of correlations between the dimensions of different traits of an organism, has long been an important issue in the evolutionary biology of animal design (LaBarbera, 1989; Harvey and Pagel, 1991). However, allometry in plants has been relatively little studied (Li et al., 2005). Although vascular plants span many orders of magnitude in size, vascular plants share essentially the same anatomical and physiological design (Enquist, 2002). So there will be a universal scaling across species differing in phyletic affiliation and growth habit. Since leaf size is positively correlated with twig size in both intra-specific and interspecific comparisons (Corner, 1949; White, 1983a, b; Brouat et al., 1998; Preston and Ackerly, 2003; Westoby and Wright, 2003; Sun et al., 2006), the leaf size–twig size spectrum recently has been recognized as one of the leading dimensions and is still being explored (West et al., 1997; Preston and Ackerly, 2003; Westoby and Wright, 2003; Sun et al., 2006; Liu et al., 2008). This variation in twig and leaf size is substantial between species coexisting at a site (Westoby and Wright, 2003). But compared with the other dimensions in the leaf-height-seed (LHS) system (Niklas, 1995; Westoby, 1998), the adaptive significance of this spectrum has not been extensively studied in relation to plant life history strategies (Westoby and Wright, 2003; Sun et al., 2006; Yang et al., 2009).

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Abbreviations: SMA, Standardized major axis; **HSD,** honestly significant difference; LMA, leaf dry mass per unit area.

Although several studies have described the variation in twig and leaf size of a broad spectrum of species of different life forms growing in a wide range of environments (White 1983a, b; Preston and Ackerly, 2003; Westoby and Wright, 2003; Sun et al., 2006), only few studies analyzed the spectrum in evergreen broad leaved forest (Xiang et al., 2009), providing some evidence of relationships among leaf and twig in evergreen broad leaved species. Leaf habit and habit type may greatly influence the leaf-stem relationship. In particular, a general tendency for evergreen species to havehigh leaf dry mass per unit area (LMA) has been reported (Westoby et al., 2002; Wright et al., 2004). In general, building high LMA leaves needs more investment per unit leaf area (Cavender-Bares and Holbrook, 2001; Cavender-Bares et al., 2005). Therefore, compared to deciduous species, evergreen species with a high LMA have to support a larger mechanical loading on twig stems for a given leaf area. Thus, it can be expected that evergreen broad leaved species may support higher leaf mass for a given leaf area and a smaller leaf area for a given stem investment given that they are usually smaller in vessel size and in stem conductive capacity than deciduous species. In addition, drought, low soil fertility and low temperature usually lead to a low transporting efficiency and a tendency of embolism and thus, may require a high investment in the transporting structure (Westoby and Wright, 2003; Sun et al., 2006). So it appears that plant at high altitude may support smaller leaf area with increasing environmental stresses. Although a scaling relationship has been found between twig sectional area and leaf area at the interspecific and intraspecific levels (Brouat et al., 1998; Brouat and McKey, 2001; Preston and Ackerly, 2003; Westoby and Wright, 2003), few studies have examined the scaling relationship in relation to environmental gradients.

In order to examine the response of the leaf size-twig size relationship to environmental variations, we investigated leaf/leaf area and mass, stem cross-sectional area and stem mass of plant twigs of 82 temperate woody species along an altitudinal gradient on Meihuashan Mountain, Southeast China. We determined the scaling relationships of leaf size versus twig size and analyzed the form of the scaling relationships in relation to leaf habit and leaf form. The following aspects are focused in this study: (1) Whether the scaling relationship between leaf size and twig size is invariant in subtropical evergreen broad-leaved forest and (2) to determine whether evergreen broad-leaved species support smaller leaf area for a given stem.

MATERIALS AND METHODS

The study site is located in Meihuashan National Nature Reserve (25° 25´ N, 116° 50´ E, 1200 m a.s.l), Fujian Province, Southeast China. The mean annual precipitation is about 1700 - 2200 mm approximately 70% of that which occurs from March to June. The, mean annual temperature is from 18.6℃ at the foot of the mountain to 7.1℃ at the top.

In this study, twig was defined as a first-year shoot of selected branches, consisting of a terminal set of internodes and the leaves borne by them. Twig samples were selected from three altitudes of the broad-leaved forests in July 2007, when leaf expands and shoot grow completely. In canopy layers, the low altitude (300 - 500 m) is predominated by *Machilus pauhoi*, the middle altitude (650 - 900 m) by *Castanopsis fargesii* and the high altitude (1,200 - 1,500 m) by *Castanopsis eyrei* and *Schima sinensis*. At each altitude, woody species including both trees and shrubs were investigated, with exception of those having fewer than three individuals.

We sampled a total of 37, 24 and 47 species at the three different altitudes, respectively. The low altitude shared 11 and 5 species in common with the middle and high altitude; and the middle altitude shared 11 species in common with the high altitude. The total species number was 82, belonging to 40 genera from 22 families, including 23 deciduous species and 59 evergreen species. For each species, five randomly selected individuals were selected and then 5 branches with tips at the outer surface of the plant's crown were randomly chosen. One twig without apparent leaf area loss was selected from each chosen branch for measurement. Twig length and diameters were measured and each twig was measured to an accuracy of 0.1 mm. Twig cross-sectional area was calculated from the diameters. All leaves on the twigs were taken off for leaf area and mass measurements. The leaves were scanned and the pictures were digitized using MOTIC software. Individual leaf area and individual leaf mass were calculated from total leaf, total leaf mass and the number of the twig. The leaves were dried to constant mass at 70℃ for 48 h and weighed.

The data of plant traits were log transformed to fit a normal distribution before analysis. The traits were arithmetically averaged within the first-year shoot and then within species. Species averages were log10 transformed to determine relationship. We conducted a hierarchical analysis of variance (ANOVA) for each variable. Model type II regression analysis was used to estimate the parameters of the allometric equations. Slopes of the allometric relationship were calculated as standardized major axis (SMA) (Falster et al., 2006). Differences in regression slopes (y-intercept) and in shifting along the common slope were tested by ANOVA. We also used Tukey's honestly significant difference (HSD) for significant differences among means. The calculation related to allometric equation parameters was conducted using (S) MATR Version 2.0 (Falster et al., 2006; Warton et al., 2006).

RESULTS

Twig cross-sectional area versus total leaf area and individual leaf area

The total leaf area supported on the twig was significantly correlated with the cross-sectional area of twigs (Figures 1a and b , Table 1, r^2 from 0.569 to 0.730, $p < 0.01$ for all altitudes and life forms) supported on the twig. SMA slopes ranged from 1.325 (EL) to1.384 (EH) for total leaf area indicating an allometric relationship. No significant difference was found in the SMA slopes between any two altitudes and life forms ($p = 0.874$). The y-intercept was found to be significantly lower in high altitude than in the other altitudes (both $p < 0.01$). These results indicate that less leaf area is supported by a given cross-sectional area for species in the high altitude. Similarly, the deciduous broad-leaved species had a significantly higher y-intercept, indicating that the deciduous broad-leaved species

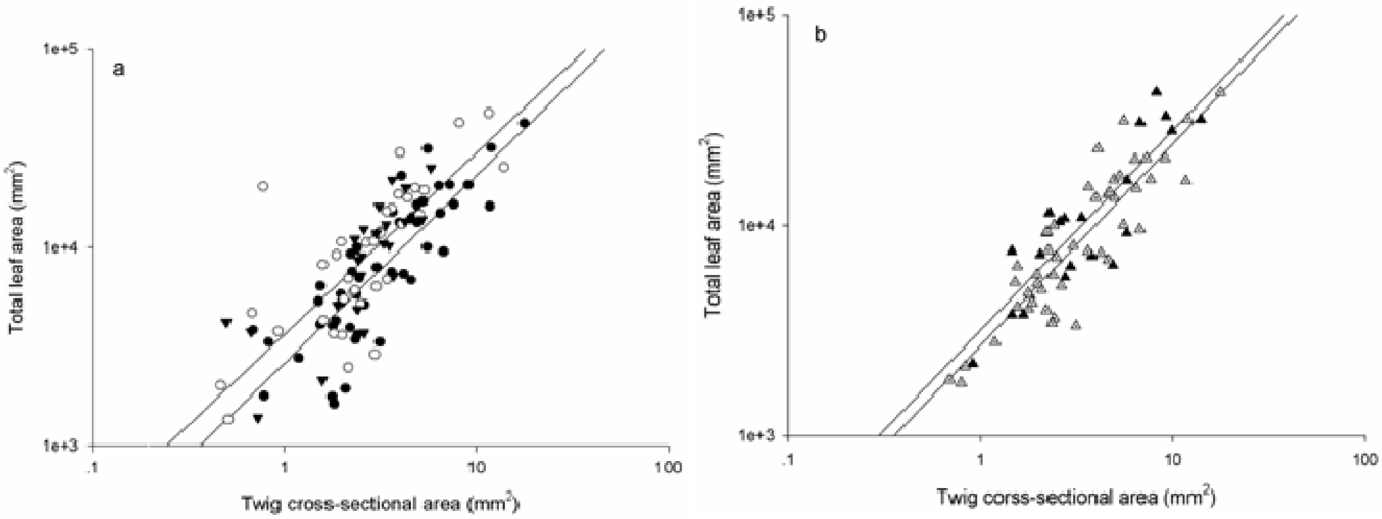

Figure 1. Relationships between twig cross-sectional area and total leaf area for evergreen woody species on Meihuashan Mountain, Southeastern China. Symbols: high altitude, closed circle; middle altitude, open circle; low altitude, closed triangle down; deciduous broad-leaved species, closed triangle up; evergreen broad-leaved species, open triangle up.

Table 1. Summary of SMA regression parameters for the scaling relationships between functional traits in plant twigs among three latitudes.

Parameters	Group	N	r^2	p	Slope	95% CI
TLA-TCSA	HA	47	0.709	<0.001	1.384	1.119 - 1.556
	MA	24	0.608	<0.001	1.380	1.096 - 1.550
	LA	37	0.569	<0.001	1.325	1.100 - 1.407
	DB	23	0.730	<0.001	1.314	1.122 - 1.520
	EB	59	0.695	<0.001	1.365	1.117 - 1.540
ILA-TCSA	HA	47	0.491	<0.001	1.284	0.996 - 1.417
	MA	24	0.647	<0.001	1.280	1.026 - 1.419
	LA	37	0.557	<0.001	1.211	1.166 - 1.519
	DB	23	0.564	0.003	1.271	1.183 - 1.429
	EB	59	0.491	<0.001	1.284	1.196 - 1.417
TLA-TSM	HA	47	0.730	<0.001	0.804	0.688 - 0.939
	MA	24	0.622	<0.001	0.851	0.650 - 1.113
	LA	37	0.459	<0.001	0.860	0.670 - 1.104
	DB	23	0.517	0.0012	0.845	0.655 - 0.964
	EB	59	0.730	<0.001	0.904	0.688 - 0.039
TLM-TSM	HA	47	0.810	<0.001	0.888	0.799 - 0.997
	MA	24	0.825	<0.001	0.937	0.780 - 1.126
	LA	37	0.663	<0.001	0.972	0.797 - 1.185
	DB	23	0.617	<0.001	0.933	0.727 - 1.108
	EB	59	0.830	<0.001	0.927	0.820 - 1.049
ILM-ILA	HA	47	0.736	<0.001	0.992	0.891 - 1.156
	MA	24	0.817	<0.001	1.090	0.925 - 1.322
	LA	37	0.730	<0.001	1.038	0.870 - 1.240
	DB	23	0.817	<0.001	1.052	0.890 - 1.113
	EB	59	0.814	<0.001	1.017	0.970 - 1.134

TCSA = Twig cross-sectional area; ILA = individual leaf area; TLA = total leaf area; TSM = twig stem mass; ILM = individual leaf mass; TLM = total leaf mass; LA = low altitude; MA = middle altitude; HA = high altitude; DB = deciduous broad-leaved species; EB = evergreen broad-leaved species.

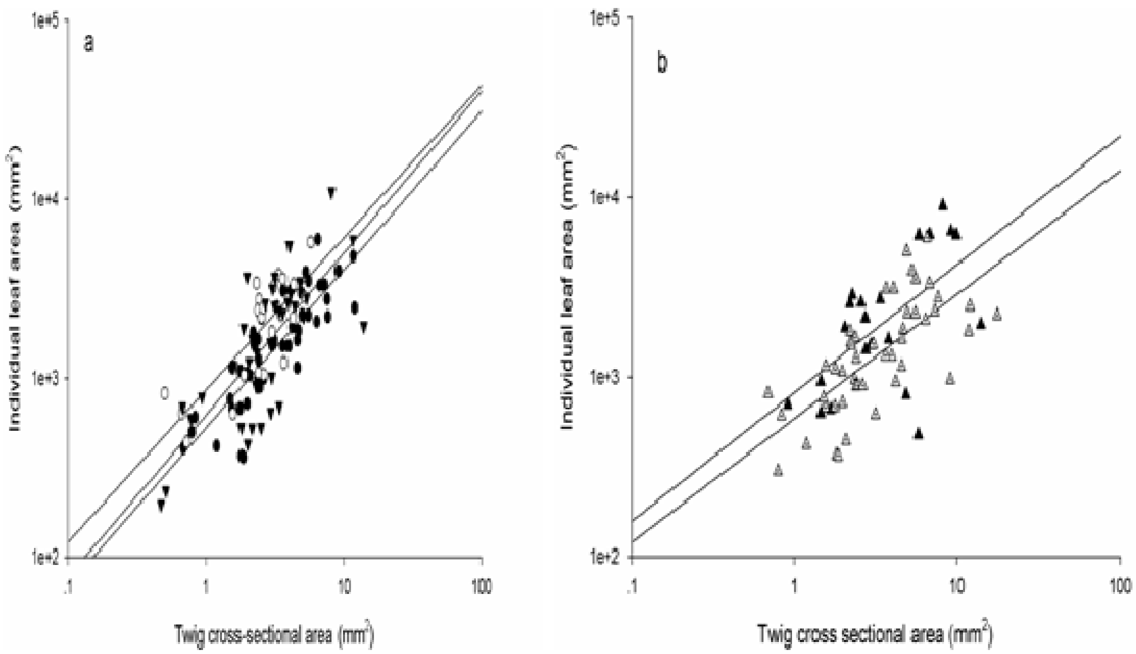

Figure 2. Relationships between individual leaf area across and twig cross-sectional area for evergreen woody species on Meihuashan Mountain, Southeastern China. Symbols: high altitude, closed circle; middle altitude, open circle; low altitude, closed triangle down; deciduous broad-leaved species, closed triangle up; evergreen broad-leaved species, open triangle up.

support a larger leaf for a given cross-sectional area than evergreen species.

A strong relationship was also found between individual leaf area and twig cross-sectional area (r^2 ranging from 0.491 to 0.647, $p < 0.01$ for all altitudes and life forms, Figures 2a and b, Table 1), with a common slope of 1.261 (CI = 1.152 – 1.414), significantly steeper than 1.0. A significant shifting in the y-intercept was also found between the high altitude species and the other two latitudes, as well as between deciduous and evergreen broad-leaved species ($p < 0.01$ for comparisons), indicating that thin stems support small individual leaves.

Stem mass versus total leaf area and total leaf mass

Total leaf area was strongly correlated with stem mass, with a common slope of 0.822 (CI = 0.725 – 0.932; $p < 0.01$, Table 1), which marginally but significantly deviated from 1.0 ($p < 0.01$). Significant differences were also found in the y-intercept between the high altitude and the other two altitudes and between different life forms ($p < 0.001$) (Figures 3a and b). This indicates that the high altitude species and deciduous broad-leaved species could support a smaller leaf area for the same stem investment than the low and middle altitude species and evergreen species.

A significant scaling relationship was found between leaf mass and stem mass (r^2 = 0.663 to 0.830, Figures 4a and b, Table 1), with a common slope of 0.906 (CI = 0.802–1.049), not significantly different from 1, suggesting the relationship perhaps is isometric. The difference in y-intercept was found to be significant between any two species groups, in which at a given stem mass the high altitude species had the largest leaf mass, followed by the middle altitude and then by low altitude (Figure 4a). Significant differences were found in the y-intercept between the high altitude and the other two altitudes ($p < 0.01$). The y-intercepts of the common slopes were also significantly lower in the evergreen species than in the deciduous broad-leaved species ($P < 0.01$).

Individual leaf mass versus individual leaf area

Individual leaf area was highly correlated with individual leaf mass (r^2 from 0.730 (EL) to 0.817 (EM), $p < 0.001$ for all the three altitudes, Figures 5a and b, Table 1), with a common slope of 1.078 (CI = 0.902 – 1.163) which is very close to 1, indicating these two traits are linearly related. Significant shifts were found in y-intercept, which was lower at low altitude than at middle and high altitudes (both $p < 0.01$). Although all the three altitudes shared a common slope, species in the high altitude were smaller in leaf area, but higher in leaf mass, suggesting that they were characterized by a high specific leaf area (LMA). Similar result is also found between deciduous and evergreen broad-leaved species, the evergreen broad-leaved species had a higher leaf mass per leaf individual than that of deciduous species.

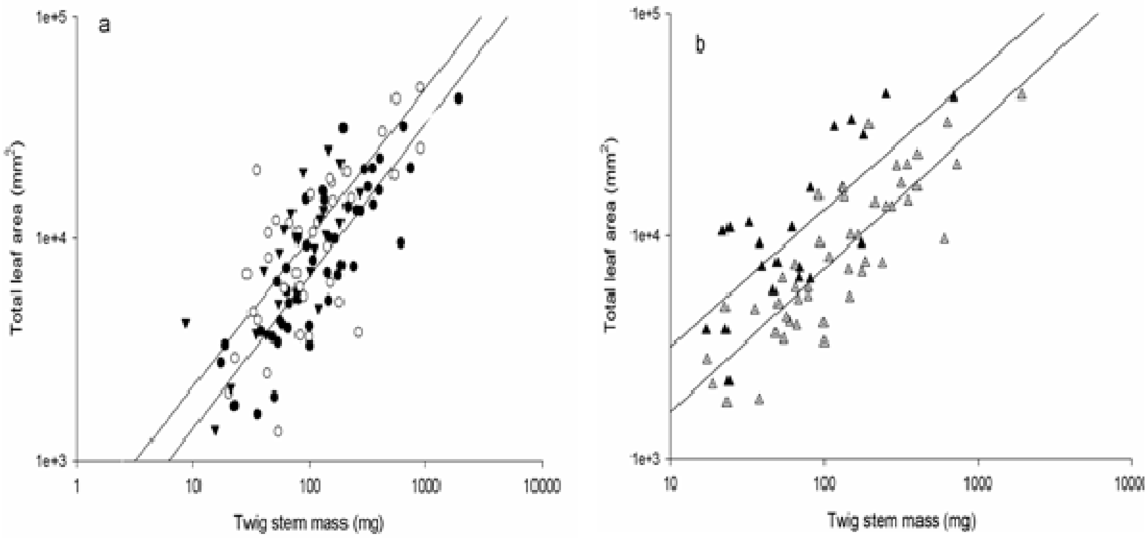

Figure 3. Relationships between total leaf area and stem mass for evergreen woody species on Meihuashan Mountain, Southeastern China. Symbols: high altitude, closed circle; middle altitude, open circle; low altitude, closed triangle down; deciduous broad-leaved species, closed triangle up; evergreen broad-leaved species, open triangle up.

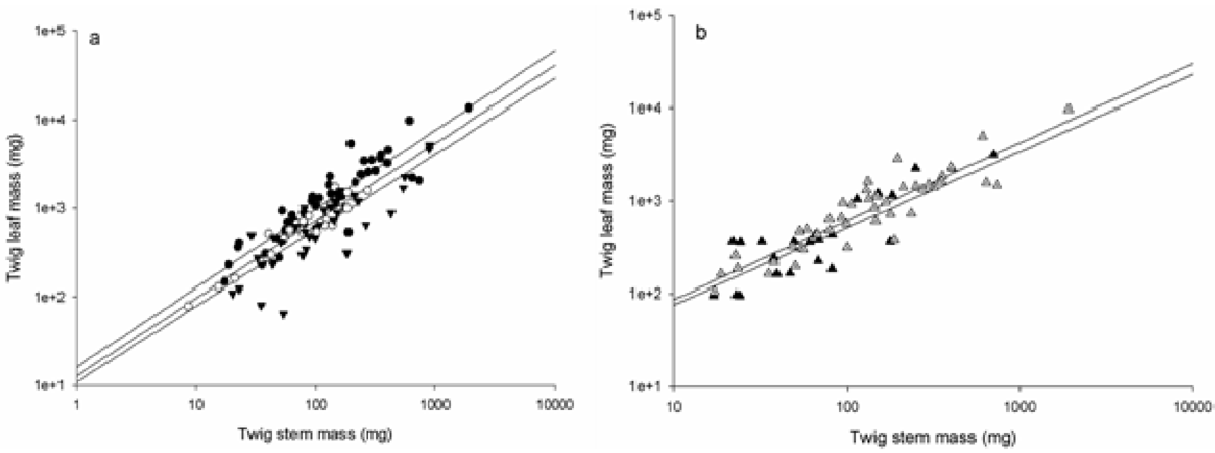

Figure 4. Relationships between total leaf mass and stem mass for evergreen woody species on Meihuashan Mountain, southeastern China. Symbols: high altitude, closed circle; middle altitude, open circle; low altitude, closed triangle down; deciduous broad-leaved species, closed triangle up; evergreen broad-leaved species, open triangle up.

DISCUSSION

It has been shown that total area should be coordinated with twig cross-sectional area for both mechanical and hydraulic (Niklas, 1992; Enquist et al., 1999). Although almost current researches claimed an invariant scaling relationship between total area and twig size, it is a dilemma whether is an isometric relationship. In the present study, the scaling relationship was found to be constant along the gradient and in different life forms, indicating that the relationship was invariant. In the present research, we found that twig cross-sectional area was related to total leaf area and individual leaf area with a common slope of 1.365 and 1.261, much higher than 1.0. The results is consistent with the studies of Westoby and Wright (2003), who reported a common slope of 1.45 in an interspecific comparison and of Preston and Ackerly (2003), who reported a common slope of 1.46 in an intra-specific comparison. However, the exponent constant of the relationship found by Brouat et al. (1998) is significantly close to 1.0. A possible reason is that twig diameter was measured with different method. Preston and Ackerly (2003) indicates that thick-twigged/large-leaf species can support a disproportionately larger leaf area than thin-twigged species. However, the isometric relationships of twig stem mass versus total leaf mass supported by the

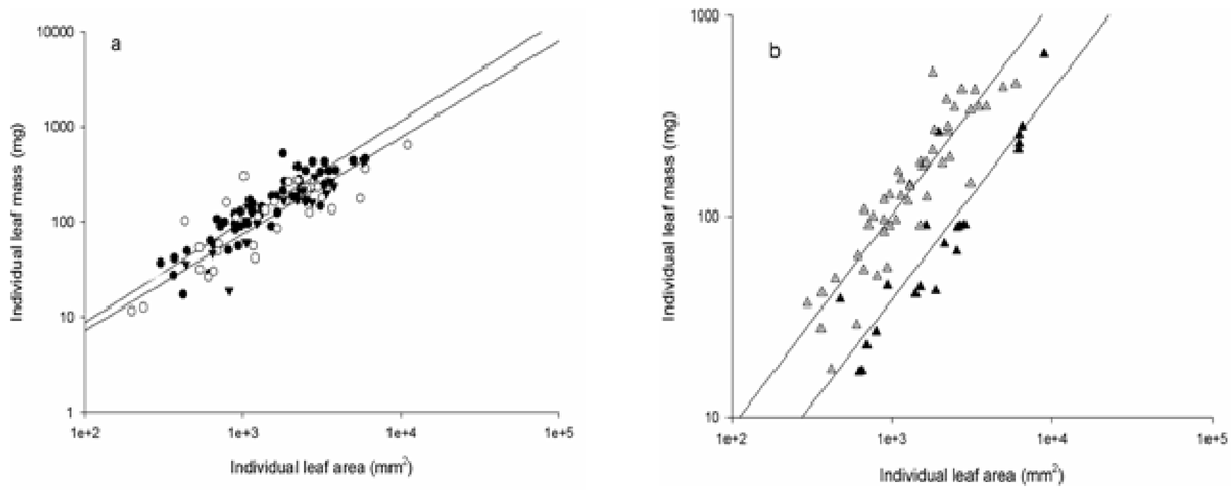

Figure 5. Relationships between individual leaf mass and individual leaf area for evergreen woody species on Meihuashan Mountain, Southeastern China. Symbols: high altitude, closed circle; middle altitude, open circle; low altitude, closed triangle down; deciduous broad-leaved species, closed triangle up; evergreen broad-leaved species, open triangle up.

twig do not confirm this advantage in the large-leaf/thick-twigged species, suggesting that leaf mass is independent of leaf size and twig size. The common slope of the relationship between total leaf area and stem mass was <1.0. That may imply large-leaf species were less efficient in deploying leaf area. Thus compared with small-leaf species, thick-twigged/large-leaf species do not have any advantage in supporting leaf area and leaf mass for the same size twig stem.

Although the scaling relationship between the cross-sectional area and leaf area was constant, the y-intercept decreased significantly with increasing altitude in the present research that can be interpreted by the hydraulic models (Harvey and Pagel, 1991; Brouat et al., 1998). Hydraulic conductance is often maximized presumably to maximize gas exchange and plant growth. The species at high altitude tend to have narrow vessels thereby decreasing the transporting efficiency of water and nutrients in cold winter. The species at high altitude require large transporting investments in stems to decrease transporting efficiency. In addition, species at high altitude may suffer greater physical loading of leaves at a given twig size than at low altitude. The high-altitude site is often windy in winters and suffers from winter snow cover. Windy environments necessitate a high investment in stems. So we observed that the y-intercept of the scaling between twig cross-sectional area and total leaf area at the high altitude was significantly lower than those at the lower altitude.

Our result also showed that the evergreen species support a smaller leaf area for the same twig in terms of both cross-sectional area and stem mass than the deciduous species. This may be due to the difference of leaf properties between the evergreen and deciduous species. The evergreen species in this study had a higher LMA (Figure 4b), than that of the deciduous species. The increase in LMA may decrease stem efficiency in deploying leaf area. LMA was found to be negatively correlated with the ratio of total leaf area to stem mass within each species group. This may imply that LMA can influence the spectrum between leaf size and twig size. In addition, evergreen species are usually characterized by narrower conducting elements than deciduous species (Cavender-Bares and Holbrook, 2001). This may permit evergreens to be more resistant to freezing-induced xylem cavitations in winter than deciduous species (Davis et al., 1999), but would decrease their conducting efficiency.

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