

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 longer, narrower isobilateral adult leaves with the characteristic lanceolate or falcate shape of eucalypts, alternate and pendulous on stems (Penfold and Willis 1961). Despite the visibility and frequency of this phenomenon in *Angophora*, *Corymbia* and *Eucalyptus*, the adaptive function of leaf heteroblasty among eucalypts is not well understood, as is true of heteroblasty generally (Zotz *et al.* 2011).

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, arborescent 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.

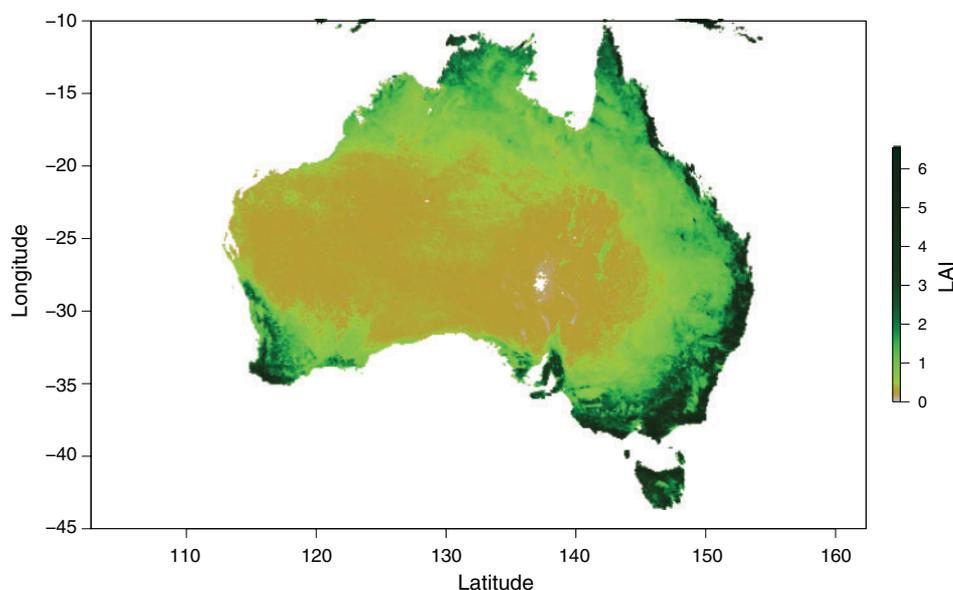


Fig. 1. Distribution of mean maximum leaf area index (LAI), measured as leaf area per unit ground area ($\text{m}^2 \text{m}^{-2}$), across Australia for the years 2000–2015, calculated from NASA Earth Observations data (<http://neo.sci.gsfc.nasa.gov>, accessed 3 November 2017). The distribution of LAI is shown by the scale bar.

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* spp. 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 glomerosa* Brooker & Hopper, *Eucalyptus gongylocarpa* Blakely, *Eucalyptus lucasii* Blakely, *Eucalyptus striaticalyx* 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 deuaensis* 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. We 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 where the juvenile value is larger than the adult value; DI > 0 where the adult value is larger than the juvenile value) species (Fig. 3). Some degree of dimorphism in leaf aspect ratio is common in the group, and in nearly all cases adult leaves have a higher aspect ratio (are longer and narrower) than juvenile leaves (DI_{aspect ratio} > 0) (Fig. 3a), confirming the widely noted pattern (Penfold and Willis 1961). In contrast, strong dimorphism in overall leaf size seems to be an exception rather than the rule, and the direction of the juvenile-adult difference is approximately equally balanced between species with larger juvenile leaves and those with larger adult leaves (Fig. 3b). Nearly all (87%) species had positive values of DI_{LMA} (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

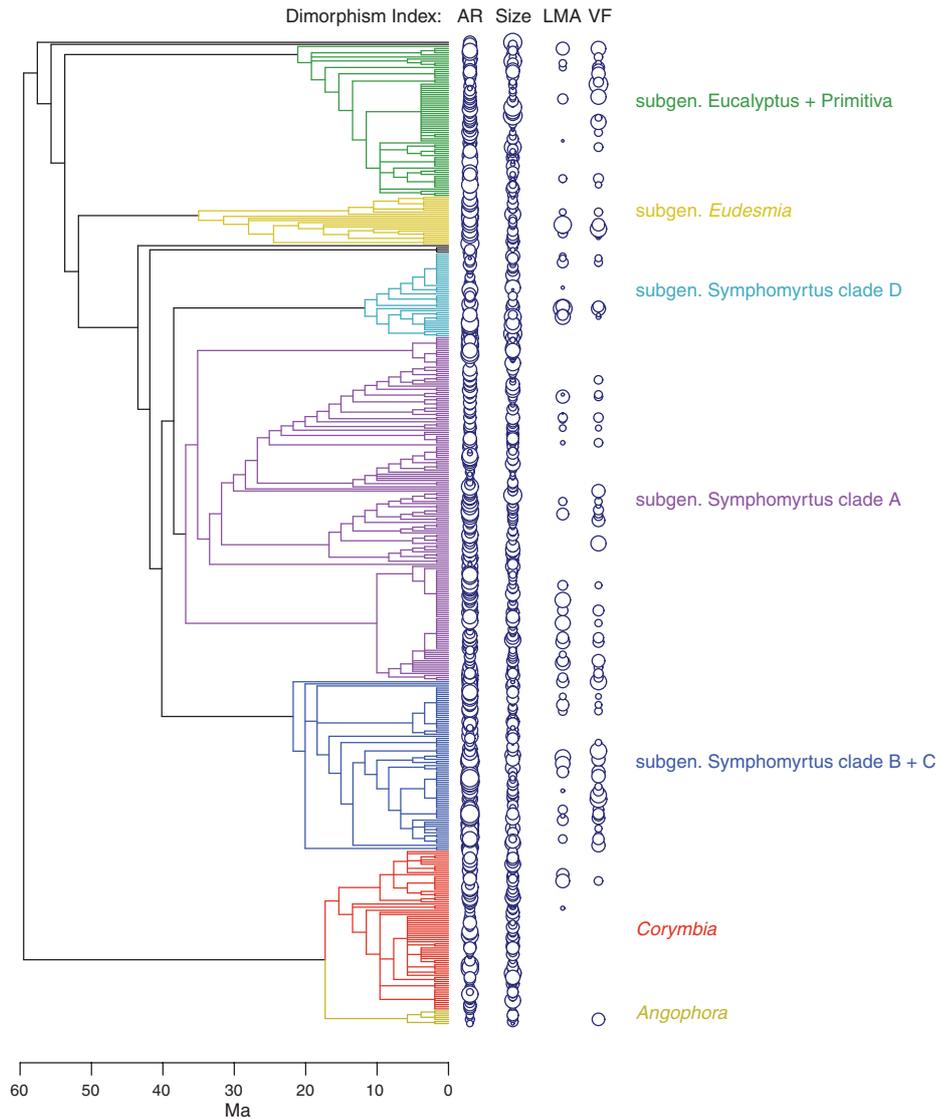


Fig. 2. Phylogeny of *Angophora*, *Corymbia* and *Eucalyptus* containing 469 terminal taxa. Abbreviations: AR, leaf aspect ratio; size, leaf size; LMA, leaf mass per area; VF, leaf vein frequency. Areas of blue circles correspond to dimorphism indexes (DI) for each trait (AR, size, LMA and VF) in each species for which data was available.

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 $DI_{\text{vein frequency}}$ 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 $DI_{\text{aspect ratio}}$ (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

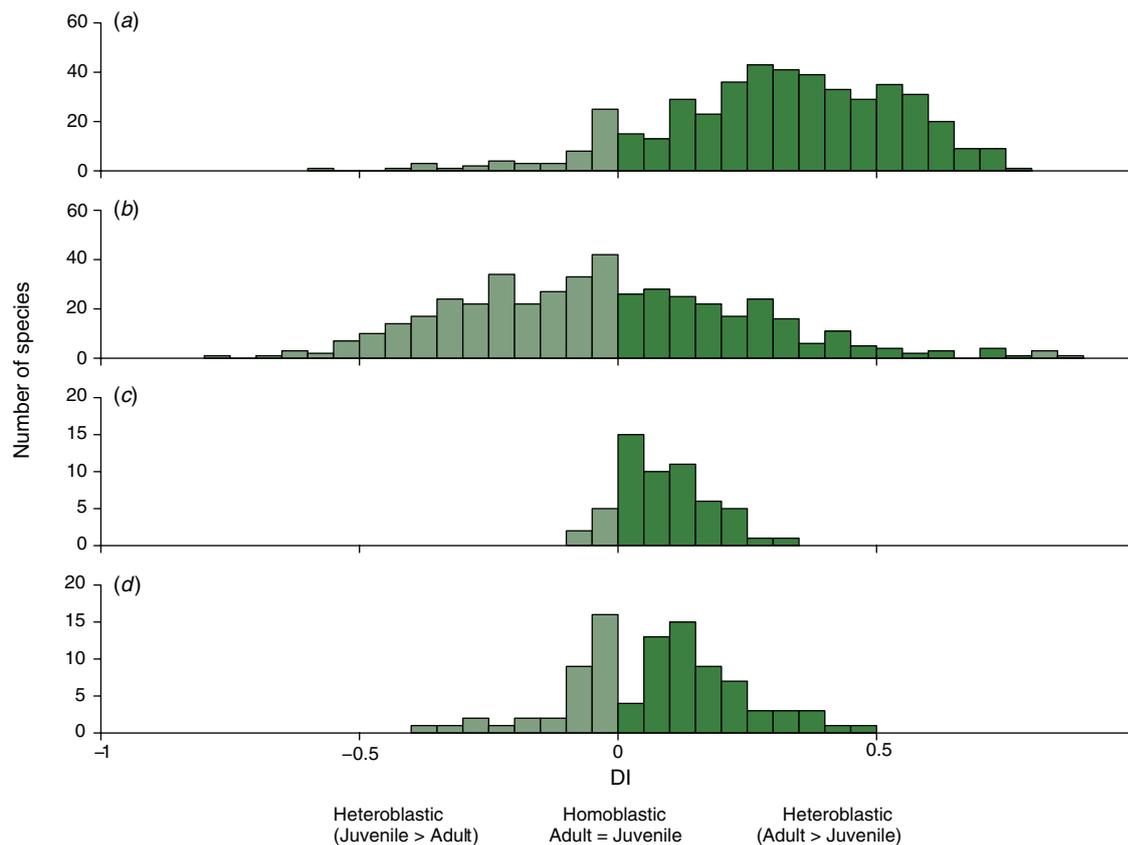


Fig. 3. Histograms of frequency distribution among species for DI (dimorphism index = (adult value – juvenile value)/(adult value + juvenile value)) in the four leaf traits: (a) aspect ratio ($n = 457$); (b) size ($n = 457$); (c) LMA ($n = 56$); (d) vein frequency ($n = 93$) (DI < 0, the juvenile value is higher than the adult value; DI > 0, the adult value is higher than the juvenile value).

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 (Percy *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, Percy *et al.* (2005)

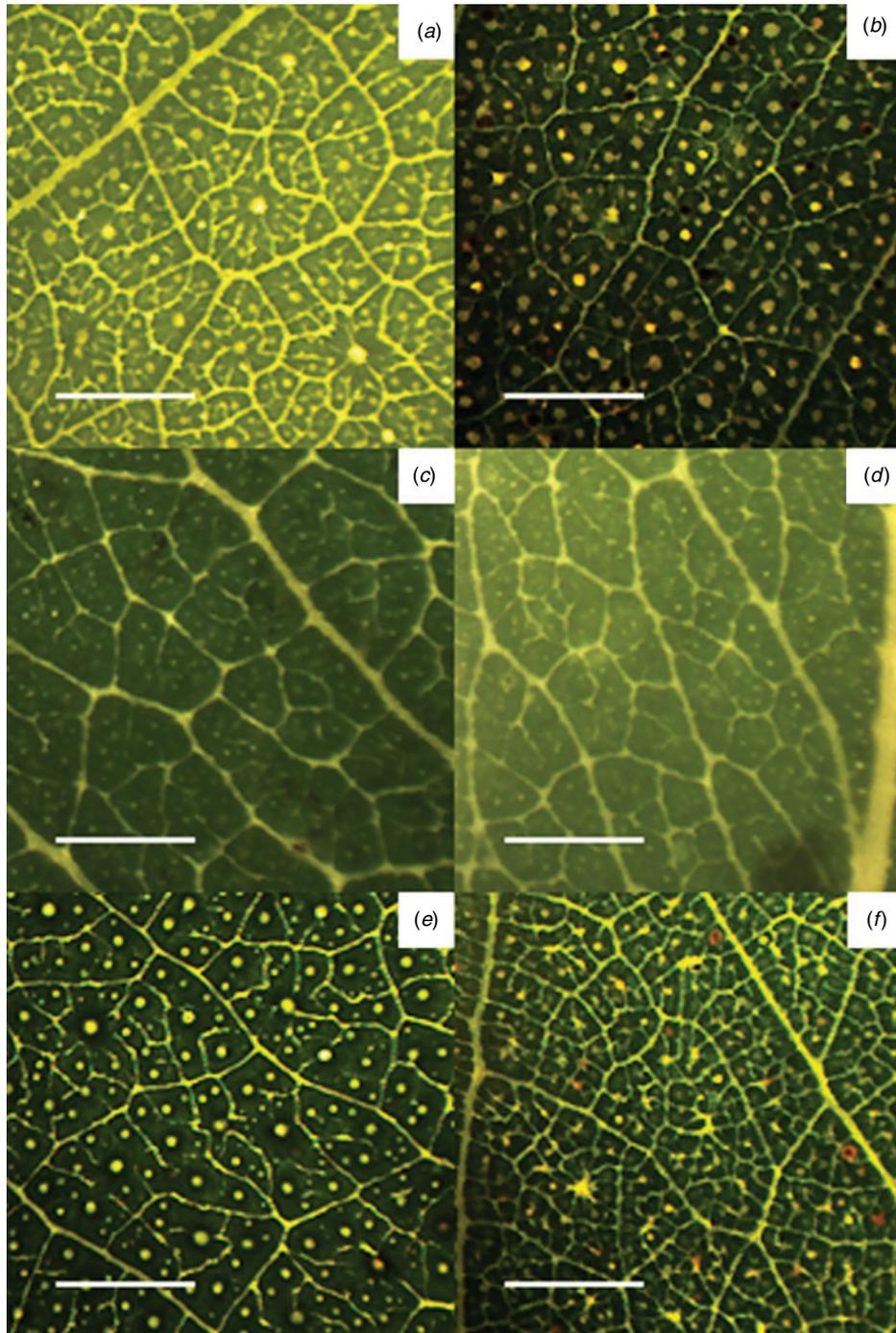


Fig. 4. Micrographs showing juvenile and adult leaf venation of three species: *Eucalyptus globulus* subsp. *pseudoglobulus* ($DI_{\text{vein frequency}} = -0.26$; heteroblastic; juvenile veins are more frequent than adult veins) juvenile (a) and adult (b); *Eucalyptus gongylocarpa* ($DI_{\text{vein frequency}} = 0$; homoblastic) juvenile (c) and adult (d); *Eucalyptus strzleckii* ($DI_{\text{vein frequency}} = 0.40$; heteroblastic; adult veins are more frequent than juvenile veins) juvenile (e) and adult (f). All scale bars = 2 mm.

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

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

Dependent variable	Slope co-efficient	d.f.	<i>t</i> -statistic	<i>R</i> ²	<i>P</i> -value
DI _{aspect ratio}	0.020	455	1.6921	0.006	0.091
DI _{size}	0.0013	455	0.0853	<0.001	0.932
DI _{LMA}	-0.00029	54	-0.032	<0.001	0.982
DI _{vein frequency}	0.065	91	3.8321	0.139	0.0002
Juvenile vein frequency (veins mm ⁻¹)	-0.11	91	-2.1940	0.050	0.031
Adult vein frequency (veins mm ⁻¹)	0.16	91	2.0877	0.046	0.040

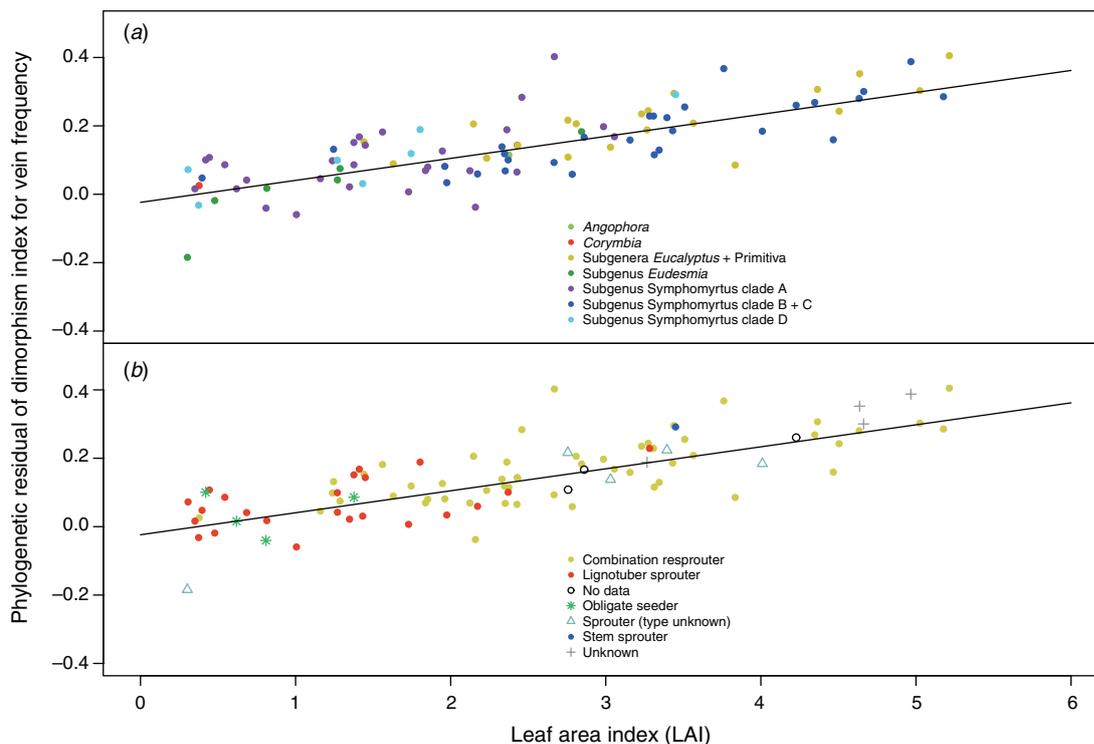


Fig. 5. Phylogenetically adjusted value of dimorphism index (DI) for vein frequency versus leaf area index (LAI). Phylogenetic least-squares (PGLS) model $F_{1,44} = 14.69$, $P = 0.0002$, $R^2 = 0.139$. Species are labelled by taxonomic group in the upper panel (a), and by regeneration niche (data from 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.

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.

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 $DI_{\text{vein frequency}}$ 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 (Brodrribb 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 $DI_{\text{vein frequency}}$, 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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References

- AVH (2017) ‘Australia’s virtual herbarium.’ (Council of Heads of Australasian Herbaria: Canberra) Available at <http://avh.chah.org.au> [Verified 10 April 2018]
- Brodrribb T, Hill RS (1993) A physiological comparison of leaves and phyllodes in *Acacia melanoxylon*. *Australian Journal of Botany* **41**, 293–305. doi:10.1071/BT9930293
- Brodrribb TJ, Jordan GJ (2011) Water supply and demand remain balanced during leaf acclimation of *Nothofagus cunninghamii* trees. *New Phytologist* **192**, 437–448. doi:10.1111/j.1469-8137.2011.03795.x
- Brodrribb TJ, Feild TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**, 1890–1898. doi:10.1104/pp.107.101352
- Brooker MIH (2000) A new classification of the genus *Eucalyptus* (Myrtaceae). *Australian Systematic Botany* **13**, 79–148. doi:10.1071/SB98008
- Brooker MIH, Kleinig DA (1990) ‘Field guide to eucalypts.’ (Inkata Press: Melbourne)
- Brooker I, Nicolle D (2013) ‘Atlas of leaf venation and oil gland patterns in the eucalypts.’ (CSIRO Publishing: Melbourne)
- Carins Murphy MR, Jordan GJ, Brodrribb TJ (2014) Acclimation to humidity modifies the link between leaf size and the density of veins and stomata. *Plant, Cell & Environment* **37**, 124–131. doi:10.1111/pce.12136
- Carr DJ (2000) The stomata of bluegums (*Eucalyptus* spp.) *Muelleria* **14**, 33–40.
- Crisp M, Cook L, Steane D (2004) Radiation of the Australian flora: what comparisons of molecular phylogenies across multiple taxa can tell us about the evolution of diversity in present-day communities. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **359**, 1551–1571. doi:10.1098/rstb.2004.1528

- Darrow HE, Bannister P, Burritt DJ, Jameson PE (2002) Are juvenile forms of New Zealand heteroblastic trees more resistant to water loss than their mature counterparts? *New Zealand Journal of Botany* **40**, 313–325. doi:10.1080/0028825X.2002.9512790
- Day JS (1998) Light conditions and the evolution of heteroblasty (and the divaricate form) in New Zealand. *New Zealand Journal of Ecology* **22**, 43–54.
- Day JS, Gould KS, Jameson PE (1997) Vegetative architecture of *Elaeocarpus hookerianus*. Transition from juvenile to adult. *Annals of Botany* **79**, 617–624. doi:10.1006/anbo.1996.0369
- Duursma RA, Falster DS, Valladares F, Sterck FJ, Pearcy RW, Lusk CH, Sendall KM, Nordenstahl M, Houter NC, Atwell BJ, Kelly N, Kelly JW, Liberloo M, Tissue DT, Medlyn BE, Ellsworth DS (2012) Light interception efficiency explained by two simple variables: a test using a diversity of small- to medium-sized woody plants. *New Phytologist* **193**, 397–408. doi:10.1111/j.1469-8137.2011.03943.x
- Gamage HK (2011) Phenotypic variation in heteroblastic woody species does not contribute to shade survival. *AoB Plants* **2011**, plr013. doi:10.1093/aobpla/plr013
- Gamage HK, Jesson L (2007) Leaf heteroblasty is not an adaptation to shade: seedling anatomical and physiological responses to light. *New Zealand Journal of Ecology* **31**, 245–254.
- Gibbons JW (1992) A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging* **56**, 269–281.
- Gibbs AK, Udovicic F, Drinnan AN, Ladiges PY (2009) Phylogeny and classification of *Eucalyptus* subgenus *Eudesmia* (Myrtaceae) based on nuclear ribosomal DNA, chloroplast DNA and morphology. *Australian Systematic Botany* **22**, 158–179. doi:10.1071/SB08043
- Gill AM (1997) Eucalypts and fires: interdependent or independent? In 'Eucalypt ecology: individuals to ecosystems'. (Eds JEWilliams, JCZ Woinarski) pp. 151–167. (Cambridge University Press: Cambridge, UK)
- Gleason SM, Butler DW, Ziemińska K, Waryszak P, Westoby M (2012) Stem xylem conductivity is key to plant water balance across Australian angiosperm species. *Functional Ecology* **26**, 343–352. doi:10.1111/j.1365-2435.2012.01962.x
- Hijmans RJ, van Etten J (2014) raster: Geographic data analysis and modeling. R package ver. 2, 15. Available at <https://cran.r-project.org/web/packages/raster/index.html> [Verified 10 April 2018]
- James SA, Bell JT (2000) Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. *Tree Physiology* **20**, 1007–1018. doi:10.1093/treephys/20.15.1007
- Johnson ED (1926) A comparison of the juvenile and adult leaves of *Eucalyptus globulus*. *New Phytologist* **25**, 202–212. doi:10.1111/j.1469-8137.1926.tb06691.x
- Jones CS (1999) An essay on juvenility, phase change, and heteroblasty in seed plants. *International Journal of Plant Sciences* **160**, S105–S111. doi:10.1086/314215
- Jones CS (2001) The functional correlates of heteroblastic variation in leaves: changes in form and ecophysiology with whole plant ontogeny. *Boletín de la Sociedad Argentina de Botánica* **36**, 171–184.
- JSTOR (2014) Global plants. Available at <https://plants.jstor.org/> [Verified 10 April 2018]
- King DA (1999) Juvenile foliage and the scaling of tree proportions, with emphasis on *Eucalyptus*. *Ecology* **80**, 1944–1954. doi:10.1890/0012-9658(1999)080[1944:JFATSO]2.0.CO;2
- Knyazikhin Y, Glassy J, Privette JL, Tian Y, Lotsch A, Zhang Y, Wang Y, Morisette JT, Votava P, Myneni RB, Nemani RR, Running SW (1999) 'MODIS leaf area index (LAI) and fraction of photosynthetically active radiation absorbed by vegetation (FPAR)' (Product (MOD15) algorithm theoretical basis document) Available at http://eosps.nasa.gov/sites/default/files/atbd/atbd_mod15.pdf [Verified 10 April 2018]
- Ladiges PY, Udovicic F, Nelson G (2003) Australian biogeographical connections and the phylogeny of large genera in the plant family Myrtaceae. *Journal of Biogeography* **30**, 989–998. doi:10.1046/j.1365-2699.2003.00881.x
- Li Y, Kröber W, Bruelheide H, Härdtle W, von Oheimb G (2017) Crown and leaf traits as predictors of subtropical tree sapling growth rates. *Journal of Plant Ecology* **10**, 136–145. doi:10.1093/jpe/rtw041
- McLean EH, Prober SM, Stock WD, Steane DA, Potts BM, Vaillancourt RE, Byrne M (2014) Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant, Cell & Environment* **37**, 1440–1451. doi:10.1111/pce.12251
- Meers TL, Bell TL, Enright NJ, Kassel S (2010) Do generalisations of global trade-offs in plant design apply to an Australian sclerophyllous flora? *Australian Journal of Botany* **58**, 257–270. doi:10.1071/BT10013
- Nicolle D (2006) A classification and census of regenerative strategies in the eucalypts (*Angophora*, *Corymbia* and *Eucalyptus* – Myrtaceae), with special reference to the obligate seeders. *Australian Journal of Botany* **54**, 391–407. doi:10.1071/BT05061
- Nicolle D (2013) 'Native eucalypts of South Australia.' (Dean Nicolle: Melrose Park, SA)
- Nunn C (2011) 'The comparative approach in evolutionary anthropology and biology.' (University of Chicago Press: Chicago, IL, USA)
- Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* **18**, 137–149. doi:10.1111/j.1466-8238.2008.00441.x
- Orme D (2013) The caper package: comparative analysis of phylogenetics and evolution in R. Available at <https://cran.r-project.org/web/packages/caper/vignettes/caper.pdf> [Verified 10 April 2018]
- Ostria-Gallardo E, Paula S, Corcuera LJ, Coopman RE (2015) Light environment has little effect on heteroblastic development of the temperate rain forest tree *Gevuina avellana* Mol. (Proteaceae). *International Journal of Plant Sciences* **176**, 285–293. doi:10.1086/680230
- Pagel M (1992) A method for the analysis of comparative data. *Journal of Theoretical Biology* **156**, 431–442. doi:10.1016/S0022-5193(05)80637-X
- Parra-O C, Bayly MJ, Drinnan A, Udovicic F, Ladiges P (2009) Phylogeny, major clades and infrageneric classification of *Corymbia* (Myrtaceae), based on nuclear ribosomal DNA and morphology. *Australian Systematic Botany* **22**, 384–399. doi:10.1071/SB09028
- Pasquet-Kok J, Creese C, Sack L (2010) Turning over a new 'leaf': multiple functional significances of leaves versus phyllodes in Hawaiian *Acacia koa*. *Plant, Cell & Environment* **33**, 2084–2100. doi:10.1111/j.1365-3040.2010.02207.x
- Pearcy RW, Muraoka H, Valladares F (2005) Crown architecture in sun and shade environments: assessing function and trade-offs with a three-dimensional simulation model. *New Phytologist* **166**, 791–800. doi:10.1111/j.1469-8137.2005.01328.x
- Penfold AR, Willis JL (1961) 'The eucalypts: botany, cultivation, chemistry and utilization.' (Leonard Hill: London)
- Pfautsch S, Harbusch M, Wesolowski A, Smith R, Macfarlane C, Tjoelker MG, Reich PB, Adams MA (2016) Climate determines vascular traits in the ecologically diverse genus *Eucalyptus*. *Ecology Letters* **19**, 240–248. doi:10.1111/ele.12559
- Pickup M, Westoby M, Basden A (2005) Dry mass costs of deploying leaf area in relation to leaf size. *Functional Ecology* **19**, 88–97. doi:10.1111/j.0269-8463.2005.00927.x
- Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**, 565–588. doi:10.1111/j.1469-8137.2009.02830.x

- Russell-Smith J, Yates CP, Whitehead PJ, Smith R, Ron Craig R, Allan GE, Thackway R, Frakes I, Cridland S, Meyer MCP, Gill AM (2007) Bushfires 'down under': patterns and implications of contemporary Australian landscape burning. *International Journal of Wildland Fire* **16**, 361–377. doi:10.1071/WF07018
- Sack L, Holbrook NM (2006) Leaf hydraulics. *Annual Review of Plant Biology* **57**, 361–381. doi:10.1146/annurev.arplant.56.032604.144141
- Sack L, Scoffoni C (2013) Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytologist* **198**, 983–1000. doi:10.1111/nph.12253
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–675. doi:10.1038/nmeth.2089
- Slee A, Connors J, Brooker M, Duffy S, West J (2006) 'EUCLID Eucalypts of Australia. Centre for Plant Biodiversity Research.' (CD-ROM) (CSIRO Publishing: Melbourne)
- Steane DA, Nicolle D, Sansaloni CP, Petroli CD, Carling J, Kilian A, Myburg AA, Grattapaglia D, Vaillancourt RE (2011) Population genetic analysis and phylogeny reconstruction in *Eucalyptus* (Myrtaceae) using high-throughput, genome-wide genotyping. *Molecular Phylogenetics and Evolution* **59**, 206–224. doi:10.1016/j.ympev.2011.02.003
- Stoneman GL (1994) Ecology and physiology of establishment of eucalypt seedlings from seed: a review. *Australian Forestry* **57**, 11–29. doi:10.1080/00049158.1994.10676109
- Valladares F, Brites D (2004) Leaf phyllotaxis: does it really affect light capture? *Plant Ecology* **174**, 11–17. doi:10.1023/B:VEGE.0000046053.23576.6b
- Williams DR, Potts BM, Smethurst PJ (2004) Phosphorus fertiliser can induce earlier vegetative phase change in *Eucalyptus nitens*. *Australian Journal of Botany* **52**, 281–284. doi:10.1071/BT03135
- Woodhams M, Steane DA, Jones RC, Nicolle D, Moulton V, Holland BR (2013) Novel distances for Dollo data. *Systematic Biology* **62**, 62–77. doi:10.1093/sysbio/sys071
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M (2004) The worldwide leaf economics spectrum. *Nature* **428**, 821–827. doi:10.1038/nature02403
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets U, Oleksyn J, Osada N, Poorter H, Warton DI, Westoby M (2005) Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* **14**, 411–421. doi:10.1111/j.1466-822x.2005.00172.x
- Yu H, Li JT (2007) Physiological comparisons of true leaves and phyllodes in *Acacia mangium* seedlings. *Photosynthetica* **45**, 312–316. doi:10.1007/s11099-007-0053-x
- Zhao WL, Chen YJ, Brodribb TJ, Cao KF (2016) Weak co-ordination between vein and stomatal densities in 105 angiosperm tree species along altitudinal gradients in Southwest China. *Functional Plant Biology* **43**, 1126–1133. doi:10.1071/FP16012
- Zotz G, Wilhelm K, Becker A (2011) Heteroblasty – a review. *Botanical Review* **77**, 109–151. doi:10.1007/s12229-010-9062-8

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