

## Longevity, Lignin Content and Construction Cost of the Assimilatory Organs of *Nepenthes* Species

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- **Background and Aims** This study examined level of causal relationships amongst functional traits in leaves and conjoint pitcher cups of the carnivorous *Nepenthes* species.
- **Methods** Physico-chemical properties, especially lignin content, construction costs, and longevity of the assimilatory organs (leaf and pitcher) of a guild of lowland *Nepenthes* species inhabiting heath and/or peat swamp forests of Brunei, northern Borneo were determined.
- **Key Results** Longevity of these assimilatory organs was linked significantly to construction cost, lignin content and structural trait of tissue density, but these effects are non-additive. Nitrogen and phosphorus contents (indicators of Rubisco and other photosynthetic proteins), were poor predictors of organ longevity and construction cost, suggesting that a substantial allocation of biomass of the assimilatory organs in *Nepenthes* is to structural material optimized for prey capture, rigidity and escape from biotic and abiotic stresses rather than to light interception. Leaf payback time – a measure of net carbon revenue – was estimated to be 48–60 d. This is in line with the onset of substantial mortality by 2–3 months of tagged leaves in many of the *Nepenthes* species examined. However, this is a high ratio (i.e. a longer minimum payback time) compared with what is known for terrestrial, non-carnivorous plants in general (5–30 d).
- **Conclusions** It is concluded that the leaf trait bivariate relationships within the *Nepenthes* genus, as in other carnivorous species (e.g. Sarraceniaceae), is substantially different from the global relationship documented in the Global Plant Trait Network.

**Key words:** Botanical carnivory, carbon gain, functional traits, leaf chemistry, leaf lifespan, leaf mass per unit area, *Nepenthes*, pitcher, payback time.

### INTRODUCTION

A large body of studies exists on factors influencing life-span of plant assimilatory organs (Kikuzawa, 1991) and the link of these factors with the physiological and growth/structural traits of the organs (Reich *et al.*, 1997, 2003). For example, leaf longevity (LL) is related to various leaf traits including nitrogen (N) and phosphorus (P) contents, leaf mass per unit area (LMA, or its derivatives of thickness and tissue density) and maximum photosynthetic capacity ( $A_{max}$ ). It is also entrenched in the literature that LL increases with increasing leaf toughness/sclerophylly and defence due to the presence of complex, non-palatable, structural carbohydrate compounds including tannins, lignin, wax and cuticles (Kikuzawa, 1991; Turner, 1994; Wright *et al.*, 2004); these are products requiring high cost to acquire, and hence tissue construction cost (CC) often varies positively with LL. It is now increasingly being widely accepted that variation amongst these traits is captured by a single axis in multidimensional space (Reich *et al.*, 1997; Westoby *et al.*, 2002; Wright *et al.*, 2004). What is not yet clear is how applicable these correlated traits are across and within life-form categories. For example, the carnivorous plants typically grow in high light and high moisture conditions, but are

extremely constrained in terms of nutrient availability and uptake (Clarke and Leen, 2004; Ellison, 2006). Does such a constraint affect their leaf physico-chemical properties, the interrelationship of these traits, and consequently the positions of carnivorous plants on the global leaf economic spectrum axis? Studies by Knight (1992), Chapin and Pastor (1995), Ellison and Gotelli (2002), Mendez and Karlsson (2005), Wakefield *et al.* (2005), Ellison (2006), Osunkoya *et al.* (2007) and Farnsworth and Ellison (2008) suggest that the some scaling relationships among leaf traits in carnivorous species (mainly members of Sarraceniaceae, Nepenthaceae and Urticulariaceae) diverge from the worldwide leaf economics spectrum. However, in the above studies on carnivorous plants not all the links of leaf functional traits, especially those of photosynthetic physiology, CC, defense and lifespan, were examined.

In an earlier paper (Osunkoya *et al.*, 2007), it was reported that tissue CC of members of the genus *Nepenthes*, whose abundance and centre of origin is in South-east Asia, varied appreciably across species, but by far the greatest difference was detected between the pitcher and the leaf. That study also detected a weak link between CC and N or P, but a strong one with potassium (K), although N serves as one of the direct input variables to the derivation of CC. It was then hypothesized that for *Nepenthes* species, factors other than the production of photosynthetic machinery (which calls for high N and P inputs), including accumulation of compounds to provide greater resistance against herbivore damage, tearing and

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bending and hence organ deformation (e.g. lignin) or to reduce water loss/increase prey-capture (e.g. wax/lipids), may better explain CC values. Indeed work by Pavlovic *et al.* (2007) has shown a much lower photosynthetic rate in *Nepenthes* leaves compared with the global value – further proof that machineries of photosynthesis may not necessarily predict CC in this group of plants. Hence here (a) the lignin content and lifespan of the assimilatory organs of eight *Nepenthes* species of Brunei, northern Borneo, that were the focus of the investigation in Osunkoya *et al.* (2007), are reported, and (b) a search was conducted to find links of these two functional traits with those of earlier reported physico-chemical properties, especially with CC, nutrient composition (N and P) and mechanical/structural traits (LMA and its derivatives). In addition, because of the cup-like nature and liquid retention (reservoir) capacity of the pitcher, it was hypothesized that this organ should contain more structural materials for minimizing organ deformation/deterioration, such as higher lignin (fibre) content, than those of the conjoint flat photosynthetic lamina.

## MATERIALS AND METHODS

### *Filed sites and study species*

Data for this comparative study come from five species (*Nepenthes albomarginata* Lobb, *N. ampullaria* Jack, *N. bicalcarata* Hook.f, *N. gracilis* Korth and *N. mirabilis* Druce) and three distinct varieties of *N. rafflesiana* Jack (var. *typical*, var. *elongata* and var. *gigantia*); the latter, because their gross differences are regarded as ‘species’ (see Phillipps and Lamb, 1996; Clarke and Leen, 2004; Osunkoya *et al.*, 2007). These species occur sympatrically in heath/Kerangas forests of Brunei, northern Borneo, South-east Asia. *Nepenthes* produces two organs of assimilation: (1) a main photosynthetic flat base (henceforth called the leaf), and (2) a modified non- or limited-photosynthetic, jug-like leaf blade at the end of the leaf tendril/petiole (henceforth called the pitcher) – a passive trap that can catch/digest prey and absorb the breakdown products. The pitcher is a modified epiascidiate leaf blade, in which the adaxial surface curls around and fuses to form the inner wall of the pitcher tube (Owen and Lennon, 1999). Fuller description, including morphology, growth habit and extent of distribution of these species can be found in Phillipps and Lamb (1996) and Clarke and Leen (2004).

Detailed description of the collection sites of the leaf and pitcher samples of these *Nepenthes* species, the subsequent morphometric measurements taken [i.e. organ area, dry mass and thickness for estimation of tissue mass per unit area (TMA) and density], as well as sample initial preparation for the chemical analyses are fully reported in Osunkoya *et al.* (2007). In summary, plant materials were collected in sunny heath and peat swamp habitats from four to six localities in Brunei, northern Borneo, in South-east Asia. The materials were washed in distilled water, dried at 40 °C and subsequently milled to fine powder. Samples for individual sites were then stored

over silica gel in desiccators to maintain dryness prior to nutrient analyses.

### *Foliar and pitcher chemistry and longevity*

The following analyses were carried out on samples collected per site, per species. Total nitrogen (N) was determined in triplicate on 1.0-g samples per site (replicate) by the micro-Kjeldahl method (Helrich, 1990). Ash content ( $\text{g g}^{-1}$ ) was determined in triplicate by incinerating 1 g samples of the dried leaves in a muffle furnace at 500 °C until a white-grey residue remained (3–4 h). Heat of combustion was measured in triplicate per site using 1 g, with a Gallenkamp bomb calorimeter (model CRA-305, UK) calibrated against benzoic acid pellets of known energy value. Ash-free heat of combustion was calculated by converting the heat of combustion on a total dry weight to the corresponding ash free weight. Leaf construction cost (mass), defined as grams of glucose (+ minerals) required to synthesize 1 g of carbon skeleton (leaf tissue) (Poorter *et al.*, 2006), was calculated using a formula based on the growth efficiency of leaf tissue, heat of combustion, ash and nitrogen content according to Williams *et al.* (1987; for the formula, see Osunkoya *et al.*, 2007).

Lignin content was assayed by the Klason procedure (Rowland and Roberts, 1994; Hatfield and Fukushima, 2005) involving 0.5 g of the dried and milled sample refluxed for 4 h with 72 %  $\text{H}_2\text{SO}_4$  followed by vacuum filtration of the insoluble lignin residue in a washed pre-weighed sintered crucible. It must be noted that this procedure does not measure the exact amount of lignin in a plant tissues as the residues obtained contain other non-hydrolysable complex compounds including wax/cutin and suberin (Jin *et al.*, 2003; Hatfield and Fukushima, 2005) However, because these products are all associated with leaf sclerotization under the physiologically stressing conditions such as in heath/Kerangas soils of low inherent fertility in which the *Nepenthes* species inhabit, the Klason lignin assay should give an indication of strength and stiffness (sometimes called modulus of elasticity) of the test material.

Organ longevity was determined in the field where materials were collected for leaf trait measurements. In November 2005 through to July 2006, for each species, except *N. rafflesiana* var. *typical*, organ longevity was estimated at each of four to six sites by tagging and following the fates of four young, healthy and fully developed leaves and pitchers with the lid still closed in each of five individual plants. Thus 4 leaves and 4 pitchers  $\times$  5 individual plants  $\times$  (4–6 sites) = 80–120 leaves or pitchers were tagged per species. The tagged organs were observed every month for a period of 8 months (until July 2006). At each census period, the organs were recorded as alive or dead, based on colour and visual appearance. A leaf was considered dead when it had turned completely brown or had fallen off. A pitcher was considered dead when a significant proportion or the whole cup became dry, turned brown and/or had fallen off. The census data collected is an indication of the operational lifespan (in terms of photosynthesis/and or

nutrient capture) as the organs were not tagged at their primordial stages. Census data collected were used to estimate the organ mortality rate ( $\lambda$ ) and operational half lifetime ( $t_{0.5}$ ) for each species according to Lieberman (1985) and Suarez (2003). The mortality rate ( $\lambda$ ) was estimated for each species through the non-linear adjustment of the survival probability as a function of time,  $\lambda = (\log_e N_o - \log_e N_f) / t$ , where  $\lambda$  is considered constant through time, and  $N_o$  and  $N_f$  are counts at the beginning and at the end of the census interval  $t$ . The half lifetime [ $t_{0.5} = (\log_e 2) / \log_e \lambda$ ] is used as a surrogate of lifespan, and is defined as the time it would take a leaf or pitcher population to lose 50 % of its members.

### Statistical analysis

The data were analysed using SPSS software (ver. 16.0). The data were confirmed for homogeneity of variances and hence there was no need for transformation prior to parametric analysis. Differences between sites (replicates), species and assimilatory organs for all measured variables were tested using multi-factorial ANOVA. A preliminary analysis indicated that within species, site differences were not significant for the majority of the traits examined. Hence data for all sites were pooled. Using species means, correlations were carried out between all measured variables. Multiple linear regressions were also carried out to explore: (a) to what extent the physical and chemical traits can predict longevity of the organs, and (b) to see if the effects of these explanatory traits are additive on longevity.

## RESULTS

Figure 1 shows the patterns of organ survival with time. At 8 months, organ longevity varied appreciably across species ( $F_{6,457} = 2.05$ ;  $P < 0.001$ ) and between the two organs ( $F_{1,481} = 13.89$ ;  $P < 0.001$ ; Fig. 1). As expected, longevity was significantly lower in the pitcher cup relative to the leaf (Table 1). In most species close to 100 % of the tagged leaves and cups were still alive and functional by 2 months; thereafter they all experienced near linear loss with time (a type I survivorship pattern), with the exception of leaves of *N. gracilis* and *N. rafflesiana* var. *gigantia* which did not start senescence until 3 and 4 months, respectively. It is noteworthy that pitchers of *N. mirabilis* experienced total loss by 2 months. It appeared that *N. bilcalacarta* pitchers lasted the longest with an estimated half lifetime of about 6 months (Fig. 1 and Table 1), while those of the remaining species were much lower ranging between 1 and 3.5 months. In leaves, half lifetime ranged from 1.5 months (*N. mirabilis*) to between 4–8 months (*N. rafflesiana* var. *gigantea*, *N. rafflesiana* var. *elongata* and *N. albomarginata*) to >10 months (*N. gracilis*, *N. bilcalcarata* and *N. ampullaria*).

In all cases lignin content was significantly higher in the leaf relative to the cup ( $F_{1,89} = 109.18$ ;  $P < 0.001$ ; Table 1), with *N. gracilis* exhibiting the highest value both for the leaf and the cup (36.80 % vs. 32.90 %, respectively). In leaves the lowest lignin is in *N. albomarginata* (26.57 %); the remaining six species had a lignin content

of 30–33 % in their leaves. In the pitcher cup, the lowest lignin contents were recorded in the three varieties of *N. rafflesiana* (19–22 %).

Trait differences between organs and amongst species for CC, N, P, TMA, thickness and tissue density have been fully reported elsewhere (Osunkoya et al., 2007). Cross-species relationships between organ longevity, lignin content, N, P, CC and physical traits of TMA, density and thickness are given in Table 2. As in previous studies (Osunkoya et al., 2007), a greater number of the bivariate relationships were significant ( $P < 0.05$ ) when the leaf and pitcher data are used as two independent data points (nine out of 28 with additional three pair-wise comparisons being marginally significant at  $P < 0.10$ ; Table 2, right upper section) than when using either the leaf (one out of 28 with an additional three significant at  $P < 0.10$ ) or the pitcher (four out of 28 at  $P < 0.05$ ; Table 2, left lower section). The well-known frequent exchange of biochemical materials between the leaf and its conjoint pitcher (see Schulze et al., 1999; Osunkoya et al., 2007) may have been responsible for the fewer numbers of significant links between traits at the individual organ levels. Overall, trends can be summarized as follows: across species and organs, longevity and lignin content are positively correlated ( $r = 0.54$ ,  $n = 14$ ;  $P = 0.04$ ; Fig. 2A); organ longevity also increased significantly with CC mass ( $r = 0.69$ ,  $n = 14$ ;  $P = 0.008$ ; Fig. 2B) but not with leaf nutritional value (e.g. N and P) or with leaf economic spectrum trait of TMA (Table 2 and Fig. 2C–E). Out of the three physical/structural traits examined, variation in tissue concentration (density) was the most influential on organ longevity and chemical properties (Fig. 2E–G and Table 2; for details, see Osunkoya et al., 2007); tissue density was positively related to CC mass ( $r = 0.64$ ,  $n = 16$ ;  $P = 0.01$ ), lignin content ( $r = 0.51$ ,  $n = 16$ ;  $P = 0.05$ ) and organ longevity ( $r = 0.59$ ,  $n = 14$ ;  $P = 0.03$ ). Interestingly, N and lignin content appeared positively related, though the trend is only marginally significant ( $r = 0.44$ ,  $n = 16$ ;  $P = 0.09$ ; Fig. 2H). Stepwise multiple regression analyses showed that the effects of CC, lignin, N content and tissue density, though influential individually, are non-additive on organ longevity (Table 3), i.e. once any of these four traits, especially CC, is incorporated into the model, the influence of the others is dampened. Thus it appears that there is a high level of collinearity amongst these traits and consequently the inclusion of other traits in the model does not explain any additional variability in organ longevity beyond that which is explained by CC. Most of the changes in variance explained (i.e. change in  $R^2$ ) are <2 % and are non-significant, except N inclusion with CC where approx. 10 % change in  $R^2$  was observed, though still only marginally significant ( $P = 0.06$ ) (Table 3).

## DISCUSSION

Concentration of lignin in green leaves has received less attention in the literature (see also Poorter et al., 1997; Diehl et al., 2008) and it appears that there are no studies reporting this trait for carnivorous plants. Overall, the average leaf lignin in the *Nepenthes* species studied

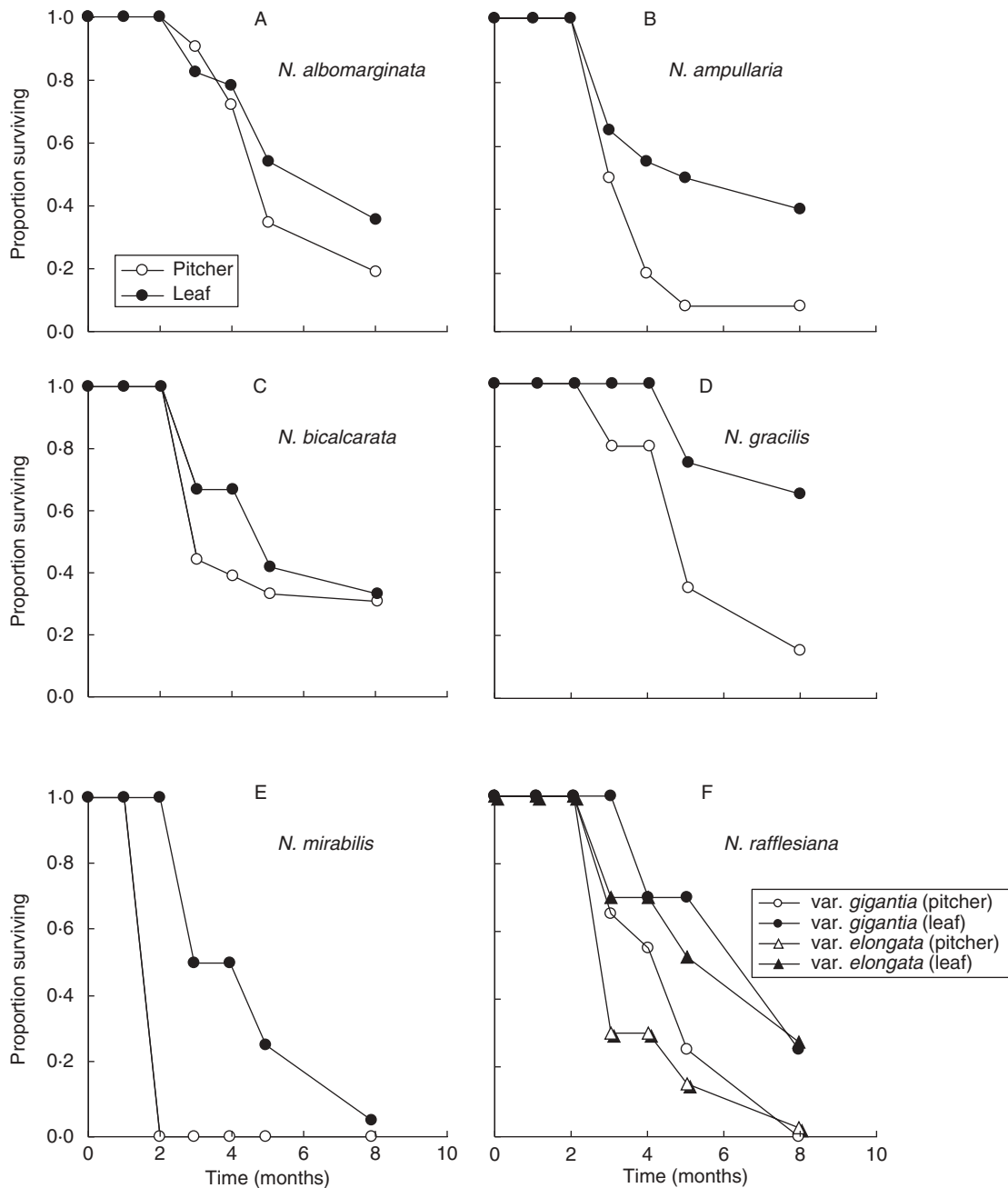


FIG. 1. Patterns of survival of leaves (closed symbols) and pitchers (opened symbols) of *Nepenthes* species over an 8-month period in heath and peat swamp forests of Brunei, northern Borneo. For each species, data have been pooled over all (four to six) sites surveyed. Note that the two varieties of *N. rafflesiana* (*var. elongata* and *var. gigantia*) are plotted on the same graph in (F).

(mean =  $31.30 \pm 0.76\%$ ) though higher, is not significantly different from those detected in the leaves of non-carnivorous plants cohabiting with them (e.g. species of *Alphitonia*, *Commersonia*, *Ploarium*, *Symplocos* and *Syzygium*; mean lignin content:  $27.32 \pm 4.56\%$ ;  $F_{1,12} = 1.31$ ;  $P = 0.28$ , O. O. Osunkoya, unpubl. res.). However, within the time interval of the survey, *Nepenthes* species did experience a significantly lower leaf survival (overall mean survival:  $33.04\%$ ; 95% confidence interval:  $25.55\text{--}40.53\%$ ) than those of non-carnivorous, small–medium-size plant species listed above (overall mean survival:  $43.13\%$ ,

95% confidence interval:  $20.50\text{--}80.25\%$ ; Omar-Ali, 2006). *Nepenthes* growth habit (being a herb/small-climbing shrub) could have been a contributing factor to the observed lower survival as they are less likely to escape trampling by ground animals and other forms of biotic attack compared with their non-carnivorous counterparts.

TMA, a product of thickness and tissue density, has been identified as a crucial trait of the leaf economic spectrum (Wright *et al.*, 2002, 2004). It was expected that a positive relationship would be detected between lignin and the structural trait of TMA (or its components) because a high lignin



TABLE 1. Mean ( $\pm$  s.e.) estimated half lifetime and lignin content of the assimilatory organs of *Nepenthes* species

<i>Nepenthes</i> species	Longevity, $t_{0.5}$ (months)		Lignin (%)	
	Leaf	Pitcher	Leaf	Pitcher
<i>N. albomarginata</i>	7.69 $\pm$ 1.37	3.46 $\pm$ 0.98	26.57 $\pm$ 0.53	23.45 $\pm$ 0.80
<i>N. ampullaria</i>	14.10 $\pm$ 3.90	2.40 $\pm$ 0.99	32.57 $\pm$ 2.09	24.39 $\pm$ 1.45
<i>N. bicalcarata</i>	9.71 $\pm$ 1.18	6.01 $\pm$ 1.85	30.09 $\pm$ 2.67	26.25 $\pm$ 0.67
<i>N. gracilis</i>	13.97 $\pm$ 2.34	2.96 $\pm$ 0.99	36.80 $\pm$ 0.55	32.90 $\pm$ 0.81
<i>N. mirabilis</i>	1.54 $\pm$ 0.43	0.93 $\pm$ 0.00	32.89 $\pm$ 0.57	31.23 $\pm$ 0.31
<i>N. rafflesiana</i> var. <i>typical</i>	NA	NA	31.87 $\pm$ 1.34	22.02 $\pm$ 0.12
<i>N. rafflesiana</i> var. <i>elongata</i>	5.59 $\pm$ 1.88	0.95 $\pm$ 0.31	31.09 $\pm$ 1.04	20.57 $\pm$ 0.29
<i>N. rafflesiana</i> var. <i>gigantia</i>	4.21 $\pm$ 0.78	1.10 $\pm$ 0.01	29.28 $\pm$ 0.39	17.92 $\pm$ 0.94
Mean	7.95 $\pm$ 1.25	2.77 $\pm$ 0.40	31.30 $\pm$ 0.76	24.81 $\pm$ 1.03
F-ratio amongst species within organs	6.58**	2.41*	4.42**	31.74***
F-ratio between organs		9.78***		109.18***

For longevity, at the initiation of the survey, sample size ( $n$ ) was  $\geq 80$  leaves or pitchers per species. For lignin content, data are based on leaves and pitchers from six sites per species with at least three readings per site.

\*  $P < 0.05$ ; \*\*  $P < 0.02$ ; \*\*\*  $P < 0.01$ ; NA, data not available.

concentration, a major constituent of cell walls and vascular bundles, will lead to more robust, sturdy leaves (Westoby *et al.*, 2002). Only tissue density upheld this hypothesis, suggesting that the thicker leaf in *Nepenthes* does not necessarily translate to a tougher organ, perhaps due, in part, to the presence of scattered palisade layers, large intercellular spaces (especially in the leaves; Muntassir, 2007; Pavlovic *et al.*, 2007) and/or storage of non-structural carbohydrate in the protoplasm of their cells. In fact, in some studies (e.g. Wilson *et al.*, 1999; Westoby *et al.*, 2002), components of TMA (i.e. lamina depth and tissue density or measures closely related to them, e.g. cell volume) have been advocated as better indices of plant strategies than TMA.

The significant interspecific positive relationship between lignin and CC in the *Nepenthes* studied has validated that lignin – an expensive, protective compound – is a better predictor of investment cost than any of the nutritional traits (especially N and P) in this carnivorous guild (see also Osunkoya *et al.*, 2007). Lignin also has a positive correlation with N, albeit marginally significant (Fig. 2H). This suggests that the lignin relationship with N (a nutritive trait) may be more of a co-ordination rather than of a physically enforced trade-off in contrast to what prevails in other plant species (Turner, 1994; Westoby *et al.*, 2002). A lignin–N trade-off would have implied a causal effect in which a greater concentration of fibres, cell walls and hence lignin, etc. will leave less room for N-rich mesophyll, but this trend was never observed in the present study. Thus in *Nepenthes*, it is likely that the higher lignin content serves as a protective function in organs with higher N concentration so as to confer (on the organ) a prolonged lifespan (for further explanation, see below). Indeed Westoby *et al.* (2002), Reich *et al.* (2003) and Wright *et al.* (2005) argued that some functional ecological traits are substantially independent of each other (as seen here for N vs. lignin and N vs. CC), expressing different aspects of a plant's ecology, and may thus not have strong causal, unidirectional relationships. Rather such traits may exhibit a co-ordination (reciprocal) effect in which trait values

function more successfully as a combination, rather than because one trait drives another mechanically or physiologically. It remains to be seen how consistent and widespread the lignin–N positive relationship is in other carnivorous species.

In all species, lignin content, tissue density, tissue mass per unit area, CC, and longevity were higher in the leaf relative to the pitcher (Table 1; see also Osunkoya *et al.*, 2007). Lower longevity in the pitcher is probably related to its high physiological function, especially its frequent production of digestive enzymes via secretory glands (Schulze *et al.*, 1999). This activity inevitably makes the pitcher more liable to a higher risk of self damage compared with the leaf. Interestingly, because of the intricate and fibrous appearance of the pitcher, it had been hypothesized (see Introduction) that the pitcher would have a much higher lignin content than the leaf, but the opposite trend was detected. This is probably due to the need for longer retention of the leaf as it contains more expensive compounds, like N and P, than the pitcher cup. The positive and significant correlation between longevity and lignin content reflects reduced palatability of the leaves and pitchers to herbivores with increasing structural materials. Indeed, it was observed that the level of herbivory on leaves of *N. gracilis* is minimal, in line with its relatively higher lignin content and higher longevity. In contrast, pitchers of *N. mirabilis* and *N. rafflesiana* var. *typical* were observed to be riddled with insect holes in view of their relatively higher N content (see table 2 of Osunkoya *et al.*, 2007) and/or low lignin value (Table 1).

Westoby *et al.* (2002) and Wright *et al.* (2004) contend that increased leaf longevity is of paramount importance for plants in a resource-limited environment (such as the heath/Kerangas forest) as it means longer residence time of nutrients in the plant and allows payback of the initial cost of production of the organ itself. A corollary to this is that if organ longevity is to increase, the structure needs to be protected over time to minimize all forms of damage (herbivores, pathogens and physical forces), thus requiring extra structural materials (see Kikuzawa, 1991;

TABLE 2. Matrix of correlation coefficients ( $r$ ) between pairs of physico-chemical properties, construction costs and longevity of the assimilatory organs of eight *Nepenthes* species

	Longevity ( $t_{0.5}$ )	Lignin	Nitrogen	Phosphorus	Tissue mass per area	Tissue density	Tissue thickness	Construction cost
Longevity ( $t_{0.5}$ )	—							
Lignin	0.41, 0.21	<b>0.54*</b>	0.04	-0.13	0.35	<b>0.59*</b>	-0.20	<b>0.69**</b>
Nitrogen	-0.17, -0.38	—	<b>0.44†</b>	0.12	0.05	<b>0.51*</b>	<b>-0.42†</b>	<b>0.59**</b>
Phosphorus	-0.28, -0.39	-0.09, 0.50	—	<b>0.72***</b>	-0.16	0.19	-0.30	0.18
Tissue mass per area	0.15, 0.59	0.04, 0.14	<b>0.76*</b> , <b>0.73</b>	—	-0.17	0.03	-0.22	-0.30
Tissue density	0.09, 0.42	-0.35, -0.16	-0.56, -0.18	-0.26, -0.20	—	<b>0.50*</b>	<b>0.48†</b>	0.25
Tissue thickness	0.15, 0.43	0.12, -0.11	0.22, -0.29	-0.02, -0.06	0.14, <b>0.72*</b>	—	<b>-0.49*</b>	<b>0.64**</b>
Construction cost	0.38, <b>0.75*</b>	-0.26, -0.14	<b>-0.62†</b> , 0.02	-0.18, -0.24	<b>0.62†</b> , <b>0.80*</b>	<b>-0.68†</b> , 0.18	—	-0.37
		-0.36, 0.59	0.15, -0.08	-0.41, -0.49	-0.03, 0.10	0.56, 0.02	-0.49, 0.11	—

The right upper section of the matrix are the correlations across species and organs using mean values of the leaf and pitcher of each species as two independent data points for the bivariate relationships;  $n = 16$ , except for correlation with longevity where data are not available for *N. rafflesiana* var. *typical* and hence  $n = 14$ . The left lower section of the matrix are the correlations across species within the leaf and pitcher, respectively;  $n = 8$  for each case, except for correlation with longevity where  $n = 7$ . Significant trends are highlighted in bold: †  $P < 0.10$ ; \*  $P < 0.05$ ; \*\*  $P < 0.02$ ; \*\*\*  $P < 0.01$ .

Turner, 1994; Reich *et al.*, 1997; Shiodera *et al.*, 2008), and hence the reason why longevity also correlates positively with lignin and TMA (or for *Nepenthes* with its component of tissue density). Consequently longevity should increase with CC, as found in the *Nepenthes* species studied, and in other non-carnivorous species as well (e.g. Eamus and Prichard, 1998; Suarez, 2003). Others (e.g. Villar and Merino, 2001; Poorter *et al.*, 2006) have argued that often it is not CC that varies with longevity, but the ratio of CC to carbon gain (i.e. CC: maximum photosynthetic rate,  $A_{max}$ ) because this currency (often called payback time) measures net carbon revenue. Currently, there are no data on the photosynthetic performance of the species studied, but it is possible to draw on the limited data in the literature to evaluate this interesting hypothesis. Pavlovic *et al.* (2007) measured  $A_{max}$  of one of the species in this study, *N. mirabilis*, and obtained a value close to 0 and  $0.0244 \mu\text{mol CO}_2 \text{g}^{-1} \text{d. wt s}^{-1}$  for the pitcher and the leaf, respectively. A leaf CC value  $1.46 \text{g g}^{-1}$  was obtained for the same species (Osunkoya *et al.*, 2007). This suggests, for the leaf, a ratio of CC:  $A_{max}$  of  $1.46/0.0244 = 60 \text{d}$ . Similarly, using all available  $A_{max}$  and CC data in the literature for *Nepenthes* leaf (Osunkoya *et al.*, 2007; Pavlovic *et al.*, 2007; A. M. Ellison and J. D. Karagatzides, Harvard Forest, Harvard University, Petersham, MA, USA, unpubl. res.), a ratio of  $1.52/0.0313 = 48 \text{d}$  is obtained. This minimum payback time of 48–60 d is in line with the onset of substantial mortality by 2–3 months of tagged leaves of many of the *Nepenthes* species in this study (see Fig. 1). However, this is a high ratio (i.e. a longer minimum payback time) when compared with what is known for terrestrial non-carnivorous plants in general (5–30 d; Saeki and Nomoto, 1958; Jurik and Chabot, 1986; Williams *et al.*, 1989; Villar and Merino, 2001; Poorter *et al.*, 2006). The payback time is even higher for the pitcher cup (because of its low  $A_{max}$  and if contribution from trapping is excluded), and one can only wonder if the minimum critical point (i.e. marginal dividend) is ever attained for the pitcher before being discarded. Alternatively, it could be argued that the trapping capability of the pitcher contributes indirectly to the plant carbon and nitrogen gain potential (Schulze *et al.*, 1997) thus helping to lower critical payback time, and may account for earlier pitcher senescence compared with the leaf as observed in this study. *Nepenthes gracilis* leaves experienced the highest survival, with 65 % of the leaves still present at 8 months (i.e. 240 d; Fig. 1D) implying a lifespan that is thrice beyond the mean critical payback time. It is not inconceivable that prior to this time (i.e. 240 d), the leaf net dry mass return per time per leaf area (i.e. net carbon revenue) is already optimized, but the organ is still being retained: (a) because of nutrient withdrawal to newly formed assimilatory organs, and (b) because net revenue, even though decelerating, had not deteriorated to zero (Westoby *et al.*, 2002). This strategy of retention beyond payback time may help explain the widespread nature and high density of *N. gracilis* compared with the other species in this study (Clarke and Leen, 2004; O. O. Osunkoya, pers. obs.). A shoot carbon budget model favoured keeping leaves this long to maximize extension growth (Ackerly, 1999).

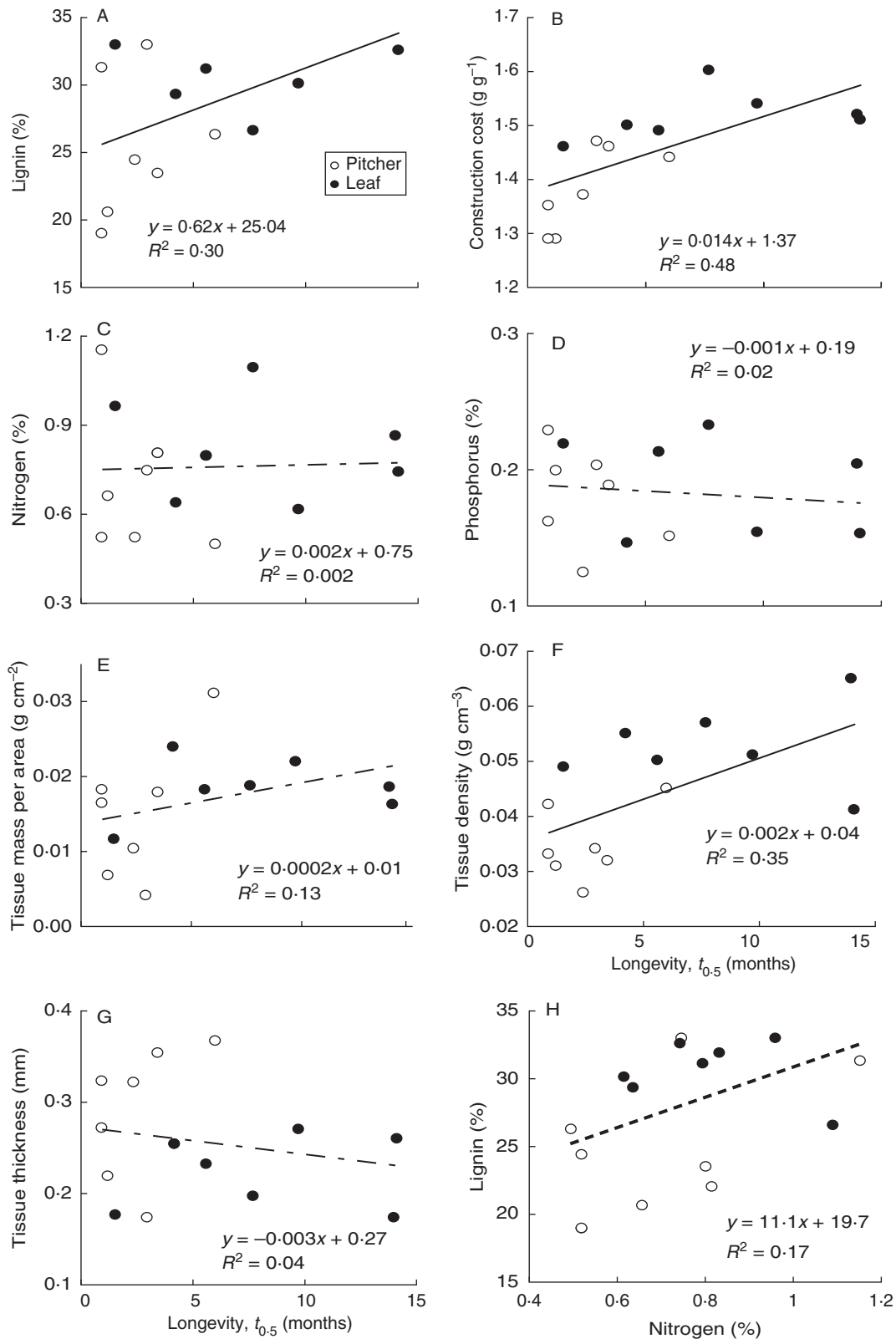


FIG. 2. Relationships between longevity as determined by half lifetime ( $t_{0.5}$ ) vs. physico-chemical properties (A–G) and between nitrogen vs. lignin content (H) in eight *Nepenthes* species from Brunei, Borneo. Each point is the mean values from four to six sites; leaf (closed circles) and pitcher (open circles). Continuous lines indicate significant correlation across species and assimilatory organs at  $P < 0.05$ ; faint dashed lines indicate non-significant trends at  $P > 0.10$ ; the bold dashed line for nitrogen–lignin relationship indicates a marginally significant ( $P = 0.08$ ) trend. For significance of trends within organs, see Table 2.

TABLE 3. Summary statistics of stepwise multiple regression analyses of likely factors influencing organ longevity in eight *Nepenthes* species in Brunei, northern Borneo

Model fit	Intercept	Slope	Variance explained ( $R^2$ )	Prob	Change in $R^2$	Significance of $R^2$ change
Construction cost (CC)	-1.369	12.066	0.696	<b>0.001</b>	–	–
TMA*	3.384	1.419 (1.62, 1.82)	0.179	0.132	–	–
Tissue density (D)	3.316	2.000	0.332	<b>0.031</b>	–	–
Lignin (L)	-4.958	3.496	0.275	<b>0.05</b>	–	–
Nitrogen (N)	0.573	0.051 (-2.39, -2.14)	0.001	0.961	–	–
CC + D	-1.094	11.651, 0.15	0.697	<b>0.001</b>	0.001	0.849
CC + L	-1.078	12.364, -0.21	0.697	<b>0.001</b>	0.001	0.886
CC + N	-1.781	13.690, -1.126	0.785	<b>0.001</b>	0.089	<b>0.056</b>
CC + D + L	-0.762	11.95, 0.159, -0.234	0.698	<b>0.006</b>	0.002	0.971
CC + D + L + N	-2.878	12.38, 0.098, 0.899, -1.271	0.795	<b>0.004</b>	0.099	0.293

All traits were log transformed prior to regression analysis in order to compare allometry exponents with the global data set of Wright *et al.* (2004). In the multiple regressions, each trait was inserted in order of magnitude of its explanatory power ( $R^2$  value) when only the term is in the model. CC-mass had the highest  $R^2$  value and was inserted first, followed by the term with the second highest  $R^2$  value and so on. The 95 % confidence interval values for the global data are given in parentheses in the Slope column for organ longevity vs. nitrogen content and organ longevity vs. tissue mass per unit area.

\* TMA, Tissue mass per unit area.

## CONCLUSIONS

The links between CC, tissue density, lignin and longevity as seen here exemplify the direct and indirect causal relationships in leaf economic spectrum traits (Wright *et al.*, 2002, 2004; Reich *et al.*, 2003). In *Nepenthes*, as in other carnivorous species (e.g. *Sarraceniaceae*; see Farnsworth and Ellison, 2008), causal relationships amongst leaf traits are apparent, but the scales or extent of the relationships definitely differ significantly from that described by the universal spectrum of leaf traits found in non-carnivorous plants. For example, in *Nepenthes* species a weak link and hence an insignificant scaling relationship exists between CC and nutrient contents, especially N (a measure of Rubisco and other photosynthetic proteins), probably due to the dual function of their assimilatory organs and a substantial allocation of their biomass to structural leaves optimized for prey capture rather than light interception. There appears to be a positive link rather than a trade-off between lignin and N which may reflect an evolutionary co-ordination and/or adaptation to protect acquired but limited nitrogenous compounds that are chronically lacking in the soils of *Nepenthes* habitats. Organ lifespan is definitely a function of CC and structural materials (e.g. lignin and tissue density), but the effects of these explanatory traits are non-additive on the response variable (i.e. longevity). Minimum payback time is longer than that estimated for non-carnivorous plants, but may be in line with *Nepenthes* low photosynthetic rate, long leaf lifespan and consequently longer revenue amortization.

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