Leaf heteroblasty in eucalypts: biogeographic evidence of ecological function

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Abstract. Leaves that develop on seedlings, young saplings or regenerative shoots of many eucalypt species are strikingly different in morphology from the typical leaves of more mature plants; a developmental pattern known as heteroblasty. We measured dimorphism between juvenile and adult leaves in shape and size, leaf mass per unit area, and vein frequency in a continent-wide sample of Angophora, Corymbia and Eucalyptus species. We tested whether heteroblasty in this group is an adaptation to shading by comparing the degree of juvenile–adult leaf dimorphism with the canopy closure (measured by the leaf area index) of the habitat in which species occurred. No pattern emerged for heteroblasty in leaf shape and size or leaf mass per unit area, but there was a significant relationship (accounting for phylogenetic relationships) between the degree of juvenile–adult dimorphism in vein frequency and habitat leaf area index. Juvenile leaves tended to have more widely spaced veins than adult leaves of the same species, in regions with more closed vegetative canopies. This evidence suggests that eucalypt heteroblasty is, at least in part, a hydraulic adaptation to the different conditions faced by younger and older plants in higher productivity regions with denser vegetation.

Additional keywords: Angophora, biogeography, Corymbia, eucalypt, Eucalyptus, leaf morphology, leaf venation.

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Introduction

Leaf heteroblasty is a prominent feature of eucalypts, the lineage comprising the genera Angophora, Corymbia and Eucalyptus (Myrtaceae) (Ladiges et al. 2003). Many eucalypt species exhibit distinctive size and shape differences between juvenile and adult foliage. A typical pattern of heteroblasty involves the development of wide, oval, dorsiventral juvenile leaves, often sessile and held perpendicular to the stem in a decussate phyllotaxy, giving way to juvenile–adult dimorphism in vein frequency and habitat leaf area index. Juvenile leaves tended to have more widely spaced veins than adult leaves of the same species, in regions with more closed vegetative canopies. This evidence suggests that eucalypt heteroblasty is, at least in part, a hydraulic adaptation to the different conditions faced by younger and older plants in higher productivity regions with denser vegetation.

There has been discussion in the literature about the terminology used to describe leaf heteroblasty (Jones 1999; Zotz et al. 2011). It is not our intent to address such issues here. We recognise that a variety of patterns of ontogenetic variation in leaf traits exist in vascular plants, so that the term ‘heteroblasty’ encompasses distinct developmental patterns and independent evolutionary origins. We recognise also that the conventional reference to ‘juvenile’ and ‘adult’ leaves does not necessarily coincide with the meaning of ‘reproductive maturity’ usually applied to adulthood. We employ these terms nonetheless because they are conventional and because our taxonomic focus is sufficiently narrow that the nature of the phenomenon can be understood with little ambiguity. We acknowledge, however, that heteroblasty has variations even within the eucalypt lineage. Indeed, as we show below, there is a continuum of quantitative difference between juvenile and adult leaf traits, and thus no distinct border that delimits heteroblasty in the group.

Juvenile–adult leaf dimorphisms are usually thought to be an adaptation to predictable environmental heterogeneity experienced during the ontogeny of an individual (Zotz et al. 2011), particularly heterogeneity in the light environment (Day et al. 1997; Day 1998). More specifically, juvenile–adult leaf dimorphisms may be an adaptation to the ontogenetic heterogeneity in limiting environmental factors. For example, for a particular species, light could be the most limiting factor during the juvenile phase, whereas water could be the most limiting factor during the adult phase, and this could cause each phase to adapt using different leaf morphologies. Although the particular form and strength of a light gradient will depend on the height and degree of closure of a canopy, arboreal species establishing under intact canopies will necessarily encounter some vertical gradient of access to sunlight. Yet despite the intuitive appeal of the argument that heteroblasty is an adaptation to a vertical light gradient, empirical
evidence is meagre and equivocal. In support, it has been noted that the morphology and anatomy of juvenile leaves of heteroblastic species are often similar to the typical leaf traits of shade-adapted species (Day et al. 1997; Jones 2001) or that higher light environments enhance the sun-adapted morphological features of adult leaves (Ostria-Gallardo et al. 2015). Measurement of physiological performance suggests that juvenile pinnate leaves are adapted for higher photosynthetic rates while adult phyllodes are adapted for water stress tolerance in *Acacia melanoxylon* R.Br. in Australia (Brodribb and Hill 1993), *Acacia mangium* Willd. in China (Yu and Li 2007), and *Acacia koa* A.Gray in Hawaii (Pasquet-Kok et al. 2010). However, juvenile leaves failed to show superior photosynthetic performance in shade among four heteroblastic species in New Zealand (Gamage and Jesson 2007; Gamage 2011). Hydraulic performance was also found to be inconsistent, with the juvenile form more resistant to water loss than the adult form in some New Zealand heteroblastic species but not others (Darrow et al. 2002).

A limitation of quantifying the effects of heteroblasty by short-term photosynthetic or hydraulic responses to juvenile environments is that such responses are translated to growth and ecological advantage only over long time frames not captured in the physiological measurements. Here we use an alternative, biogeographic approach to address the functional significance of heteroblasty, and its relationship to light environments in particular. *Angophora* (13 species), *Corymbia* (~100 species) and *Eucalyptus* (~600 species) inhabit diverse environments, from closed forests to open woodlands and savannas (Ladiges et al. 2003). This habitat diversity can be quantified by the leaf area index (LAI), a measure of the average number of leaf layers in the vegetation above a given point on the ground surface (Fig. 1). If heteroblasty were an adaptation to changing light environments that a plant experiences during growth, one would expect the most leaf-dimorphic species to be associated with the most closed habitats having the highest LAI.

However, it is not obvious that this hypothesis can be extended to the case of eucalypt heteroblasty. Eucalypts frequently regenerate following fire (Stoneman 1994; Gill 1997), which can expose seedlings and saplings or vegetative shoots from regenerative lignotubers to a variety of light gradients and environmental conditions, depending on the severity of the fire and the extent of canopy destruction (Nicolle 2006). However, seed reproduction does occur under established canopies (Stoneman 1994), and rare seed establishment even among species typically regenerating from lignotubers may be ecologically important (Gill 1997), hence relevant to the evolution of heteroblasty. It cannot, therefore, be assumed either that juvenile leaf forms in eucalypts would or would not be adapted to shade. However, before adapting our hypothesis to what we expect based on eucalypt ecology, we are first investigating whether eucalypt heteroblasty has any association with open or closed environments at all.

In order to look for associations of eucalypt heteroblasty with habitat, we quantified the degree of difference in gross morphology and anatomy between juvenile and adult leaves for a large sample of species. The leaf traits we examined were leaf aspect ratio (length to width ratio) and size, because these undergo strikingly obvious ontogenetic changes in many eucalypt species (Penfold and Willis 1961), and vein frequency and leaf mass per area (LMA), because these are closely associated with leaf function in relation to the environment (Wright et al. 2004; Brodribb et al. 2007; Sack and Scoffoni 2013). Therefore, investigating ontogenetic changes in these traits should help in understanding the ecological function of eucalypt heteroblasty. We created a composite phylogeny for the eucalypts based on previous systematic studies of the group in order to account for phylogenetic relationships in our examination of the association between leaf traits and habitat LAI.

![Fig. 1. Distribution of mean maximum leaf area index (LAI), measured as leaf area per unit ground area (m² m⁻²), across Australia for the years 2000–2015, calculated from NASA Earth Observations data (http://neo.sci.gsfc.nasa.gov, accessed 3 November 2017). The distribution of LAI is shown by the scale bar.](http://example.com/figure1.png)
Materials and methods

Sampling and measurement of traits

We quantified juvenile and adult leaf morphology by the aspect ratio (leaf length divided by maximum width), and an index of leaf size (the product of length and width), the most obvious features of heteroblasty in the eucalypts. Leaf anatomy was quantified by leaf mass per unit area (LMA) and the vein frequency. Variation in LMA summarises much of the interspecific spectrum of ecological strategies of leaf design (Wright et al. 2004; Poorter et al. 2009), whereas vein density often differs between species in shaded and non-shaded environments (Sack and Scoffoni 2013) and so could be reflected also in heteroblastic leaves. Trait values were taken from published data and from our own measurements (see below). Our final dataset contained morphological data for 457 species (six Angophora spp., 76 Corymbia spp. and 375 Eucalyptus spp.), LMA for 56 species (three Corymbia spp. and 53 Eucalyptus spp.), and vein density (measured as vein frequency) for 93 species (one Angophora sp., two Corymbia sp., and 90 Eucalyptus spp.).

We used the leaf dimensions reported in the EUCLID Eucalypts of Australia database (Slee et al. 2006) to calculate aspect ratio and size index (midpoints of linear dimensions were used when a range was reported). Where juvenile data were unavailable, measurements were taken from cultivated seedlings. For five species lacking adult leaf dimensions (Eucalyptus ceracea Brooker & Done, Eucalyptus cordata Labill., Eucalyptus mooreana Maiden, Eucalyptus pulverulenta Sims and Eucalyptus risdonii Hook.f.), leaves were measured from calibrated images in JSTOR Global Plants (JSTOR 2014) using ImageJ (Schneider et al. 2012). The magnitude of intraspecific trait plasticity is likely to be small compared with the interspecific variation. For example, McLean et al. (2014) sampled Eucalyptus tricarpa (L.A.S.Johnson) L.A.S.Johnson & K.D.Hill from nine sites over a precipitation gradient of 460–1120 mm year$^{-1}$, and found maximum differences among sites in adult specific leaf area (the reciprocal of LMA) that were only 12.4% of the interspecific range of the equivalent measure in our data. Measurements of vein frequency were taken from Brooker and Nicolle (2013), and supplemented with additional measurements of samples we took in 2015 from the Currency Creek Arboretum, South Australia, the same source of the samples used by Brooker and Nicolle (2013). In most species, juvenile and adult vein images were taken from leaves of the same individual. Otherwise, they were taken from the leaves of juvenile and adult individuals growing in close proximity to each other (<50 m). All trees sampled from Currency Creek Arboretum have been planted in evenly spaced rows. In most species, intra-species variation in vein frequency is restricted enough to be used as a diagnostic tool for species identification (Brooker and Nicolle 2013). Juvenile leaves were taken from seedlings, the basal nodes of small saplings, or epicormic growth on mature trees. Leaves of coppice growth and seedling growth generally both take the form of juvenile leaves (Brooker and Kleinig 1990; Nicolle 2013). Small differences may exist between resprouting foliage and seedling foliage in some species, and this requires investigation in the future. For a given species, juvenile and adult material were obtained from a single individual tree when possible. A range of 1–10 leaves were taken from each individual, totalling 2015 leaves. Each leaf was stored in a sealed plastic pocket at 4°C and later micrographed on its adaxial surface under a dissecting microscope with transmitted light to reveal the veins. Vein frequency (veins mm$^{-1}$) was measured by counting the number of veins intersecting the perimeter of a square (2 or 4 mm on each side, depending on the size of the leaf) drawn on each micrograph using ImageJ. Each square was placed at the approximate midpoint between the midrib and margin, and at the middle of the leaf length, in accordance with the measurements taken by Brooker and Nicolle (2013). No leaves were chemically cleared in the micrographs used for vein frequency measurements. Veins were always clearly visible up to the third order, at the least. Exceptions to this were in only a few samples, from which measurements were not taken (this was mostly due to damage by fungal growth). Juvenile and adult samples taken from Currency Creek Arboretum in 2015 were also used to measure and calculate LMA. To measure LMA, leaves were scanned at 600 dpi and area was measured using ImageJ (Schneider et al. 2012). Each leaf was oven dried at 60°C for 72 ± 2 h and weighed. LMA was calculated as dry mass per unit area (g m$^{-2}$), which included the petiole if present. Averages from multiple leaves were taken to characterise the species.

Dimorphism index (DI)

The degree of juvenile–adult leaf dimorphism (dimorphism index (DI)) in each of the four leaf traits was quantified by the formula $DI = (\text{adult value} - \text{juvenile value})/(\text{adult value} + \text{juvenile value})$. DI was adapted from a formula used by Gibbons (1992) to quantify sexual size dimorphism in animals. It reflects both the direction and magnitude of a dimorphism. Negative values indicate that the trait value is greater for juvenile than for adult leaves, and positive values indicate that the adult trait value is greater than that of the juvenile. Larger absolute values of DI indicate a more extreme difference between juvenile and adult leaves.

Species geography and habitat LAI

Geographical occurrences for the species in our dataset were obtained from the Australasian Virtual Herbarium (AVH 2017). Occurrences of individual plants were excluded if they were cultivated, inconsistent with EUCLID species distributions (Slee et al. 2006), or duplicate records at the same geographical coordinates.

To characterise habitat closure, we obtained data on global patterns of leaf area index (LAI) from the NASA Earth Observations (NEO) database (http://neo.sci.gsfc.nasa.gov, accessed 3 November 2017). LAI represents the number of leaf layers in the vegetation above a point on the earth’s surface. Global patterns of LAI in the NEO database were estimated from satellite observations of surface reflectance spectra in conjunction with a model of expected radiances from canopy structures over the range of natural conditions (Knyazikhin et al. 1999). LAI is not as seasonally variable in Australia as in some other regions of the earth. Nonetheless, to account for seasonal variation we obtained monthly LAI data at 0.1 degree resolution for every month from 2000 through 2015, used the maximum value that occurred at a given location...
within a calendar year, and averaged the 16 yearly maxima. We refer to this as the mean maximum LAI (Fig. 1).

We then used the R package raster (Hijmans and van Etten 2014) to extract the mean maximum LAI value corresponding to each geographic occurrence record of a species in the dataset. We averaged these LAI values among all occurrences for a given species to obtain a measure of the characteristic canopy closure for the habitat it occupies. The lowest means (0.30) were obtained for a suite of species (Eucalyptus canescens D.Nicolle., Eucalyptus globulosa Brooker & Hopper, Eucalyptus gongylocarpa Blakely, Eucalyptus lucasii Blakely, Eucalyptus striatallyx W.Fitzg.) found in the Gibson Desert and Great Victoria Desert regions of the Western Australia and South Australia interior; the highest values (5.18–5.32) were obtained for species in the subtropical coastal regions of New South Wales (Eucalyptus dunnensis Boland & P.M.Gilmour, Eucalyptus scopulorum K.D.Hill) or the wet highlands of Victoria (Eucalyptus strzeleckii K.Rule).

**Phylogenetic tree**

To undertake a phylogenetically informed analysis, we used a composite tree constructed from multiple phylogenetic studies of the eucalypts. A scaffold topology was derived from the 94-taxon strict consensus tree inferred by Steane et al. (2011). Subgeneric detail and topology for Corymbia and Angophora were added to this scaffold using the analyses of Gibbs et al. (2009), Parra-O. et al. (2009) and Woodhams et al. (2013). This resulting tree, based on molecular sequence data, contained 229 species. To this tree, additional species were added using the morphologically based taxonomic series and subseries of (Brooker 2000). Species in a series or subseries were added as a terminal polytomy to the 229-species tree if a member of the taxonomic series also occurred on the sequence-based tree. The final composite tree contained 469 species (Fig. 2). Branch lengths in the tree were provisionally set according to Pagel’s (1992) rule, which adjusts node heights based on the number of descendent taxa arising from the node. Then set the basal split between Angophora/Corymbia and Eucalyptus at 59.5 Ma following Crisp et al. (2004), and scaled all branch lengths to this basal age, maintaining the relative lengths determined by Pagel’s (1992) rule. The resulting tree agreed well with ages for four internal nodes based on divergence time intervals estimated by Crisp et al. (2004). Nexus code for the full tree (Text S1), and a graphical display of the tree with terminal taxon names (Fig. S1) are available as Supplementary Material to this paper. The leaf trait data were then pruned to include only species also appearing in the final phylogeny. For analysis pertaining to the dimorphism of each trait, the tree was pruned a second time to only include species for which dimorphism data was available for that trait.

**Data analysis**

Phylogenetic generalised least-squares (PGLS) regression as implemented in the R package caper (Orme 2013) was then used to test the relationship between habitat LAI and the dimorphism indices for the four leaf traits. PGLS accounts for the varying degrees of relatedness among species in multispecies regression analysis by using phylogenetic branch lengths to estimate the expected pattern of covariance between trait values. A matrix of these covariance values is then used in the calculation of regression parameter estimates (in contrast to ordinary least-squares regression in which all co-variances are assumed to be zero) (Nunn 2011). We visualised the results of the PGLS analysis by plotting phylogenetically adjusted DI values (for a given species, the prediction of the regression equation plus the phylogenetic residual) in relation to LAI.

**Results**

There is a continuous range among the eucalypts in juvenile-adult dimorphism for all four leaf traits, with no discrete breaks between homoblastic (DI ~ 0) and heteroblastic (DI < 0) (Fig. 3). Nearly all (87%) species had positive values of DI;MA (Fig. 3c), that is juvenile leaves were thinner and less dense than adult leaves, a pattern thought often to be true of the ontogeny of plants (Jones 2001). Finally, most species in the sample (63%) had a positive DI; vein frequency, i.e. adult vein frequency is greater than juvenile vein frequency (Fig. 3d). Some visual examples of the disparities in vein frequency between juveniles and adults of the same species, can be seen in Fig. 4. The apparent bimodality in the histogram was strongly reduced when different bin ranges were chosen, although there is room for further exploration of the distribution of vein frequency and its relation to geography.

Of the four leaf traits we measured, only dimorphism in vein frequency had a significant relationship to habitat LAI in the interspecific PGLS analysis (Table 1; Fig. 5). The slope of this relationship is positive (i.e. the degree to which adult leaves have greater vein frequency than juvenile leaves tends to increase in more closed habitats). Over the range of habitat LAI encountered by the species sampled (roughly 0.3–5.2), the average DI; vein frequency increases from 0.04 to 0.12, although there is considerable variation in the relationship ($R^2=0.13$). The change in the dimorphism index could be due to increases in adult vein frequency, decreases in juvenile vein frequency, or both, as habitat LAI increases. To dissect the underlying effects, we performed PGLS analyses of vein frequency in relation to LAI and found that both effects occurred: adult leaves tended to have significantly higher and juvenile leaves significantly lower vein frequency among species inhabiting more closed habitats, although individual ontogenetic stages were not strongly bound to the trends ($R^2=0.050$ for juvenile leaves, $R^2=0.046$ for adult leaves: Table 1). In the positive relationship between LAI and DI; vein frequency, there is no visually obvious difference between the different subclades of the eucalypts (Fig. 5a). Lignotuber sprouters and obligate seeders appear to be restricted to low-LAI environments, while
combination resprouters appear to occur across the whole eucalypt LAI range (Fig. 5b). However, eucalypts of all regeneration types appear to follow the same trend, of higher DI in high-LAI environments. See Fig. S2 (available as Supplementary Material to this paper) for visual representations of the relationships between LAI and dimorphisms for the other three leaf traits, which were non-significant.

Discussion

A continuum of leaf heteroblasty occurs in the eucalypts, for two gross morphology traits and two important anatomical traits (Fig. 3). Thus, there is no clear border between homoblastic and heteroblastic species. The continuum in leaf dimorphism likely reflects a continuum in ecological strategy, and perhaps a summation of multiple dimensions of ecological strategy.

We do confirm, however, that the presence of juvenile leaves with a low aspect ratio and long, narrow adult leaves with a high aspect ratio (Penfold and Willis 1961) is overwhelmingly the typical pattern of heteroblasty in the group, as shown by positive values of DI (Fig. 3a). However, leaf size seems to not have a typical pattern of heteroblasty. Juvenile leaves are larger than adult leaves at about equal frequency among species to the reverse pattern of larger adult than juvenile leaves (Fig. 3b).

The working hypothesis that heteroblasty is an adaptation to predictable ontogenetic shifts in light environment under vegetative canopies was not supported for gross leaf morphology (Table 1). On this biogeographic evidence, we can reject the idea that heteroblasty in the eucalypts involves juvenile leaf sizes and shapes that are adapted to shady understories while adult leaves are adapted to illuminated canopies, an idea that is suspect anyway, given the role of fire in regeneration of many
eucalypt species (Stoneman 1994; Gill 1997). Our evidence does not imply that there is no adaptive function of leaf size and shape dimorphism in eucalypts, only that such function is not strongly related to vegetation closure as measured by LAI.

This finding led us to consider a different model of heteroblasty from King (1999), which emphasises the distribution of biomass between support and photosynthetic tissues, and the changing allometric balance between these investments as a tree grows. His model suggests that heteroblasty in eucalypts could be a strategy to maximise crown area in juveniles (when relatively little support is needed to expand the crown) but maximise leaf density within crowns of adults (when crown area is constrained by large support costs and competition from neighbouring trees). He concluded that such morphology could afford seedlings and saplings rapid growth in high light environments. That is, King’s (1999) argument addresses heteroblasty at the level of whole shoot and plant geometry, rather than just leaf traits. Oval, sessile, decussate juvenile leaves would tend to maximise the light interception of crowns in seedlings or small saplings, given the small stem investment such plants can afford (King 1999). Lower LMA among juvenile leaves (positive values for DI_{LMA} in Fig. 3c) would reinforce this tendency by increasing leaf surface areas for a given biomass investment. Juvenile shoot geometry may also enhance the biomechanical stability of shoots by reducing and balancing bending moments (juvenile leaves with low aspect ratios, decussate phyllotaxy), thus reducing the need for investment in supporting tissue. The mechanical advantages of this geometry might outweigh the possible inefficiency of decussate phyllotaxy for light interception (Valladares and Brites 2004).

These considerations suggest a research strategy examining the role of foliage display, crown architecture, and support costs in the ecology of heteroblasty in eucalypts. Such approaches are gaining favour in the study of plant functional ecology generally (Pearcy et al. 2005). Pickup et al. (2005) noted, for example, that the leaf mass fraction of branches (the proportion of total branch biomass devoted to leaf tissue) tends to increase with leaf size among woody species of New South Wales, a pattern that would provide a growth advantage for larger leaves. Small-leaved species persist, probably due to associations of leaf size with specific leaf area and the consequences of leaf size for thermal regulation. The density and arrangement of foliar surfaces within crowns accounted for 85% of the variation in light interception efficiency in a large sample of plants whose three-dimensional geometry had been measured (Duursma et al. 2012), and a combination of crown architecture, leaf morphology and stomatal conductance best explained sapling growth rates among 37 species in regrowth forest in subtropical China (Li et al. 2017). Similar perspectives may be quite helpful in understanding heteroblasty. For example, Pearcy et al. (2005)
explained the contrasting distributions of *Arbutus menziesii* and *Heteromeles arbutifolia* in, respectively, cooler and warmer habitats in California by the heat load effects of their different crown projection efficiencies. The difference in their foliage display – low aspect ratio leaves held horizontally in *A. menziesii* and high aspect ratio leaves held more vertically in *H. arbutifolia* —is reminiscent of heteroblasty in eucalypts.

As with gross leaf morphology, DI_{LMA} had no relation to habitat LAI (Table 1), despite the documented importance of this feature (or its reciprocal, specific leaf area) in plant ecology.
worldwide (Wright et al. 2005; Ordoñez et al. 2009; Poorter et al. 2009; Meers et al. 2010). This is not to say that LMA is unrelated to eucalypt ecology, only that canopy closure fails to explain the degree of juvenile-adult differences in LMA. However, habitat LAI was significantly related to juvenile–adult dimorphism in leaf vein frequency (Table 1; Fig. 5). Specifically, higher LAI habitats tended to favour species with greater disparity between less vein-dense juvenile leaves and more vein-dense adult leaves (Table 1; Fig. 5). Increased vein presence affects leaf hydraulic resistance by reducing the path length from veins to the site of evaporation in the leaf mesophyll, enabling greater rates of evapotranspiration and thus photosynthetic assimilation (Brodribb et al. 2007; Sack and Scoffoni 2013). Therefore, adult leaves in heteroblastic eucalypts appear typically to be structured for higher transpiration rates than their juvenile counterparts, a feature of heteroblasty documented decades ago in the strongly dimorphic leaves of *Eucalyptus globulus* (Johnson 1926). Meanwhile, juvenile leaves of *E. globulus* have several features of typically sun-adapted leaves (James and Bell 2000). This appears to be the reverse of heteroblasty in *Acacia*, with shade-adapted pinnate leaves in juveniles and sun-adapted phyllodes in adults (Brodribb and Hill 1993).

Vegetation with high LAI occurs primarily along the eastern and south-eastern margins of the continent (Fig. 1), regions that tend to have relatively high rainfall but are also subject to high fire frequency (Russell-Smith et al. 2007). Our data on mean maximum LAI do not account for canopy removal after a fire.

Table 1. Results from phylogenetic generalised least-squares regression (PGLS) with mean maximum leaf area index (LAI) as the independent variable

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Slope co-efficient</th>
<th>d.f.</th>
<th>t-statistic</th>
<th>( R^2 )</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( DI_{aspect\ ratio} )</td>
<td>0.020</td>
<td>455</td>
<td>1.6921</td>
<td>0.006</td>
<td>0.091</td>
</tr>
<tr>
<td>( DI_{size} )</td>
<td>0.0013</td>
<td>455</td>
<td>0.0853</td>
<td>&lt;0.001</td>
<td>0.932</td>
</tr>
<tr>
<td>( DI_{LMA} )</td>
<td>-0.00029</td>
<td>54</td>
<td>-0.032</td>
<td>&lt;0.001</td>
<td>0.982</td>
</tr>
<tr>
<td>( DI_{vene\ frequency} )</td>
<td>0.065</td>
<td>91</td>
<td>3.8321</td>
<td>0.139</td>
<td>0.0002</td>
</tr>
<tr>
<td>Juvenile vein frequency (veins mm(^{-1}))</td>
<td>-0.11</td>
<td>91</td>
<td>-2.1940</td>
<td>0.050</td>
<td>0.031</td>
</tr>
<tr>
<td>Adult vein frequency (veins mm(^{-1}))</td>
<td>0.16</td>
<td>91</td>
<td>2.0877</td>
<td>0.046</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Fig. 5. Phylogenetically adjusted value of dimorphism index (DI) for vein frequency versus leaf area index (LAI). Phylogenetic least-squares (PGLS) model \( F_{1,44} = 14.69, P = 0.0002, R^2 = 0.139 \). Species are labelled by taxonomic group in the upper panel (a), and by regeneration niche (data from Nicolle (2006)) in the lower panel (b). Regeneration niche categories are from Nicolle (2006): a ‘combination resprouter’ has the ability to regenerate via seeding, lignotuber resprouting or stem resprouting; a ‘lignotuber sprouter’ has the ability to regenerate via lignotuber resprouting or seeding only; a ‘stem sprouter’ has the ability to regenerate by stem resprouting or seeding only; an ‘obligate seeder’ has the ability to regenerate via seeding only; and a ‘sprouter (type unknown)’ has the ability to regenerate only from an unknown form of sprouting and seeding.
Further biogeographic investigation of heteroblasty will need to account for the LAI (or other environmental factors) particular to the regeneration niche of each species. Nonetheless, the association of high LAI vegetation with high DIvein frequency paints a picture of hydraulically conservative seedlings or shoots regenerating in full sunlight following destruction of a canopy, followed by an eventual transition to adult leaves capable of taking advantage of high growth potential due to (mostly seasonal) moisture availability, in order to be competitive in closed environments. Less developed root systems in seedlings and saplings could also be a reason for hydraulically conservative leaves – leaves need not conduct water quickly until the root system can supply water at a faster rate. That is, heteroblasty in the eucalypts may be more of a hydraulic adaptation than an accommodation to juvenile shade and adult insolation.

With the possibility of heteroblasty being a hydraulic adaptation, the next step is to explore heteroblasty in other traits that influence leaf hydraulics, especially those pertaining to stomata, since vein and stomatal densities are functionally linked through their contribution to the evapotranspiration rate of the whole leaf (Sack and Holbrook 2006). Also, both traits are often positively correlated within and among species (Brodribb and Jordan 2011; Carins Murphy et al. 2014; Zhao et al. 2016). Our results support the notion of hydraulically conservative juvenile leaves and hydraulically non-conservative adult leaves in closed environments. Therefore, in closed environments, we might expect to find larger or denser stomata in adult leaves. Indeed, in several species from south-eastern Australia in relatively closed environments, stomatal diameter is different in juvenile and adult leaves, with a juvenile/adult ratio ranging from 0.4 to 0.804, except one single individual with a ratio of 1.04 (Carr 2000). This did not usually fluctuate too greatly among individuals within species. Further investigation is required by obtaining the same measurements from species in both open and closed environments. In addition to observing patterns in other hydraulic traits, another possibility to gain more clarity is to experiment directly with the hydraulic capabilities of juvenile and adult leaves from species indigenous to both open and closed environments (for example, hydraulic conductance or vulnerability to cavitation under a low supply of water).

The ‘common garden’ source of our LMA and vein frequency data has advantages and disadvantages. Variation in the environmental contribution to trait values is reduced, but clinal variation within a species is unavailable. Because we address a continent-wide sample of species, we believe the advantages prevail. Phenotypic plasticity in functional traits may play a role in allowing species to persist in a range of environments, and this is an avenue for continuing research, but we present our common-garden dataset mostly as a means of initially examining broad continental patterns.

Another more minor avenue for investigation is the possibility that juvenile-adult dimorphism in leaf vein density is an alternative strategy to plasticity in adult leaf vein density. Intraspecific variation in vein frequency is usually quite restricted (Brooker and Nicolle 2013). But plasticity in vein frequency may occur alternatively via the timing vegetative phase change, from the juvenile to the adult form (if the juvenile and adult vein frequencies are functionally different in a given species). For example, addition of phosphorus can induce earlier vegetative phases change in Eucalyptus nitens (Williams et al. 2004).

Although our results point to an important role for venation, we note two qualifications: the PGLS regression accounts for only 13% of variance in DIvein frequency and vein frequency cannot be isolated from other functional characteristics of leaves. These two points are undoubtedly related: some of the shortcoming in the statistical explanatory power of vein frequency must arise from variation in interacting traits we did not consider. For example woody stem vascular traits in Eucalyptus species are associated with climate and may compensate some of the effects of leaf venation on hydraulic conductivity (Gleason et al. 2012; Pfautsch et al. 2016). Nonetheless, juvenile and adult leaf venation seem to play a pivotal role in the ecology of heteroblasty at the broad interspecific and geographic scales we have examined.

Conflicts of interest

The authors declare that they have no conflicts of interest regarding the work presented here.

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