

SPECIAL FEATURE

THE TREE OF LIFE IN ECOSYSTEMS

Leaf life span and the leaf economic spectrum in the context of whole plant architecture

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Summary

1. The leaf economics spectrum (LES) has been an organizing framework of plant functional ecology for the past decade. The LES describes a set of trade-offs among traits related to plant carbon balance. Species with a long leaf life span (LLS) invest additional material for leaf protection and structural support and consequently tend to have a lower leaf photosynthetic rate per unit mass than species with a shorter LLS.

2. While the LES is most apparent in comparing species with extreme differences in their traits, it has nonetheless been adopted as a general explanation of leaf trait variation at all scales and in all plants. It highlights the ‘trait-based’ approach to plant ecology, which has generally used a small set of traits to predict whole organism and even whole ecosystem attributes. Few studies have investigated the relationships between LES traits and organismal attributes not directly related to carbon economy.

3. We explored the LES in 32 deciduous woody species of *Viburnum* (Adoxaceae). We found no evidence for any mass-based LES trade-offs. Rather, on an area basis, photosynthetic rates were positively correlated with leaf mass per area (LMA); higher LMA was associated with greater investment in photosynthetic tissue, with most of the variation due to changes in the thickness of photosynthetic mesophyll.

4. Species’ mean LLS varied between 19 and 26 weeks and was not correlated with other LES traits. Instead, LLS was strongly associated with the diverse set of whole-plant branching patterns in *Viburnum*. In the most common growth pattern, LLS was significantly correlated with flowering time, because branches end in terminal inflorescences, and all leaves and inflorescences are performed in overwintering buds.

5. *Synthesis*. Plants may recover the cost of their leaves early in the growing season, allowing LLS to vary independently of the plant carbon budget. In deciduous species, LLS may be strongly influenced by whole plant architecture, which, in *Viburnum*, is evolutionarily conserved. In general, positive area-based LES trait relationships will limit the relevance of LLS to this spectrum and allow LLS to vary for reasons that are not directly related to carbon economy.

Key-words: deciduous leaf habit, determinants of plant community diversity and structure, eco-physiology, evolution, leaf economic spectrum, phylogeny, whole plant architecture

Introduction

Clouds of leaves, flowers, fruits, trichomes and other diagnostically useful parts do not hang somewhere in the air but form part of whole organisms.

Halle, Oldeman & Tomlinson (1978), p. 330.

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As the leaf is the primary site of photosynthesis in most plants, it is intimately connected to a plant’s carbon budget. Variation in many leaf traits is often interpreted within a framework referred to as the ‘leaf economics spectrum’ (LES) (Wright *et al.* 2004). The LES describes a set of relationships between leaf traits that are connected to how plants both acquire and spend carbon, including light-saturated photosynthetic rates (A), dark respiration rates (R), leaf mass per area

(LMA), leaf life span (LLS) and foliar nitrogen concentrations (N). Relationships among these traits are typically examined on either a leaf mass or area basis, with each uncovering different yet important axes of trait integration. On an area basis, for instance, A_{area} , N_{area} and LMA can be positively correlated, because LMA is determined in part by leaf thickness and, to the extent that a thicker leaf contains more photosynthetic tissue than a thinner leaf on a per area basis, A_{area} and N_{area} will also be higher (Niinemets 1999; Poorter *et al.* 2009).

However, in larger data sets that span the full range of variation in LMA, area-based positive correlations tend to be weak and are replaced by strong mass-based trade-offs, with a negative relationship between LMA and A_{mass} (Reich *et al.* 1999; Wright *et al.* 2004). Interpreted from a biological point of view, this trade-off could reasonably arise due to the non-photosynthetic components of increasing LMA, including non-photosynthetic carbon compounds in the thicker cell walls and protoplasm, and greater cell packing, all of which will 'dilute' a given photosynthetic capacity on a dry mass basis. The dominance of mass-based relationships in large data sets has generally been interpreted in terms of alternative ecological strategies. A leaf with a high LMA and lower A_{mass} will typically have a longer LLS, giving the leaf more time to achieve a positive carbon balance. This end of the spectrum represents the 'heavy investment, long time to return' strategy, best exemplified by a slow-growing evergreen shrub. Alternatively, a leaf with a lower LMA will typically have a shorter LLS, as the leaf is not 'built to last', with a higher A_{mass} to assure a positive carbon balance. This is the 'live fast, die young' strategy, adopted at the extreme by weedy annuals. These alternative strategies, and even the LES that underlies them, have been acknowledged for many years (Small 1972; Chabot & Hicks 1982; Field & Mooney 1986; Reich *et al.* 1991, 1999), but rose to prominence after Wright *et al.*'s (2004) analysis of a global data set identified this single axis of variation across thousands of species from all of the world's biomes. LES has grown in recent years to be the defining element of plant functional strategy, and has even percolated into micro-evolutionary studies seeking genetic correlations among these traits (Donovan *et al.* 2011).

The LES has recently been interpreted by several authors as a mathematical artefact derived from the transformation of area-based photosynthesis measurements to mass-based estimates, which is achieved by normalizing measurements by LMA (Lloyd *et al.* 2013; Osnas *et al.* 2013). As LMA typically varies by orders of magnitude more than A_{area} , A_{mass} (the ratio $A_{\text{area}}/\text{LMA}$) will decline as LMA increases, and thus, a negative A_{mass} versus LMA relationship will exist when measured photosynthetic rates and LMA vary completely independently of one another. Lloyd *et al.* (2013) illustrated this point by simulating two data sets in which A_{area} and LMA varied independently with variances similar to the Wright *et al.* (2004) data set, and used these data to generate an A_{mass} -LMA relationship identical to the one recovered by Wright *et al.* However, although a negative A_{mass} -LMA relationship may be nothing more than a mathematical repercussion that can be generated with random data, such a relationship remains biologically relevant with conse-

quences for the plant carbon budget (Sack *et al.* 2013; Westoby, Reich & Wright 2013). Furthermore, both Lloyd *et al.* and Osnas *et al.* agree that while the 'instantaneous' trade-off (A_{mass} versus LMA) may be trivial, the time-integrated trade-off is not. That is, a leaf with a higher LMA still represents a larger carbon investment and would seem to require a longer life span in order to recoup its costs.

At the extremes, there are obvious links between LMA and LLS. A very thin leaf cannot withstand the physical stresses of weather and herbivory for very long (Coley 1988; Matsuki & Koike 2006). At the other end of the spectrum, a plant could physically construct leaves that cost more carbon than they will fix, but only until its carbon reserves are depleted. But what about the middle ground? Wright *et al.* (2004) acknowledged that LES is less pronounced when comparing only deciduous species, for example – whose range of LLSs cannot exceed 1 year – but this observation is seldom referred to in the literature.

Irrespective of the recent controversy, here, we raise a different question with respect to the 'global' nature of the LES. Are LES trade-offs strong enough to have actually governed the evolutionary trajectories of the traits themselves? There are, after all, many other important interactions that these leaf traits might have with other organismal attributes, and a whole plant perspective is generally lacking in the LES literature and in the trait-based approach to plant ecology. Consider LLS in the context of a deciduous woody plant. In this case, LLS must be determined in no small part by phenological patterns of plant growth, which in turn must depend in part on the relative proportion of vegetative and reproductive branches and on the nature of branching (e.g. whether axes are monopodial or sympodial). And for branches with terminal inflorescences, the period of leaf production will likely be connected to flowering time, which could be under an entirely different set of selection pressures unrelated to a leaf's carbon budget. If LLS is at least partially controlled by such factors, it is important to ask whether the LES trade-off is significant enough to maintain the LLS-LMA connection.

We investigated the relationships between photosynthesis, LMA, foliar N concentration and LLS in 32 deciduous *Viburnum* species growing in a common garden. *Viburnum* is a lineage of woody plants that appears to have evolved a deciduous leaf habit multiple times in connection with repeated transitions to the temperate zone during the Cenozoic (Clement & Donoghue 2011; Schmerler *et al.* 2012). Our sample of species broadly spans the phylogeny and also includes distinct types of branching architecture that could influence mean LLS in these species. Previous studies of particular lineages have shown no evidence for the predicted LES-mass-based relationships (Martin, Asner & Sack 2007; Dunbar-Co, Sporck & Sack 2009), and we provide a new explanation for why these trade-offs may be largely irrelevant for deciduous woody plants.

Materials and methods

We utilized the extensive living collection of *Viburnum* species grown at Harvard University's Arnold Arboretum (Jamaica Plain, MA, USA). We included all species at the Arboretum that were repre-

sented by at least two individuals; in the majority of cases, we monitored three individuals per species.

MONITORING LEAF AND FLOWERING PHENOLOGY

In March 2009, prior to spring leaf out, we tagged five branches per individual on two or three individuals for each of 32 *Viburnum* species. We attempted to tag branches that would be most exposed to full sun, but otherwise they were chosen randomly. Beginning in April 2009, we performed a regular census of all individuals, noting both leaf out and flowering status. Flowering state was coded at an individual rather than branch level, as follows: (i) first flower – the date at which the first inflorescence was fully open; (ii) peak flower – the date at which at least 50% of inflorescences were in full bloom; and (iii) last bloom – the last date when open inflorescences were recorded.

To estimate LLS, our methods were similar to those of Reich *et al.* (1991): for each tagged branch, we drew a schematic and added leaves and inflorescences to the figure as they were formed, and then noted when these senesced. We monitored the plants weekly during the peak leaf production time between April and June and the senescence period in September–December; during July–August, we performed a bi-weekly census. At the end of the season, the life span for each leaf was calculated from its emergence and senescence dates, in units of weeks. The LLS data set included 79 individuals and 5461 leaves.

MEASURING OTHER LEAF TRAITS

During the summers of 2009 and 2010, we measured light-saturated leaf photosynthetic rate per area (A_{area}). For each of our 32 species, A_{area} was measured on five fully expanded leaves sampled from two to three individuals using a portable photosynthesis system (Li-6400; Li-Cor Inc., Lincoln, NE, USA). Measurements were made on sun-exposed leaves between 10 am and 2 pm, at a photosynthetically active radiation of $1500 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, a target temperature of $22 \text{ }^\circ\text{C}$, a CO_2 partial concentration of 400 ppm and a relative humidity between 30% and 50%. A single estimate of A_{mass} per species was estimated by dividing species mean A_{area} by species mean LMA.

To calculate LMA, between 13 and 20 mature leaves per species were collected in 2009 and 2010. Each leaf was photographed individually and leaf areas were calculated using IMAGEJ 1.47d (National Institute of Health, Washington D. C., USA). The leaves were then dried for more than 3 days in a $60 \text{ }^\circ\text{C}$ oven before being weighed on a precision balance Mettler Toledo XS603S (Mettler-Toledo Inc, Columbus, OH, USA). LMA (g m^{-2}) was calculated for each leaf and averaged per species.

Once LMA was measured, several dried leaves were chosen among the same samples for carbon and nitrogen analyses, for a total of 10 leaves per species. Each leaf was coarsely ground in a Wiley Mixer/Mill (Thomas Scientific, Swedesboro, NJ, USA). The nitrogen content of each leaf was determined on a $1000 \mu\text{g}$ dry-matter aliquot using a Carbon-Nitrogen Elemental Analyzer (CE Instruments model NC2100; CE Elantech, Inc, Lakewood, NJ, USA). The N concentration per leaf mass ($N_{\text{mass}}, \text{g g}^{-1}$) was calculated per leaf and averaged for each species. N concentration per leaf area ($N_{\text{area}}, \text{g m}^{-2}$) for each species was estimated by multiplying species mean N_{mass} (g g^{-1}) by species mean LMA (g m^{-2}).

BRANCHING ARCHITECTURE AND GROWTH PATTERNS

Donoghue (1981, 1982) analyzed the branching architecture of all *Viburnum* species growing in the Arnold Arboretum. All viburnums

are characterized by opposite (rarely whorled) leaves and the production of terminal umbel-like or panicle-like many-flowered corymb inflorescences. Different growth patterns are distinguished based on the following traits: orthotropic versus plagiotropic shoots; monopodial versus sympodial growth; determinate versus indeterminate shoots; long versus short shoots; pre-formed versus neofomed inflorescences; and naked versus scaly buds. We utilize the Donoghue growth pattern categories here, but have added an additional ‘Sieboldii’ pattern (see below).

Most viburnums produce only orthotropic (vertically oriented) shoots, but two growth forms also produce specialized plagiotropic (horizontally oriented) shoots (the Plicatum and Furcatum patterns, Fig. 1e–f). These in turn are readily distinguished from one another by the form of their plagiotropic shoots: these are monopodial with short lateral reproductive shoots in the Plicatum pattern (Fig. 1e), and sympodial with short upright reproductive shoots in the Furcatum pattern (Fig. 1f).

Among the patterns with orthotropic shoots, the Opulus pattern is easily distinguished from the others by the production of generally ephemeral reproductive shoots that bear additional branches in following years from lateral buds in the axils of the bud scales at their bases (Fig. 1b). This tends to result in a clustered, or even whorled, appearance of the branches at the major nodes, and these branches

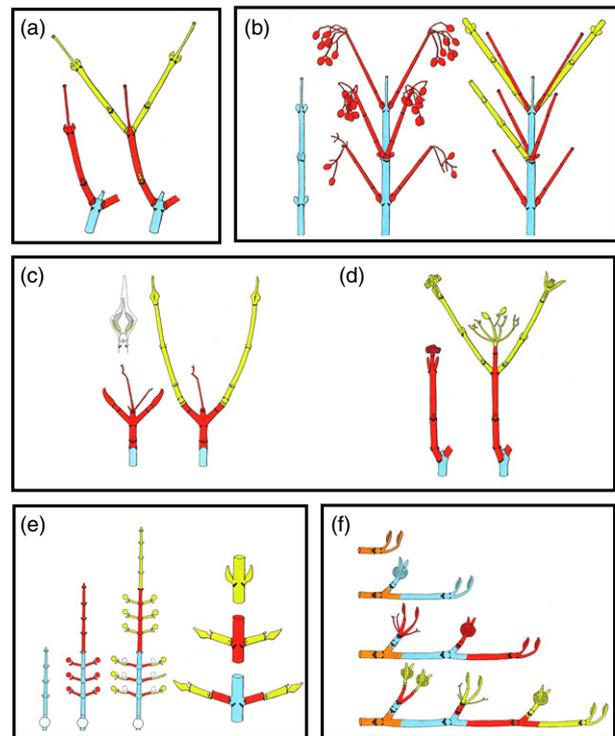


Fig. 1. Schematic of major growth patterns in *Viburnum* study species. The different colours represent sequential seasons of growth. For example, in (a), the blue shading at the base of the plant represents the growth of two seasons past, with the stub representing an old inflorescence pedicel that has since fallen off. The red branch represents the growth of one season past, which resulted in two nodes and a terminal inflorescence. The green shading represents the current year's growth, which reiterated the basic unit of two nodes and a terminal inflorescence. (a) Dentatum type. (b) Opulus type. (c) Lentago type. (d) Lantana type. (e) Plicatum type. (f) Furcatum type. The Sieboldii type is similar to (a), except that it regularly produces two flushes in 1 year, so in this case, the red and green branches would have all been produced in one season. Reprinted with permission from Donoghue (1981).

are typically a mix of new reproductive shoots (with a pre-formed, terminal inflorescence) as well as vegetative shoots (lacking a pre-formed, terminal inflorescence).

The remaining growth patterns can be differentiated by the relative timing of shoot growth and inflorescence development (Fig. 1). The primary difference between Dentatum (A), Lentago (C) and Lantana (D) is that in the Dentatum type, all of the major axes are determinate, in the sense that they are terminated by an inflorescence that had formed the previous season in an overwintering bud. Lateral buds subtending the inflorescence develop in the following year, never the current year, so the entire architectural unit is essentially pre-formed in bud. In contrast, in the Lantana and Lentago patterns, branches are produced beneath the terminal inflorescence during the same year that the inflorescence flowers. The main difference between the Lentago (C) and Lantana (D) patterns is that in the Lantana pattern, the reproductive shoots terminate in a naked inflorescence bud and renewal shoots emerge from buds on stems that were produced during the previous season. In the Lentago pattern, the inflorescence is not as well developed and is housed within a terminal bud; the lateral shoots that emerge from beneath the flowering inflorescence are born on a stem that elongated during the same season.

Donoghue (1982) commented specifically on the intermediacy of *V. sieboldii*, and our observations of *V. sieboldii* show that these plants consistently produce a second flush of growth in which lateral branches subtending the inflorescence elongate and produce multiple pairs of leaves – and occasionally additional inflorescences – during the same season (as opposed to overwintering in the bud as is typical of the Dentatum pattern). This behaviour was not unique to 2009, as we have continued to monitor phenology in these species yearly through 2013. It is also not unique to the growing conditions at the Arnold Arboretum, as we have recently noted the same behaviour in wild native populations in Kyushu, Japan (E. Edwards, pers. observation).

STATISTICAL ANALYSES

We analyzed the evolutionary relationships between our set of traits (A_{area} , A_{mass} , N_{mass} , N_{area} , LMA, LLS and leaf thickness) using phylogenetic independent contrasts (Felsenstein 1985). We utilized the *Viburnum* phylogeny from Chatelet *et al.* (2013), which is a well-supported molecular phylogeny based on nine chloroplast gene regions and one nuclear gene region. We tested several sets of branch lengths to use in our independent contrast analyses: molecular branch lengths; an ultrametric tree with root age set to 1; the log of the molecular branch lengths; and all branch lengths equal to 1. We found that the phylogeny with all branch lengths of 1 minimized the correlation between the

absolute size of a contrast and its standard deviation (Garland, Harvey & Ives 1992), so all analyses were conducted with branch lengths set to one. All data were log-transformed prior to analysis.

Because several of our major growth patterns were represented by a single species (e.g. Furcatum, Sieboldii and Plicatum), there was no straightforward way to test for the statistical significance of our observed differences in LLS among the different types using species as data points. To partially overcome this, we used individuals ($N = 79$) and leaves ($N = 5461$) rather than species ($N = 32$) as data points, to provide some estimate of variance within each growth pattern. In addition, we also performed a series of one-way analyses of variance (ANOVAS) based on several potentially important characteristics that partially define these growth patterns and could influence LLS, yet also overlap between the growth patterns so that each character state is represented by more than one species (Table 1). The first character is whether a particular growth pattern is characterized by the production of a stereotypical number of leaves during its seasonal growth. This captures one meaning of determinate versus indeterminate growth. The second character is whether the primary axes are terminated by an inflorescence that was pre-formed the previous winter in bud. This second character is a special case of the first: these branches are determinate because the meristem was committed to terminate before growth began. While this mechanism of determinism does not necessarily limit the number of nodes (and thus number of leaves) subtending the inflorescence in bud, in *Viburnum*, it is typically two, or occasionally three. In the first character, some branches are determinate by virtue of a fixed period of growth, not by the production of an inflorescence. In such cases, the termination of growth must be regulated by a different mechanism.

All data analyses were performed in R v 2.14.2, using the standard R Stats package and the phylogenetic package 'APE' (Paradis, Claude & Strimmer 2004). Data matrices and the phylogeny used for analyses can be downloaded from the Dryad Digital Repository (Edwards *et al.* 2014).

Results

EVOLUTION OF THE LES WITHIN *VIBURNUM*

Our *Viburnum* species exhibited substantial variation in all measured traits, with means of A_{area} ranging from 7.0 to 16.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$, LMA from 39.6 to 91.5 g m^{-2} and LLS from 18.5 to 27.9 weeks, occupying a substantial area of the 'quick return' end of the global LES data set (Fig. 2). Species

Table 1. Defining features of growth patterns in *Viburnum* that influence LLS

Architecture type	Stereotypical number of leaves?	Main growth axes terminate in pre-formed inflorescence?	Average LLS (weeks)	Species included in study
Dentatum	Yes	Yes	26	<i>betulifolium</i> , <i>bracteatum</i> , <i>cassinoides</i> , <i>corylifolium</i> , <i>dentatum</i> , <i>dilatatum</i> , <i>erosum</i> , <i>hupehense</i> , <i>ichangense</i> , <i>lobophyllum</i> , <i>molle</i> , <i>rafinesquianum</i> , <i>setigerum</i> , and <i>wrightii</i>
Furcatum	Yes	No	25.4	<i>furcatum</i>
Lantana	Intermediate	No	24.4	<i>bitchiuense</i> , <i>burejaeticum</i> , <i>carlesii</i> , and <i>lantana</i>
Opulus	Yes	Mixed	23.4	<i>opulus</i> , <i>trilobum</i> , and <i>sargentii</i>
Sieboldii	No	No	22	<i>sieboldii</i>
Plicatum	No	No	21.8	<i>plicatum</i>
Lentago	Intermediate	No	20.9	<i>lentago</i> , <i>prunifolium</i> , and <i>rufidulum</i>

LLS, leaf life span.

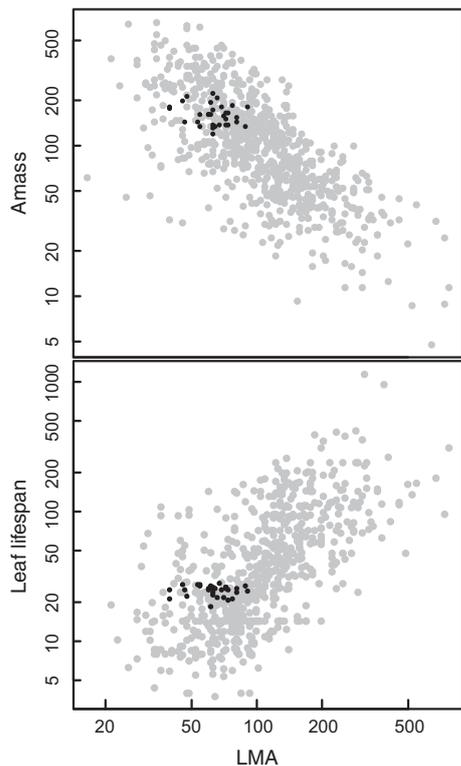


Fig. 2. *Viburnum* leaf economics spectrum (LES) traits within the global LES trait space. Black dots are the *Viburnum* data generated during this study; grey dots are the original 'gloptnet' data set used in Wright *et al.* (2004). LMA, leaf mass per area (g m^{-2}); A_{mass} , maximum photosynthetic rate per mass tissue, $\text{nmol g}^{-1} \text{s}^{-1}$; Leaf life span = weeks.

with higher LMA also had higher photosynthetic rates and greater N concentrations on an area basis; thus, we found strong and significant positive evolutionary correlations between A_{area} , N_{area} and LMA (Fig. 3). Pearson product-moment correlations were all significant at $P < 1.0 \times 10^{-5}$. LMA was determined largely by leaf thickness, which in these species is in turn determined by the thickness of the photosynthetic mesophyll layers (Chatelet *et al.* 2013). Thus, it seems that increases in LMA in these *Viburnum* species are a direct result of increased investment in photosynthetic tissue.

In contrast, there was no evidence for any mass-based relationships between these variables, with the exception of a weak positive evolutionary correlation between A_{mass} and N_{mass} (correlation coefficient: 0.47; $P = 0.007$); however, after a statistical correction for multiple comparisons, the significance of this relationship was rejected (Bonferroni correction, significant $P = 0.00625$).

In spite of the substantial variation across species in LLS, LLS showed no evolutionary correlations with any other LES trait (Fig. 3).

PHYLOGENETIC DISTRIBUTION OF *VIBURNUM* GROWTH PATTERNS

In his original descriptions, Donoghue (1981, 1982, 1983) noted that growth patterns appeared to be evolutionarily

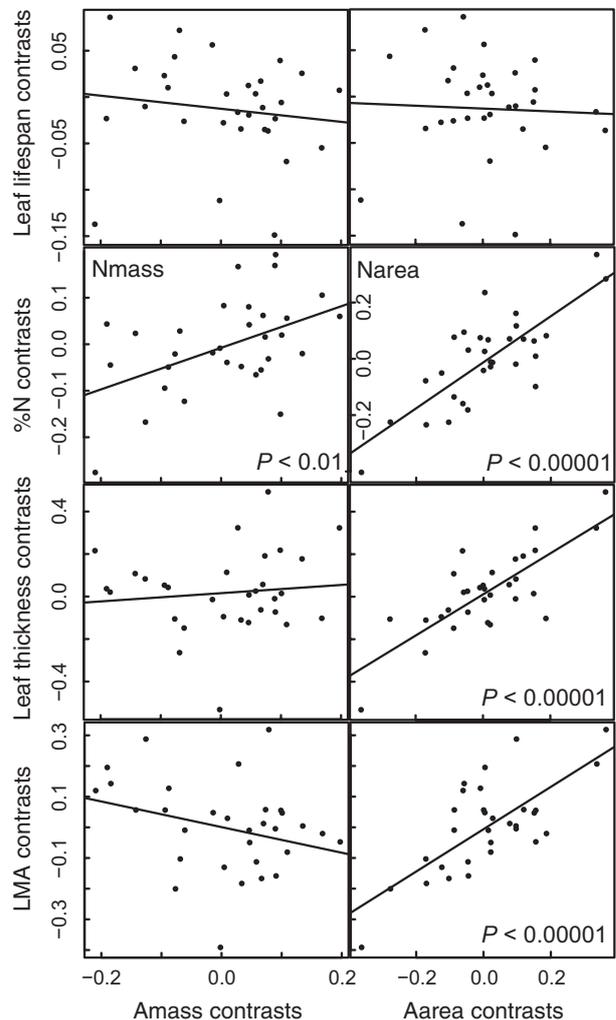


Fig. 3. Evolutionary correlations among leaf economics spectrum (LES) traits in *Viburnum*. Correlations between phylogenetically independent contrasts, utilizing a pruned phylogeny from Chatelet *et al.* (2013) with phylogenetic branch lengths set to one. All data were log-transformed prior to analysis. Significance of correlations were assessed with the Pearson's product-moment correlation; only P -values < 0.05 are indicated on the figure.

conserved, but this was based on very limited knowledge of *Viburnum* phylogeny. This conservatism is confirmed in Fig. 4, which shows the phylogenetic distribution of growth patterns for our study species based on the most recently published molecular phylogeny of *Viburnum* (Chatelet *et al.* 2013). Our seven architectural types characterize major *Viburnum* clades and show no unequivocal homoplasy, and this result holds true when additional species are included. For example, the three additional species recorded by Donoghue (1982) as having the Furcatum pattern (*V. lantanoides*, *V. sympodiale* and *V. nervosum*) are united in all recent phylogenetic studies with *V. furcatum*. Likewise, our recent field studies in southern China and Southeast Asia indicate that the species most closely related to *V. plicatum* also bear long monopodial, plagiotropic shoots, including *V. amplifolium*, *V. hanceanum*, *V. lutescens* and *V. pyramidatum* (M. Donoghue, W. Clement, P. Sweeney & E. Edwards, pers.

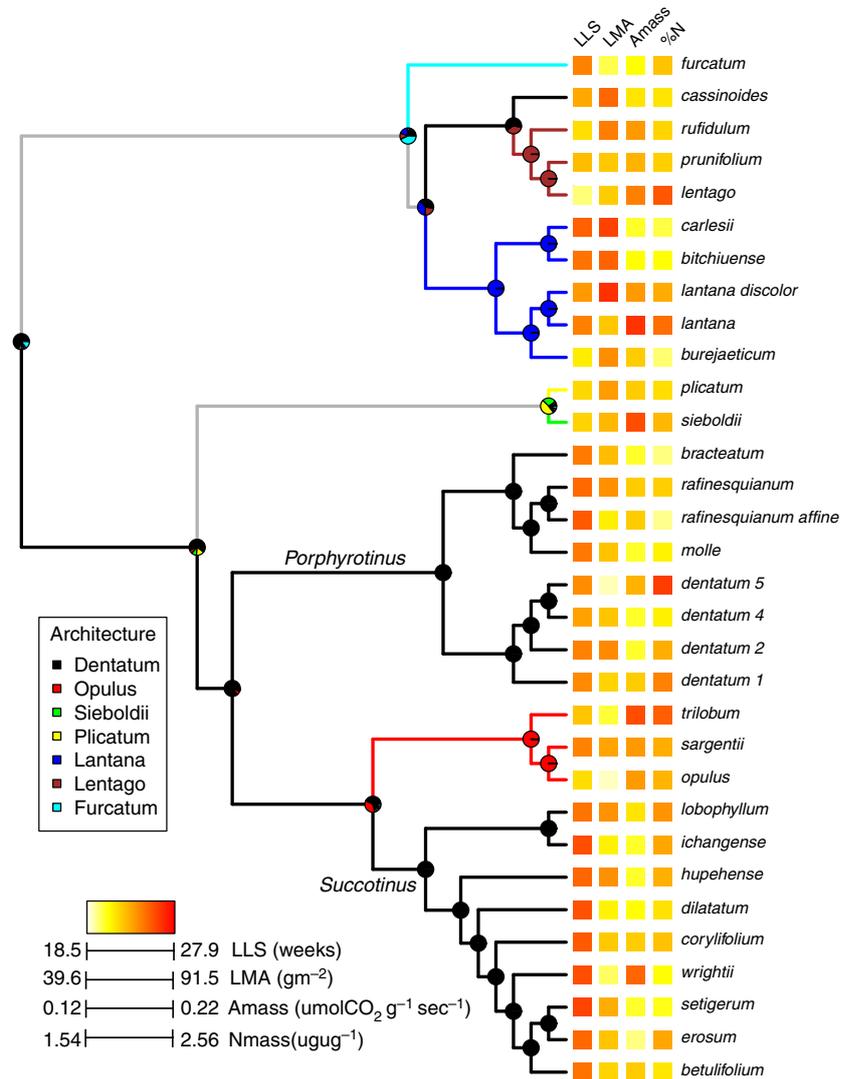


Fig. 4. Evolutionary relationships among the *Viburnum* species included in this study and phylogenetic distribution of growth patterns and LES traits. Ancestral state reconstructions of branching architecture were estimated using maximum likelihood. Branches where the reconstruction was ambiguous are coloured grey. LES, leaf economics spectrum.

observations). With our limited sample, and in the absence of growth data on most of the tropical *Viburnum* species, the precise sequence of evolutionary events is mostly ambiguous (Fig. 4).

LLS AND BRANCHING ARCHITECTURE

Growth patterns were strongly associated with differences in LLS (Fig. 5). The Dentatum and Furcatum types had the longest LLS and the smallest variance. In contrast, the Lentago, Sieboldii and Plicatum patterns showed substantially lower mean LLS values and a wider range of variation. These differences in LLS are directly accounted for by reference to the details of growth in these different patterns. In plants showing the Dentatum pattern, the reproductive shoots are entirely pre-formed in overwintering buds (Fig. 1a). These shoots emerge quickly, produce several (most often two) pairs of leaves, and then flower. As no additional leaves are produced on these shoots later in the season and the leaves all senesce at roughly the same time, LLS shows little variance (Fig. 5a,b). Although their inflorescences are not pre-formed, plants with

the Furcatum pattern are also highly constrained in terms of the number of leaves produced each season (Fig. 1f). Specifically, the sympodial plagiotropic shoots in *V. furcatum* almost always bear one pair of large leaves and one pair of small bract-like leaves before producing the characteristic overwintering naked buds. This stereotyped behaviour accounts for both the long LLS and the low variance (Fig. 5a).

The Plicatum pattern lies at the other end of the spectrum. Here, pairs of leaves are produced continuously and over quite a long time period (up to ca. 150 days; Fig. 5b) throughout the growth of the monopodial plagiotropic shoots (Fig. 1e). These shoots only rarely terminate in an inflorescence; instead, the inflorescences are borne on short lateral shoots (Fig. 1e). This results in the pattern of leaf addition shown for *V. plicatum* in Fig. 5b and consequently for a short mean LLS and high variance. Specifically, as the leaves dehiscence at roughly the same time, those that are produced later in the growing season will, by necessity, have shorter LLS.

The Sieboldii pattern likewise yields a lower mean LLS value and higher variance, but for quite different reasons. In

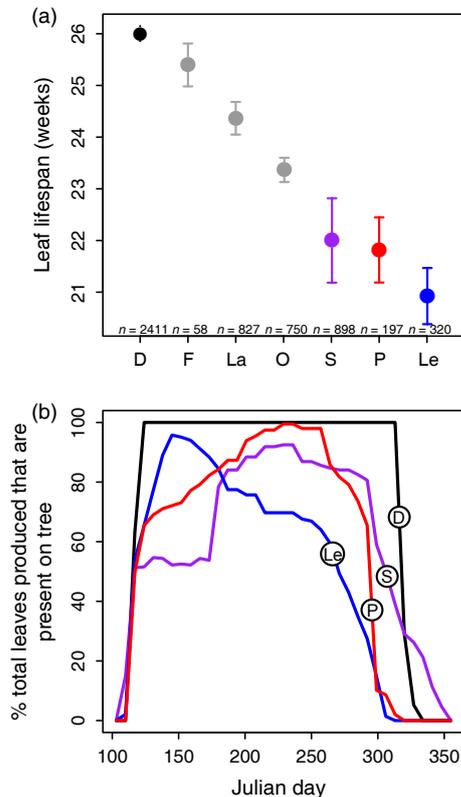


Fig. 5. Influence of growth patterns on leafing phenology and life span. (a) Mean leaf life span with 95% confidence intervals with leaves grouped according to growth pattern. N refers to the total number of leaves in each category. D, Dentatum; F, Furcatum; La, Lantana; O, Opulus; S, Sieboldii; P, Plicatum; and Le, Lentago. (b) Examples of leaf accumulation curves for each growth pattern, following the colouring in panel (a). Black = Dentatum type (represented by *V. ichangense*); purple = Sieboldii type (*V. sieboldii*); red = Plicatum type (*V. plicatum*); and blue = Lentago type (*V. lentago*). The y-axis represents the percentage of leaves present on a given day relative to the total amount of leaves produced during the growing season. For example, in the Dentatum type, all leaves produced during the growing season are made in the first 2 weeks, so they reach 100% quickly. See text for full details.

this case, all of the reproductive axes are orthotropic and sympodial, but buds beneath the terminal inflorescences often rest for only a short time before flushing. These new branches often produce an indefinite number of leaves before ending in a terminal bud (or, more rarely, in an inflorescence). This cohort of precocious branches, produced later in the growing season (at ca. 175 Julian days), is clearly evident in Fig. 5B, and is responsible for the lower mean LLS and the higher variance associated with this growth pattern.

While the Lantana and Lentago patterns are similar in many respects (especially in the production of branches of intermediate determinacy beneath the flowering inflorescences), they end up with quite different mean LLS values. This difference is not so much a function of a difference in the production of leaves on the sympodial branches, but instead is accounted for mainly by the loss of leaves in the Lentago pattern. Specifically, after the initial flush of leaf production early in the season, *V. lentago* and its relatives show a

characteristic attrition of leaves. That is, they tend to drop the first pair(s) of leaves that are produced on the primary axis (beneath the inflorescence), which is reflected in the sharp decline in leaves remaining after ca. Julian day 140 in Fig. 5B. In contrast to the Lentago pattern, in the Lantana pattern, there are only rarely leaves produced during the flowering season in a comparable position on the axis that bears the inflorescence. Consequently, they lack leaves in that position to be lost during the season.

Finally, the Opulus pattern shows intermediate LLS values, largely due to the early senescence of leaves in the late summer in *V. opulus* and *V. trilobum*, though not *V. sargentii*. We are not entirely sure of how this early senescence relates to growth form, but it might be connected with their production of 'cheap' reproductive shoots that typically die back after a single year of growth.

Although, as we have just discussed, there are clear mechanistic connections between different growth patterns and LLS, this is difficult to evaluate statistically because several of the growth patterns (Furcatum, Plicatum and Sieboldii) are represented in our current sample by only a single species. However, as noted above, we analysed growth pattern effect using both individual plants ($N = 2-43$ per growth pattern) and individual leaves ($N = 197-2411$ per growth pattern). In both cases, growth pattern had a highly significant effect (one-way ANOVA: by individual, $F = 10.23$, $P = 3.65e^{-8}$; by leaf, $F = 139.4$, $P < 2e^{-16}$). We also tested for the effect on LLS of two characteristics that partially define the different growth patterns (Table 1). Both traits independently exhibited very strong effects on LLS (one-way ANOVA, by individual: stereotypical leaf number, $F = 9.427$, $P = 0.0002$; pre-formed inflorescences, $F = 15.95$, $P = 1.65e^{-6}$).

Discussion

Similar to other studies that have investigated LES traits in plants that capture only a partial range of global LES variation, we found very strong positive relationships between A_{area} , LMA, leaf thickness and N_{area} (Niinemets & Sack 2006; Martin, Asner & Sack 2007; Dunbar-Co, Sporck & Sack 2009; Funk & Cornwell 2013). This makes perfect sense in the light of our previous anatomical work with these species, which demonstrated that leaf thickness in *Viburnum* is primarily a function of the thickness of the photosynthetic mesophyll layer (Chatelet *et al.* 2013). Evolutionary increases in LMA were direct anatomical boosts to leaf photosynthetic capacity. Such a strong positive correlation between A_{area} and LMA negates the possibility of finding any A_{mass} versus LMA trade-off, and indeed we did not find any of the 'global' mass-based trade-offs operating here.

Neither did we find an LMA versus LLS trade-off. In fact, LLS appears to have evolved quite independently of any of the LES traits included in our study, and instead appears to be directly associated with the different growth patterns. More generally, a positive correlation between A_{area} and LMA essentially removes LLS from the carbon budget equation altogether, as a leaf with a high initial investment (high

LMA) will have an instantaneous higher rate of return (high A_{area}), rendering A_{mass} independent of LMA (Niinemets & Sack 2006). Under these conditions, leaves with higher LMA may not need a longer LLS to repay themselves, leaving LLS free to vary. If this is the case, what sorts of selection pressures might be acting on LLS?

In the case of *Viburnum*, we have discovered a tight connection between LLS and distinct plant architectures. These growth patterns are evidently not very labile in *Viburnum*, and a single growth pattern characterizes each of the major lineages. Although we currently do not fully understand the mechanisms underlying this conservatism (Revell, Harmon & Collar 2008), particular growth patterns could be adaptive in particular environments and directly maintained by selection. For example, the Furcatum pattern is shared by all members of the Pseudotinus clade, which occupy understorey habitats in northern temperate forests. This pattern represents a classical temperate understorey architecture that minimizes self-shading and allows leaves to be displayed nearly horizontally, thereby maximizing light absorption (Horn 1971; King 2005). Likewise, the Opulus growth pattern, which is restricted to the Opulus clade, may be adaptive in the cold northern regions that these species occupy. Specifically, their ephemeral (throw-away) reproductive shoots and their early senescence may help to minimize frost damage in cold, unpredictable climates.

In each of these cases, particular LLS's emerge as a consequence of connections with other plant traits. In the Dentatum growth pattern, we find an especially clear case of this, where LLS is significantly correlated with peak flowering time (Fig. 6). This link arises as a function of the highly stereotyped nature of this growth pattern: all organs are pre-formed in bud, with typically two pairs of leaves subtending an inflorescence. As spring bud break occurs, leaves and inflorescences develop more or less simultaneously. This intimate connection between leaf and floral development opens the real possibility that the evolution of LLS has been influenced by

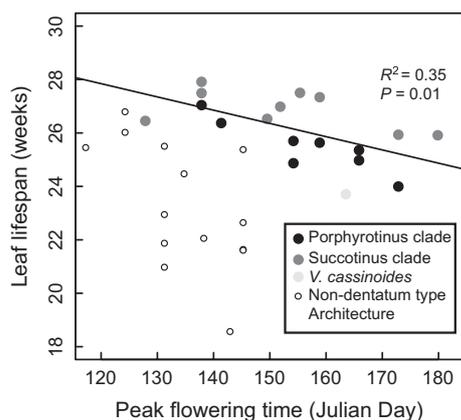


Fig. 6. Relationship between leaf life span (LLS) and flowering time. In plants with the Dentatum growth pattern, represented here by three distinct lineages (porphyrotinus, succotinus and *V. cassinoides*), there is a significant relationship between flowering time and LLS, because all of these organs are pre-formed in bud. The relationship is not significant when considering the other growth patterns.

yet other factors such as pollination, fruit maturation and dispersal.

An alternative view, of course, is that LLS is what is actually being selected for, and traits such as branching pattern and flowering phenology are just 'along for the ride'. We find this explanation less tenable in the deciduous viburnums, where, as we have argued, LLS is essentially removed from the leaf carbon balance sheet altogether. One could argue that even in this situation, a longer LLS would always be favoured by selection in that a leaf should persist for as long as its carbon gains outstrip its maintenance costs. In that case, one would imagine LLS to closely mirror the length of the growing season itself, but this was achieved in only some of our species.

Our study purposely only included deciduous *Viburnum*, though there are evergreen *Viburnum* species as well, many living in tropical and subtropical forests of Asia and high elevation cloud forests of the Andes. Including these species in our study would likely greatly increase the range of LMA and LLS, and the A_{mass} versus LMA trade-off might re-emerge. The general argument for the importance of LLS in mediating this trade-off might be more realistic in evergreen species that produce leaves with the potential to persist for many seasons. It is conceptually similar to placing a set of leaves with a wide range of LMA hung out to dry on a clothesline, and calculating their LLS in terms of how long they persist, in the face of herbivory and weather. We predict that such an experiment would yield a very significant positive relationship between LLS and LMA.

But, in nature, leaves are not borne on clotheslines – rather, as Halle, Oldeman & Tomlinson *et al.* (1978) so aptly state in our epigram, 'they form part of whole organisms'. We suspect that in most deciduous plants with relatively low LMA and a finite growing season, the LES may be more or less irrelevant and this is most acutely so when photosynthetic capacity and LMA are positively correlated. In this sense, the LES is hardly 'global'. We certainly understand the appeal of the 'trait-based' approach to plant ecology (Díaz *et al.* 2004; Westoby & Wright 2006; Ackerly & Cornwell 2007; Reich, Wright & Lusk 2007), particularly in its promise to scale up and permit grand generalizations about plant function, in both ecological and evolutionary contexts. However, we still know so little about how these basic traits are integrated – sometimes in subtle and unexpected ways – not just with other leaf traits, but with a wide range of organismal attributes. We urge for a more holistic approach to LES traits – one that takes seriously a wider variety of factors as being potentially significant in shaping their form and interdependence.

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Data Accessibility

Data available from the Dryad Digital Repository (Edwards *et al.* 2014).

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