

The evolution and functional significance of leaf shape in the angiosperms

Adrienne B. Nicotra^{A,H}, Andrea Leigh^B, C. Kevin Boyce^C, Cynthia S. Jones^D, Karl J. Niklas^E, Dana L. Royer^F and Hirokazu Tsukaya^G

^AResearch School of Biology, The Australian National University, Canberra, ACT 0200, Australia.

^BSchool of the Environment, University of Technology, Sydney, PO Box 123, Broadway, NSW 2007, Australia.

^CDepartment of the Geophysical Sciences, 5734 S. Ellis Avenue, Chicago, IL 60637, USA.

^DDepartment of Ecology and Evolutionary Biology, University of Connecticut, 75 N. Eagleville Road, Unit-3043, Storrs, CT 06269, USA.

^EDepartment of Plant Biology, Cornell University, 412 Mann Library Building, Cornell University, Ithaca, NY 14853, USA.

^FDepartment of Earth and Environmental Sciences, Wesleyan University, 265 Church Street, Middletown, CT 06459, USA.

^GGraduate School of Science, University of Tokyo, Science Build #2, 7-3-1 Hongo, Tokyo 113-0033, Japan.

^HCorresponding author. Email: adrienne.nicotra@anu.edu.au

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Abstract. Angiosperm leaves manifest a remarkable diversity of shapes that range from developmental sequences within a shoot and within crown response to microenvironment to variation among species within and between communities and among orders or families. It is generally assumed that because photosynthetic leaves are critical to plant growth and survival, variation in their shape reflects natural selection operating on function. Several non-mutually exclusive theories have been proposed to explain leaf shape diversity. These include: thermoregulation of leaves especially in arid and hot environments, hydraulic constraints, patterns of leaf expansion in deciduous species, biomechanical constraints, adaptations to avoid herbivory, adaptations to optimise light interception and even that leaf shape variation is a response to selection on flower form. However, the relative importance, or likelihood, of each of these factors is unclear. Here we review the evolutionary context of leaf shape diversification, discuss the proximal mechanisms that generate the diversity in extant systems, and consider the evidence for each the above hypotheses in the context of the functional significance of leaf shape. The synthesis of these broad ranging areas helps to identify points of conceptual convergence for ongoing discussion and integrated directions for future research.

Additional keywords: compound leaf, leaf dissection, leaf margin, leaf size, leaves, lobbing.

Introduction

A casual look at any local flora reveals great diversity in angiosperm leaf shape. 'Shape' describes a dimensionless descriptor, for example, length/width, which quantifies form in terms of the natural dimensions or size of any object. The functional significance of shape variation among leaves has been the subject of debate for many years, and there are a range of different approaches to describing leaf shape (e.g. Nicotra 2010). The diversity of shape suggests that there is no one ecological strategy that is dependent exclusively on leaf shape. Even within a single genus, leaf shape variation can be tremendous (Fig. 1). Here we provide an overview of the evolutionary history of leaf shape and then examine genetic, developmental, physiological and ecological determinants of

leaf shape to better understand the evolutionary drivers of diversification in leaf shape and the functional significance therein.

Research over the past decade has produced sound ecological explanations for the significance of some key non-shape leaf traits. The leaf economic spectrum describes a continuum ranging from leaves with low to high mass per unit area (LMA). Leaves with high LMA represent a high investment in structure and are typically long-lived with lower photosynthetic rates (Wright *et al.* 2004). Leaf size varies in a similar way: small leaves are associated with harsh conditions such as cold (Gates 1980), hot (Smith and Nobel 1977), dry (Thoday 1931; Raunkiaer 1934; Gates *et al.* 1968; Parkhurst and Loucks 1972; Specht and Specht 1999; Fonseca *et al.* 2000; McDonald *et al.* 2003), high light



Fig. 1. Leaf shape in the genus *Pelargonium* ranges from single and entire to compound and highly dissected. Species are (a) *P. bowkeri*, (b) *P. reniforme*, (c) *P. klinghardtense*, (d) *P. fulgidum*, (e) *P. carnosum*, (f) *P. cucullatum*, (g) *P. abrotanifolium*, (h) *P. citronellum*, (i) *P. australe* and (j) *P. alternans*. Photographs courtesy of Stuart Hay, ANU Photography.

(Smith and Nobel 1977; Bragg and Westoby 2002), exposed (Ackerly *et al.* 2002), nutrient poor (Beadle 1966; Cunningham *et al.* 1999; Fonseca *et al.* 2000) and saline environments (Ball *et al.* 1988), or many of these factors in combination (e.g. McDonald *et al.* 2003). Leaf shape, in contrast, has been shown to vary less predictably across environments or biomes than size or LMA (McDonald *et al.* 2003). Thus, the functional significance of leaf shape and the evolutionary drivers of its diversity remain the subject of discussion.

The theories about leaf shape are many, and not mutually exclusive: thermoregulation of leaves especially in arid and hot environments, hydraulic constraints, patterns of leaf expansion in deciduous species, mechanical constraints, adaptations to avoid herbivory, adaptations to optimise light interception and, given that leaves are hypothesised to be developmental homologues of floral organs, and it has even been suggested that leaf shape reflects the effects of selection on flower form. Finally, there is the chance that leaf shape variation has little functional or adaptive significance and instead reflects random variation within the context of phylogenetic history. However, given the importance of the leaf we believe the latter option rather unlikely. To distinguish among the former, we here synthesise the

varied fields involved so that we may assess the relative importance of each and identify the ecological situations in which one or the other of these mechanisms is likely to be significant.

In this review we first examine leaf shape from an evolutionary perspective and in the process demonstrate that shape is highly labile and widely explored in evolutionary history. We bring the focus onto the angiosperms in particular, and then consider the proximate determinants of angiosperm leaf shape. The current understanding of the genetic signals and developmental processes underlying shape differences are then reviewed to explore the genetic controls and constraints on leaf shape evolution. The hypotheses about the function of leaf shape are next reviewed with an evolutionary and genetic perspective in mind. We highlight those functional issues that we see as particularly relevant to leaf shape evolution, and then turn to three examples of leaf shape variation: across communities, within lineages and within individuals to explore how an evolutionary perspective alters understanding of the relationship between leaf form and function. We conclude with suggestions on how this perspective can be used to direct future research on the function and evolution of leaf shape.

A brief history of the angiosperm leaf and shape diversity therein

To understand the evolution of angiosperm leaf shape, some perspective on the evolution of the leaf itself is needed, as leaves of many shapes have evolved numerous times independently in evolutionary history. For the purpose of this review of angiosperm leaf shape we define a leaf as a vascular asymmetric appendicular structure initiated at the shoot apical meristem. This definition excludes the functionally analogous structures of mosses and leafy liverworts. Morphologically simple leaves, microphylls, arose in the lycopsids. Larger leaves with more complex shapes and venation networks, called megaphylls, arose in the seed plants, in the extinct progymnosperm and sphenophyll lineages, and in one or more lineages of 'ferns' (Galtier 1981; Kenrick and Crane 1997; Niklas 1997; Boyce and Knoll 2002; Tomescu 2009; Boyce 2010). Thus, the seed plants represent just one of several independent evolutionary origins of large leaves with diverse shapes among the vascular plants.

Despite the many independent origins of leaves, several traits now associated with leaves can be considered homologous across all vascular plants through a shared monophyletic origin from a leafless common ancestor. First, all vascular plant leaves are distinct from the leaf-like organs of bryophytes that rely on external surfaces for photosynthetic gas exchange (as would also be true of the external appendages independently derived in many algal lineages; Niklas 2000). Vascular plant leaves use internal airspaces regulated by stomata for gas exchange (Raven 1996; Boyce 2008a). Second, evidence from both living plants and the venation patterns of fossil leaves indicates that the ancestral form of tissue production in the growing leaves of all vascular plants was limited to discrete marginal zones of growth (Pray 1960; Zurkowski and Gifford 1988; Boyce and Knoll 2002; Boyce 2007). Other aspects of leaf organography, such as the evolution of differentiated abaxial/adaxial domains (see section on 'Proximal mechanisms underlying leaf shape diversity' below) and determinate leaf growth appear to arise independently in seed plants and other lineages, however, several of these lineages have co-opted the same underlying genetic pathways to regulate these developmental processes (see section on 'Proximal mechanisms underlying leaf shape diversity', Bharathan *et al.* 2002; Harrison *et al.* 2005; Sanders *et al.* 2007; Tomescu 2009; Boyce 2010).

Within this broader evolutionary context, angiosperm leaves represent a range of particularly aberrant variants of vascular plant leaves. Some of the distinctive characteristics of angiosperm leaves have arisen independently in other lineages, whereas other traits are unique to the flowering plants. Angiosperms have evolved leaf growth that occurs diffusely throughout the leaf without being limited to the margin (Pray 1955; Poethig and Sussex 1985a, 1985b), thus, enabling the enormous diversity and complexity of angiosperm leaf venation patterns and shapes. The phylogenetic distribution of complex venation patterns suggest that the evolutionary departures from marginal growth observed in the angiosperms have also arisen repeatedly in two or three other seed plant lineages and 10 or more groups of ferns (Boyce 2005). In these, likewise, diffuse growth and complex venation patterns can be seen to occur with diverse leaf shapes. Despite these similarities to the other seed plants, angiosperm leaf

evolution remains distinctive in that it ran counter to the general temporal trend seen in the vasculature of other seed plants. The vascular network of other seed plant lineages evolved towards simplification. After initially exhibiting the complete range of morphologies consistent with marginal growth, including many involving two vein orders with a midvein and open or reticulate networks of secondary veins (morphologies now common only among the ferns), the leaf architecture in the other major vascular plant lineages became progressively limited to a narrow subset of forms. In cycads, conifers, Ginkgo and several other extinct seed plant lineages a single order of veins lead straight, parallel courses to a distal margin. Angiosperms, however, exhibit many hierarchical orders of reticulate, internally directed veins (Boyce 2005). Further, angiosperm leaves are unique in their possession of extraordinarily high vein densities (Fig. 2): whereas all other plants, living or extinct, average ~2 mm of vein length per square mm of leaf surface, angiosperms average between 8 and 10 mm mm⁻² and can range to above 20 mm mm⁻² (Boyce 2008a, 2008b, 2009; Brodribb and Feild 2010; Brodribb *et al.* 2010).

Together these changes that accompanied angiosperm evolution represent a substantial revision of the functional possibilities of a leaf. First, the release of venation patterns from strictly marginal growth allows the production of novel leaf shapes and sizes that would not otherwise be possible. Second, differences in angiosperm venation allowed a complete revolution in how water is distributed within a leaf (Boyce 2008a). In any leaf, the tissue furthest from the leaf base has access only to that water which has not been lost to transpiration in more proximal tissues (Zwieniecki *et al.* 2004a, 2006). Reticulate venation and the minute size of the final order of veins in flowering plants leads to equitable distribution of water between successive vein orders (Zwieniecki *et al.* 2002). Finally, the high vein densities of angiosperms shorten the path length along xylem and mesophyll, thus, enabling assimilation and transpiration capacities higher than in any other group of plants (Bond 1989; Sack and Frole 2006; Brodribb *et al.* 2007; Boyce *et al.* 2009). The uniquely high transpiration capacities of angiosperms may also have influenced the evolution of leaf shape by relaxing some of the thermal constraints on leaf size and shape (see 'Temperature and water' section).

It remains controversial when, and in which lineages, these characteristics of angiosperm leaves arose. The distinctiveness of angiosperms leaves has led to polarised interpretations of their ancestry. Some researcher propose that similarities in leaf venation tie the early angiosperms to particular fossil seed plants (e.g. Melville 1969), but the group of plants with the most similar venation are the clearly unrelated dipterid ferns. The various seed plant lineages most often favoured as angiosperm relatives based upon reproductive characteristics (Doyle and Hickey 1976; Hilton and Bateman 2006; Frohlich and Chase 2007) tend to have very dissimilar leaves. Others suggest that angiosperm leaves are so distinct as to require a complete reinvention of a leaf after passing through a leafless intermediate, such as one that was aquatic- or desert-adapted (Doyle and Hickey 1976). But the appearance of angiosperm-like leaf traits in a variety of fern lineages suggest that invoking a leafless intermediate is unnecessary. Although the

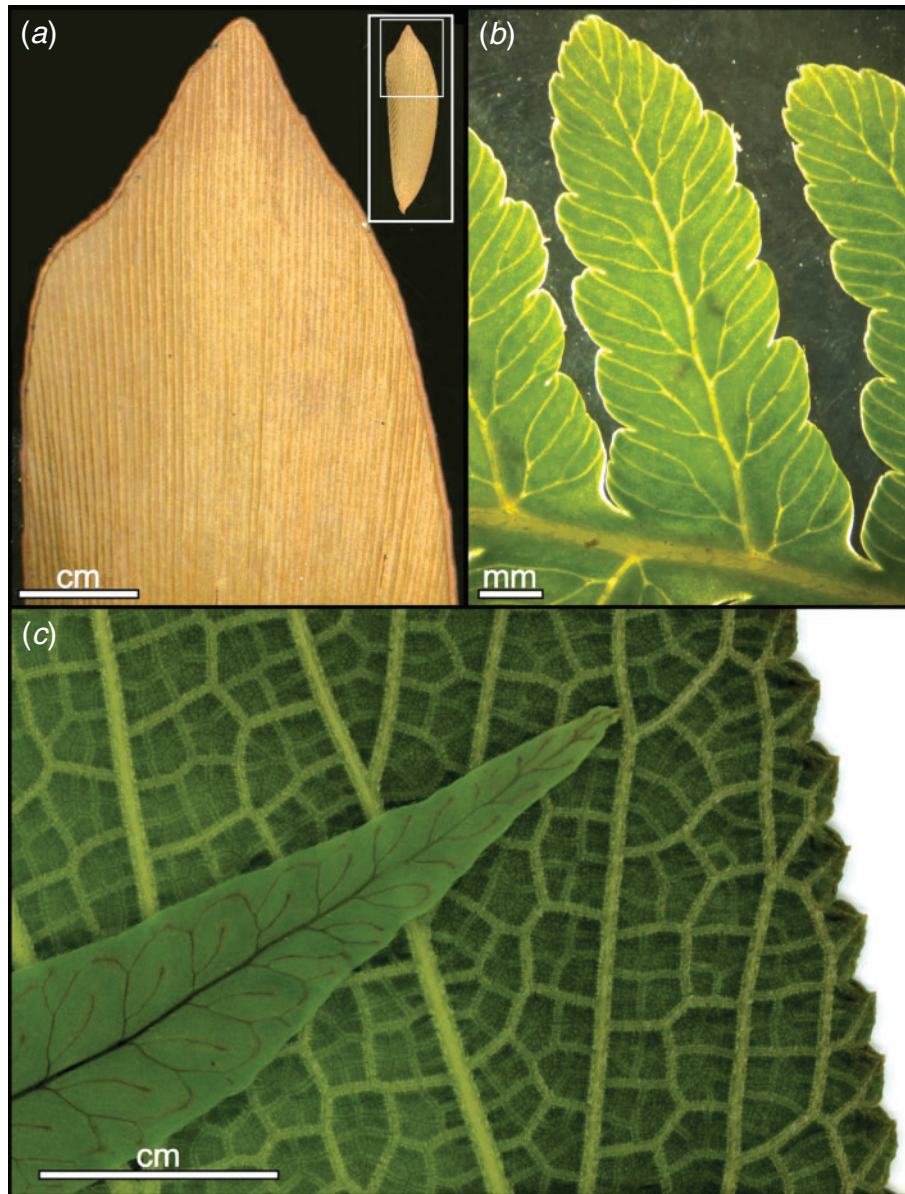


Fig. 2. Leaf venation architecture in (a) the cycad *Zamia furfuracea*, (b) the fern *Blechnum gibbum* and (c) the angiosperm *Boehmeria nivea* overlain with the fern *Polypodium formosanum*. Both the *Zamia* and the *Blechnum* have marginally ending veins suggesting marginal growth, but the single order of parallel veins in *Zamia* must distribute water over more than 10 cm of length whereas the finest veins in the *Blechnum* are a few mm. The finest veins distributing water in angiosperms can be even shorter and the density of veins much higher: the *Boehmeria* has $\sim 8 \text{ mm of vein mm}^{-2}$ of leaf versus 1 mm mm^{-2} in the *Polypodium*.

history of early angiosperm leaf evolution is still debated, we are certain that there have been numerous independent origins of diverse leaf shapes and that leaf shape diversification is associated with both high vein densities and reticulate venation patterns.

Proximal mechanisms underlying leaf shape diversity

What are the genetic mechanisms underlying this leaf shape diversification and how similar are they given the many

independent origins of diverse leaf shapes? What we know of the processes of leaf development (and leaf shape determination) is largely a product of studies on model species systems. In particular, *Arabidopsis thaliana* L. (Heynh) (hereafter referred to as *Arabidopsis*) has been widely used for the identification of genes determining leaf form. In the following sections we examine the development of the angiosperm leaf: specifically, the establishment of dorsiventral domains and flattening; expansion of the lamina, and formation of leaf margin; and discuss how these factors interact to determine leaf shape.

Establishment of dorsiventral domains and lamina flattening

Since leaf primordia arise as protrusions from shoot apical meristems (SAM), suppression of the regular SAM developmental program is the first step required for normal development of a leaf primordium. Recently, Sarojam *et al.* (2010) found that genes in the *YABBY* family suppress SAM identity and promote lamina growth in *Arabidopsis*. In most bifacial leaves, such as leaves of *Arabidopsis* and snapdragon (*Antirrhinum majus* L.), the radial primordium differentiates into abaxial (lower) and adaxial (upper) surfaces to establish dorsiventrality (Waites and Hudson 1995). Our current understanding is that the molecular genetic regulation of identification of the abaxial and adaxial domains depends on actions of both small RNAs and direct/indirect targets such as class III Zip genes, *KANADI* genes and *AUXIN RESPONSE FACTORS3* (*ARF3*)/*ETT* in *Arabidopsis* (Husbands *et al.* 2009; Floyd and Bowman 2010; Kidner 2010). Without establishment of the adaxial and abaxial domains, it is believed that leaves cannot expand in any angiosperm species; rather they become stalk like, since the so-called plate meristem is never activated to expand the laminae (Fig. 3).

The monocot clade is typified by many unifacial leaves that have only abaxial identity in the lamina, as seen in leek (*Allium ampeloprasum* var. *porrum* L.) and *Iris* spp. Leek leaves are cylindrical, since they lack adaxial identity in their leaf blade. *Iris*, in contrast, has flat leaves that expand in a distinct,

perpendicular direction relative to that of normal bifacial leaves. Likewise, the rush *Juncus prismatocarpus* R.Br. has unifacial flat leaves; the primordium of the leaf blade grows into a flat lamina as a result of extensive thickening growth. This developmental pathway is dependent on the gene, *DROOPING LEAF* (*DL*, Yamaguchi *et al.* 2010; Fig. 3), that is also a member of the *YABBY* gene family, but with a monocot-specific function. As seen in this case, diversified morphology in a particular taxon sometimes depends on some taxon-specific molecular mechanisms that are modifications of gene function from ancestral lineages.

Mechanisms for expansion of lamina

Once dorsiventral identity is generated the leaf lamina can then diversify in morphology to form simple or compound leaves with entire or serrated margins, differing in thickness, length and width, and in orientation (Tsukaya 2006). The leaf length : width ratio is regulated by polar-dependent cell expansion and cell proliferation/distribution. Several key genes for its regulation have been identified from *Arabidopsis*: *ANGUSTIFOLIA* (*AN*) and *ROTUNDIFOLIA3* (*ROT3*) regulate the shape of cells whereas *ANGUSTIFOLIA3* (*AN3*) and *ROTUNDIFOLIA4* (*ROT4*) affect the number of cells in the lamina (Tsukaya 2006). Loss-of-function mutations of *AN* and *AN3* result in narrower leaves, whereas loss-of-function of *ROT3* or overexpression of *ROT4* make leaves shorter. In addition,

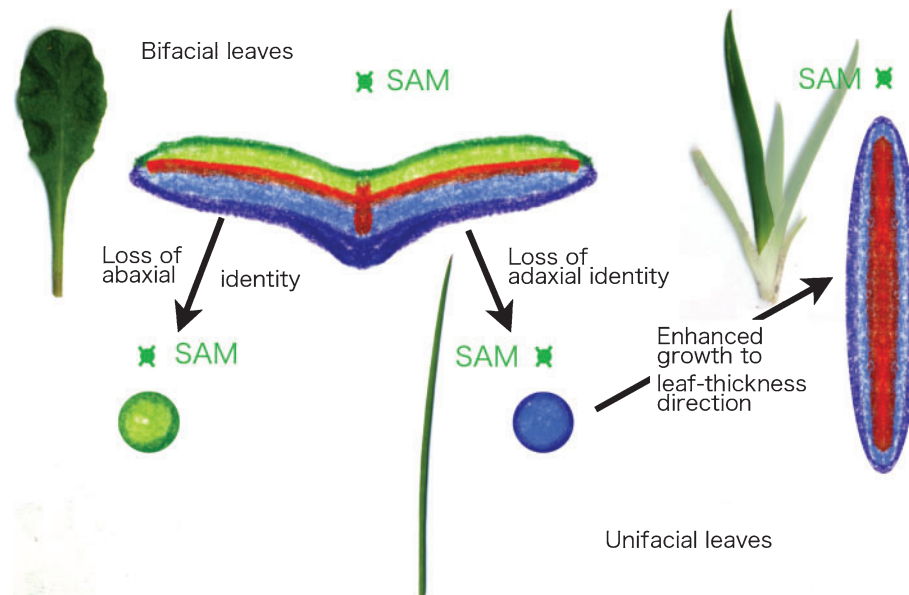


Fig. 3. Two different mechanisms of lamina growth. In most leaves such as leaves of *Arabidopsis* (upper left), dorsiventral identity (adaxial, upper side (shown in green in colour version online); and abaxial, lower side (shown in blue in colour version)) identities are established. Flattening lamina growth occurs along the junction between adaxial and abaxial domains as a result of activity of the plate meristem. Loss of either abaxial or adaxial identity results in radialised growth (lower right). Stick-like leaf morphology seen in leek and *Juncus wallichianus*, (centre) is the typical example of unifacial leaves that have no adaxial domain. In the monocot clade, however, some species develop flat lamina irrespective of lack of adaxial identity in the lamina (right, *Sisyrinchium rosulatum*), that is supported by enhanced growth in the leaf-thickness direction. Meristematic activity occurs in center region of both leaves (orange in colour version); relative position of the shoot apical meristem (SAM) is shown by the cross-hatched dot (green in colour version).

LONGIFOLIA1 (*LONI*) and *LON2* regulate the length of the leaf lamina in *Arabidopsis* (Lee *et al.* 2006). A vast array of genes is known to influence leaf area (Horiguchi *et al.* 2006). For example, heteroblastic change in leaf area is governed by the miR156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)-miR172 pathway via regulation of cell size and cell numbers in *Arabidopsis* (Usami *et al.* 2009). Alterations of activities of any of these factors could contribute to natural variation in leaf length:width ratio and area. None of these genetic variations result in loss of reproductive structures, suggesting that these genes could be under direct environmentally mediated selection. As yet, we do not know whether the above patterns hold in other non-model species.

Leaves also vary in the extent to which the lamina is flat or curved. *CINCINATA* (*CIN*) of snapdragon was once thought to function to keep the leaf flat by orchestrating cell proliferation along the medio-lateral axis of the leaf primordia (Nath *et al.* 2003). More recently, however, analyses of the *TCP* genes, which are homologues of *CIN* in *Arabidopsis*, indicate that the major role of the *TCP* is in promotion of maturation in laminar tissues, not in regulation of cell proliferation (Efroni *et al.* 2008; Sarojam *et al.* 2010). The role of *CIN* in snapdragon may also be the same as that of *TCPs* in *Arabidopsis*. The other candidate genetic mechanism for control of leaf curvature is an auxin-related pathway. Several hyponastic leaf mutants have been isolated from *Arabidopsis* and many of the responsible genes are related to auxin signalling (Perez-Perez *et al.* 2002, 2009). Since auxin signalling is also very important for venation patterning, species-specific modification of auxin signalling in leaf primordia may have caused diversification of both leaf curvature and vasculature patterning.

Genetic regulation for patterning of leaf margin: serration and leaflet formation

Variation in leaf shape, as distinct from length and area or curvature, arises from variation in leaf margin and lobe formation. Kawamura *et al.* (2010) showed that spatial patterning of serrations, or leaf teeth (see 'Leaf shape variation within lineages' section), on the leaf margin depends on formation of auxin maxima on the leaf margin in *Arabidopsis*. Location of these maxima is governed by polar auxin transport and the stabilisation of auxin maxima is maintained by prolonged expression of *CUP-SHAPED COTYLEDON2* (*CUC2*) around it (Kawamura *et al.* 2010). The mechanism of rhythmic pattern formation of the auxin maxima/minima on the leaf margin is thought to involve self-organising pattern formation mechanisms by polar auxin transport, similar to that involved in pattern formation of phyllotaxy (de Reuille *et al.* 2006; Jonsson *et al.* 2006). Either the loss of distinct auxin maxima or an instability of the auxin maxima will result in an entire leaf margin (Kawamura *et al.* 2010). Changes in functions of these genes, however, severely influence many biological processes including floral organ development as seen in the *pin-formed 1* mutant (Okada *et al.* 1991). Thus, variation among species in pattern of margin serration might accompany variation in floral organs, or more likely, reflect differential regulation for example by downstream genetic pathways.

Combined control of auxin-maxima formation by *PIN* and *CUC* genes, as described for serration above, is also known to have an important role in leaflet patterning in bittercress (*Cardamine flexuosa* With.), an *Arabidopsis* relative with compound leaves (Barkoulas *et al.* 2008). Again this control varies among species: in tomato (*Solanum lycopersicum* L.), loss of *CUC3* function does not convert compound leaves into simple leaves (Blein *et al.* 2008). Thus, it is clear that the independent evolution of compound leaves from simple leaves is likely driven by species-specific mechanisms (Efroni *et al.* 2010), though these may reflect modifications of similar pathways.

Recent studies have identified several important key genes for regulation of compound versus simple leaf forms. For example, absence or prolonged expression of class I *Knotted1*-like homeobox (*KNOX*) genes in leaf primordia is important to compound leaf formation in a wide range of angiosperms and gymnosperms (Bharathan *et al.* 2002). In some legume species, however, the *LEAFY* (*LFY*)/*UNIFOLIATA* (*UNI*) gene is the key for compound leaf formation, instead of the class I *KNOX* genes (Hofer *et al.* 1997). Changes in these genes could be linked to the evolution of compound leaves, although loss-of-function of *LFY/UNI* also causes infertility in *Arabidopsis* and, thus, it is unlikely that selection directly on these particular genes could result in the evolution of variation in leaf shape. Diversified spatial patterning of petiole/petiolule and lamina/leaflet in compound leaves have also been suggested to be linked to the distribution pattern of *AS1-ROUGH SHEATH2-PHANTASTICA* (*ARP*) expression domains (Kim *et al.* 2003). However, based on the fact that the *ARP* gene has only a limited role in compound leaf formation in some species, it is unclear whether *ARP* will turn out to be major factor in others (Efroni *et al.* 2010).

As described above, the independent evolutionary origins of several angiosperm leaf features should caution against the assumption that a single ancestral genetic system underlies leaf development. The developmental genetic approaches outlined have revealed several key mechanisms for leaf shape control, but have also demonstrated variation in the function of these genes among lineages. In a few studies researchers have gone beyond one or two species studies, and have looked for deeper evolutionary links (Illing *et al.* 2009). Studies of the role of the class I *KNOX* genes have considered not just angiosperms but other lineages. Among simple-leaved dicots and monocots (e.g. *Arabidopsis* and *Zea*), *KNOX* genes are strongly expressed during the indeterminate growth of the apical meristem, but are rapidly downregulated in the cells flanking the apical dome in regions defined by leaf primordia. Although some arginine-rich protein (*ARP*) genes repress the expression of *KNOX* transcription factors during the development of both micro- and megaphyll primordia (Harrison *et al.* 2005), the role of homeodomain-leucine zipper (*HD-ZIP*) III genes, which are involved in the specification of foliar adaxial/abaxial fate, differs in the development of these two leaf types (Floyd and Bowman 2006). *HD-ZIP III* genes have been identified in the moss *Physcomitrella* (Sakakibara *et al.* 2001) and in the fern *Ceratopteris* (Aso *et al.* 1999); they are also expressed during normal vascular tissue development in *Arabidopsis* (Zhong and Ye 1999), suggesting that they are very ancient but also multifunctional.

Accumulating knowledge on molecular genetic mechanisms of leaf morphogenesis is leading to an increasingly evo-devo approach to studies of leaf diversity (Tsukaya 2010); this progress is aided by establishing new model species (e.g. *Cardamine hirsuta* and the rush; Canales *et al.* 2010; Yamaguchi and Tsukaya 2010). Over the next decade we anticipate that genomic approaches on multiple species analysed in phylogenetic frameworks (e.g. Illing *et al.* 2009) will resolve much of the enigma regarding the evolution of diverse leaf forms.

Genetic constraints on flower petal and leaf form

One additional developmental consideration is that, as famously described by von Goethe in 1790, flower petals are derivatives of the apical meristem and serial homologues to leaves (von Goethe 2009). Thus, one might hypothesise that although the evolution of leaves substantially predates that of flowers, selection on the form of either of these organs would also result in changes in the other, such that selection on leaf shape and size subsequent to the evolution of floral organs may be constrained by pleiotropic control of floral form. Recent genetic data identifies several genes that have roles in both leaf and flower form (Dinneny *et al.* 2004; Schmid *et al.* 2005; Street *et al.* 2008). For example, genetic changes in both leaf length to width ratio (e.g. *rot3*) and leaf size (e.g. *an3*) influence not only leaf proportions, but also that of the floral organs in *Arabidopsis* (Kim *et al.* 1999; Horiguchi *et al.* 2005). The garden pea (*Pisum sativum* L.) is a good case in point: as discussed above, compound leaf formation depends on *LFY/UNI* but the same gene is also required for flowering. Thus, if current selection favoured simple leaves in garden pea changes in leaf shape could be constrained by pleiotropic control of flowering (Hofer *et al.* 1997). Likewise, Shalit *et al.* (2009) recently reported that the action of florigen – a plant hormone for flowering – influences complexity of leaves by changing numbers of leaflets in tomato. Such relationships suggest that some floral characters as well as other metabolic pathways may now be closely linked with regulation of leaf shape and size. A few ecological studies have also assessed correlations between petal and leaf form (Berg 1960; Armbruster *et al.* 1999). This question is of particular interest in lineages where animal pollination is associated with increased diversification rates either as a result of direct selection, or as a secondary reinforcing factor (van der Niet *et al.* 2006; Armbruster and Muchhala 2009; Kay and Sargent 2009).

In situations where leaf and petal form are under similar genetic control and both under selection, one of two things could happen. If leaf shape does not have substantial functional impact then one might expect leaf form to change with selection on petal form. However, given the likely importance of leaf form to photosynthetic function, it is more likely that a breakdown in genetic correlations between leaf and flower form (e.g. by alterations of downstream regulatory pathways) would evolve. Several factors argue against the hypothesis that leaf shape is frequently a product of selection on flower form. First, diverse leaf shapes were in evidence long before the origin of flowers in the angiosperms. Second, the genetic mechanisms underlying leaf shape reveal considerable evolvability with similar genes being co-opted

for different functions in different species. Finally, given the potential importance of both petal and leaf shape we suggest that genetic constraints on the independent evolution of both structures are likely to have broken down in those lineages with highly diversified leaf shapes over the many millions of years since petals first evolved.

Ecological correlates and the functional significance of leaf shape

Evolutionary history plays a part in determining leaf morphology, but our consideration of leaf shape evolution and its developmental genetic basis demonstrates that diverse lineages have repeatedly gained and lost many leaf features. Thus, developmental and genetic constraints on the evolution of leaf form, and shape in particular, have been overcome many times, or have led to new evolutionary opportunities. It is of particular interest then to ask: what are the likely ecological drivers and/or functional significance of leaf shape?

Temperature and water

Conventional wisdom would say that thermoregulation is perhaps the main driver of leaf shape evolution. The maintenance of leaf temperatures within certain limits is critical for a plant's growth and survival and although photosynthetic tissue of some species can withstand temperatures below -6°C (Ball *et al.* 2006) and above 60°C (Clum 1926; Nobel 1988), irreversible damage to leaves can occur at temperatures well within this range (Levitt 1980; Jones 1992; Ball *et al.* 2004; Groom *et al.* 2004; Sharkey 2005). Leaf size and shape potentially have large effects on leaf temperature because the two-dimensional proportions of a flattened leaf determine the rate of heat transfer between across the leaf–air interface by influencing the thickness of its boundary layer.

All else being equal, the thickness of a boundary layer increases with length from the windward edge so that heat convection from small leaves is more rapid than from large leaves (Raschke 1960; Gates 1968; Vogel 1970; Parkhurst and Loucks 1972; Grace *et al.* 1980; Geller and Smith 1982; Monteith and Unsworth 1990; Schuepp 1993). Likewise, because leaf lobing reduces the distance across the lamina, the rate of heat transfer is predicted to be greater in a lobed leaf than an unlobed leaf with equivalent area (Parkhurst *et al.* 1968; Vogel 1968; Lewis 1972; Givnish 1978; Gurevitch and Schuepp 1990a).

The rate of heat transfer from lobed metal plates or lobed leaves coated in metal is greater than from shallow-lobed or unlobed ones (Parkhurst *et al.* 1968; Thom 1968; Vogel 1970; Gottschlich and Smith 1982; Gurevitch and Schuepp 1990b; Roth-Nebelsick 2001). Artificial or coated leaves have been used because they enable boundary layer resistance to be investigated in the absence of transpiration. However, this approach is biologically flawed because it does not enable an assessment of the relative importance of a range of traits (e.g. leaf water content, absorbance) in determining the actual operating temperatures of leaves in the field.

Actual leaf temperature measurements show that large leaves can operate at below-ambient temperatures even on hot days, as a result of high rates of latent heat loss through transpiration (Drake *et al.* 1970; Gates 1980; Hegazy and El Amry 1998).

Thus, conditions of evaporative demand and the availability of soil water for transpiration are key to understanding leaf shape–temperature relationships under biologically realistic conditions. Because of their much greater transpirational capacities (see above), flowering plants have far greater leeway than other plants regarding the size and shape of their leaves (see ‘A brief history of the angiosperm leaf and shape diversity therein’ section; Boyce *et al.* 2009).

In addition to reducing heat loads, the morphology of deeply lobed leaves may also reflect direct selection for increased hydraulic efficiency. Water moving through a plant encounters numerous resistances on its path from soil to roots to stems through leaves and out to the external atmosphere. Within the liquid phase of water flow through a plant, leaf hydraulic resistance, R_{leaf} , accounts for more than 30% of total plant resistance (Sack and Holbrook 2006). The high resistance in leaves is due first to a system of veins of decreasing size, the resistance of which is inversely proportional to the fourth power of the radius of the component conduits in these veins. Therefore, as a proportion of the considerable resistance in the leaf (Zwieniecki *et al.* 2002), the minor veins provide the greatest resistance to water flow (Sack *et al.* 2004). Water also encounters high resistance as it exits veins laterally and travels to other cells, mainly via apoplastic pathways through the mesophyll. Accordingly, on the order of half of total R_{leaf} can occur outside the major veins (Sack and Holbrook 2006). As resistance increases along the pathway towards sites of evaporation, localised water potential (Ψ) becomes progressively more negative (Brodribb *et al.* 2010). The result of how this phenomenon might affect a leaf has long been observed: regions of leaves lying furthest from the main supply channels are the first to ‘wither’ when exposed to strong winds (Fig. 4; Yapp 1912). Leaf lobing represents an effective removal of this potentially stress-prone tissue and has been suggested as an adaptation to dry conditions (Thoday 1931; Givnish 1979). If deeply lobed leaves have a lower ratio of mesophyll tissue to large, highly conductive veins, they should then have reduced resistance relative to less- or un-lobed leaves (Sack and Tyree 2005).

Although leaf lobing might confer adaptive benefits with respect to maintaining stable hydraulic supply, it may also be that the extent of lobing or margin dissection is itself determined by hydraulic limitation (Sack *et al.* 2003; Zwieniecki *et al.* 2004b; Boyce 2009). For species with ‘sun’ and ‘shade’ leaves, the morphology of small, deeply lobed sun leaves at the outer canopy is proposed to result from water pressure drop across the leaf lamina caused by reduced water delivery to expanding cells during growth (Fig. 5; Zwieniecki *et al.* 2004b; Boyce 2009; Leigh *et al.* 2011). Alternatively, recent studies have suggested that many of the characteristics of ‘sun’ and ‘shade’ leaves are the result of branch autonomy within the plant canopy (Niklas and Cobb 2010).

Thus, although the effects of leaf shape on leaf thermal regulation hold when tested on model leaves, predictions are less straightforward when hydraulic function is accounted for. Additionally, consideration of the relative contribution of a range of leaf and branch properties to leaf temperature is critical. It is likely that leaf shape is only one among many factors influencing leaf thermal regulation; other factors could include water

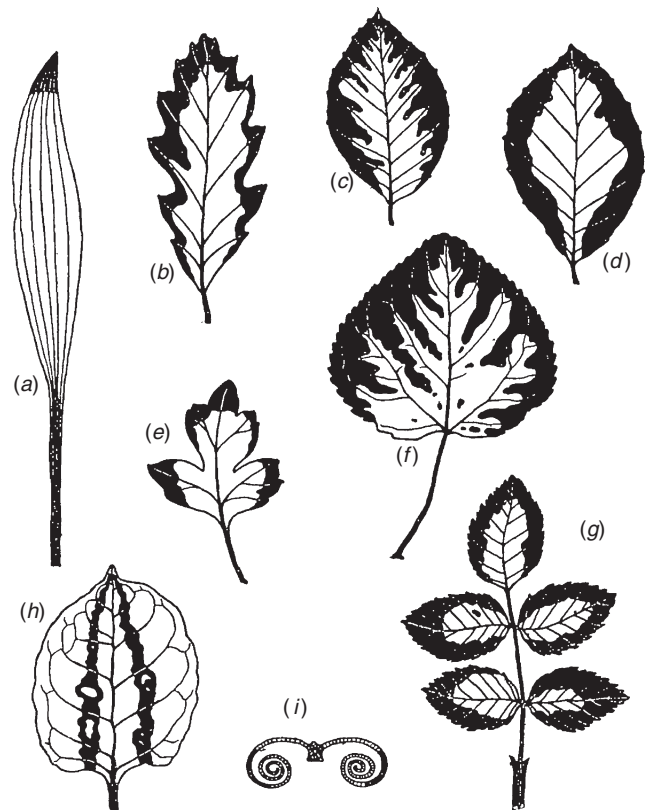


Fig. 4. Localised ‘withering’ of leaves exposed to strong wind. Reprinted from Yapp (1912) with permission from Oxford University Press.

content, leaf thickness, spectral reflectance, orientation and plant architecture. Further, given the observation of high leaf shape diversity within many plant communities from hot and arid environments, it seems likely that regulating leaf temperature is not the single most important evolutionary influence on leaf shape diversity. The association between leaf shape and hydraulic properties, which is affected by leaf temperature, in contrast, is likely to be of greater importance.

Leaf structure and functional trade-offs

Many foliar leaf traits, including shape, reflect functional trade-offs that have been resolved in various ways by different species depending on their ecological settings (e.g. hydro- vs mesophytes), physiological attributes (e.g. C_3 vs C_4 metabolism), developmental capabilities (e.g. heterobaric vs non-heterobaric leaves), or evolutionary histories (e.g. micro- vs megaphylls). Life history and optimisation theory show that the number of phenotypic solutions that allow for different equally successful trait combinations increases as the number of trade-offs increases – a conclusion that applies to traits within the leaf (e.g. for shape) as well as to leaf–branch relationships (Niklas 1988).

It is evident that leaves perform several functions simultaneously; that is, they intercept light, transport liquids and solutes, exchange gases with the atmosphere, dissipate heat, cope with externally applied mechanical forces, and

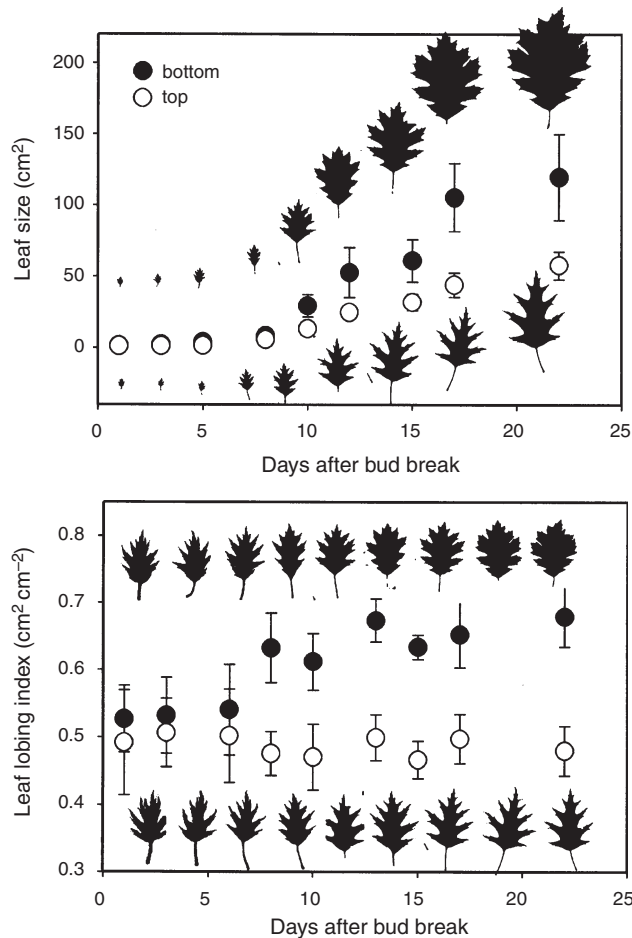


Fig. 5. Variation in (a) leaf size and (b) shape complexity in *Quercus rubra* leaves growing at the top and bottom of the tree crown. Reprinted from Zwieniecki *et al.* (2004b) with permission from John Wiley & Sons.

defend themselves from herbivores and pathogens. Importantly, many of these functions have conflicting physiological, anatomical, or morphological requirements. For example, orientations of lamina that maximise light interception also minimise the ability to dissipate heat. Further, all of these functions are intrinsically size dependent. For example, the quantity of mechanical tissues required to support laminae depends on the mass of laminae, whereas the ability to intercept light depends on laminae area.

When seen in this light, the trade-offs required for the successful functionality of foliar leaves generate rather than constrain ecological and evolutionary opportunities. This can be illustrated mathematically and empirically in the context of phyllotaxy and its consequences on light interception. Computer simulations of mathematically generated shoots to assess the influence of leaf shape, size, and phyllotactic patterns on the ability to intercept direct solar radiation show that differences in phyllotaxy significantly influence light interception (particularly for shoots with a rosette growth habit). They also show that comparatively small differences in leaf shape can compensate for the negative effects of leaf overlap resulting from virtually any phyllotactic pattern. For example, lobed leaves or pinnatifid

compound leaves facilitate light penetration through shoots bearing densely packed leaves. Thus, phyllotaxy can be viewed as a developmental limiting factor that can drive compensatory changes in morphological features such as shape, that are not directly controlled by patterns of leaf initiation. In this way, the ‘constraints’ imposed by phyllotaxy can foster evolutionary modifications at the level of the entire shoot (Niklas 1988).

One particularly important trade-off operating at the level of the architecture of a shoot is that between leaf size and number. For any given leaf biomass allocation, an individual shoot can either have a few large leaves or many smaller ones. Across different herbaceous species and juvenile specimens of tree species drawn from diverse lineages and ecological settings, the relationship between total leaf biomass per plant (M_L) and total stem mass per plant (M_S) falls within a comparatively narrow corridor of variation that obeys a positive isometric scaling relationship (i.e. $M_L \propto M_S^{-1.0}$; Niklas 2004). Accordingly, across these species, any stem mass investment yields a more or less proportional increase in leaf mass. Therefore, it follows mathematically that across these species, the relationship among the average mass of a typical leaf m_L , total leaf number N_L , and total stem mass complies with the proportionality $m_L N_L \propto M_S^{-1.0}$. When individual shoots are sampled across diverse species, the relationship between m_L and N_L per shoot is negative and isometric (i.e. $m_L \propto N_L^{-1}$; Kleiman and Aarssen 2007), a relationship that appears to be insensitive to how species are grouped according to other functional traits or according to their habitat preference (Yang *et al.* 2008). That this phenomenology reflects a biomass allocation trade-off is clear. What remains uncertain is whether it is a simple trade-off between leaf mass investment and leaf size, or a more complex set of relationships imposed by twig mass investments and hydraulics or biomechanics.

Another trade-off that appears to hold across many but not all species is the relationship between leaf surface area A and average leaf mass m_L . For more than a few species-groupings, this relationship is allometric with a slope that is less than one, which indicates that increases in leaf mass investment do not result in proportional increases in leaf area (i.e. $A \propto m_L^{<1.0}$; Niklas *et al.* 2007). This trend of ‘diminishing returns’ has also been reported when total leaf area per plant is plotted against total leaf mass for plants differing in size (Niklas and Cobb 2008, 2010). Several factors may contribute to these trends, e.g. biomechanical analyses show that disproportionately larger investments must be made to mechanically support photosynthetic tissues as leaves increase in their surface area (Niklas *et al.* 2009). In both the leaf mass to stem mass and leaf mass to area relationships described above, leaf shape variation can provide an extra degree of freedom for selection to act on at both the leaf and branch level.

Case studies of leaf shape variation at different scales

Above, we have discussed evolutionary and proximal drivers of leaf shape, as well as ecological correlates and functional significance thereof. Here, we present case studies at three scales where we can examine the ecological and evolutionary implications of leaf shape. These are the cross community scale case of leaf teeth as a subset of shape modifications, within lineage

considerations of the genus *Pelargonium* and the family Proteaceae, and finally the case of leaf shape variation within a single genetic individual: heteroblasty and heterophylly.

Leaf teeth: leaf shape variation at the community scale

Leaf teeth are conspicuous features in many plants and are notable as a leaf-shape trait that varies in predictable ways across plant communities. Nearly 100 years ago, Bailey and Sinnott (1915, 1916) observed that plant communities from regions with low mean annual temperature (MAT) have a higher site mean proportion of non-monocotyledonous species with toothed leaves. Paleobotanists quickly seized upon this relationship as an index for inferring paleotemperature. Little *et al.* (2010) recently documented the rich interest in this topic, compiling 351 literature references related to leaf physiognomy and temperature, of which the primary focus is leaf teeth. The link between teeth and temperature is observed on all vegetated continents (Greenwood 2005a; Peppe Royer *et al.* 2011) and is also seen in related metrics such as tooth size and tooth number (Huff *et al.* 2003; Royer *et al.* 2005; Peppe *et al.* 2011). Further, these tooth variables can display phenotypic plasticity to temperature change (Fig. 6; Royer *et al.* 2009b).

What is known about the functional bases of these leaf–climate relationships? Leaf teeth commonly expand and mature faster than the bulk leaf (e.g. Billings 1905; Feild *et al.* 2005) and young teeth are disproportionately vigorous in their gas exchange (Baker-Brosh and Peet 1997; Royer and Wilf 2006). From these observations, Royer and Wilf (2006) hypothesised that one role of teeth is to increase sap flow, thereby delivering nutrients and other solutes to young, emerging leaves. In habitats with low temperatures, teeth could play an important role in maximising the potential for carbon gain and growth early in the season. In warmer climates, with longer growing seasons, the carbon benefit conferred by teeth would be outweighed by their unavoidable water cost. Alternatively, Feild *et al.* (2005) hypothesised that teeth help remove freeze–thaw embolisms through positive root pressure and guttation. Importantly, the transport of fluids is common to both hypotheses. From this, it may be surmised that water-limiting conditions select against teeth (Bailey and Sinnott 1916). Indeed, this pattern is clearly seen across local water availability gradients (Burnham *et al.* 2001;

Kowalski and Dilcher 2003; Greenwood 2005b; Royer *et al.* 2009a; Peppe *et al.* 2011) but not globally against mean annual precipitation (e.g. Peppe *et al.* 2011).

If the hypothesis by Royer and Wilf (2006) is correct, toothed species should have a higher photosynthetic rate, at least at the beginning of the growing season. As such, teeth may be related to the leaf economics spectrum (Wright *et al.* 2004). Some studies find support for this idea: leaves with a short leaf lifespan (deciduous) (Peppe *et al.* 2011) and low leaf mass per area (Royer *et al.* 2005), two leaf economic variables, are more likely to be toothed. It also follows that teeth should be more functionally related to growing season climatic variables like seasonality, growing season length, and growing degree days, not mean annual temperature, however, few studies find support for this idea (Royer *et al.* 2005; Peppe *et al.* 2011). Ultimately, it would be valuable to have a better understanding of the genetic processes underlying tooth development, but presently little is known outside model species (see ‘Genetic regulation for patterning of leaf margin: serration and leaflet formation’ section). Further, phylogenetic history has typically been assumed to have only a minor role in these leaf–climate patterns, but rigorous tests demonstrate that this assumption is false (Little *et al.* 2010). This outcome is of particular concern for paleobotanists because systematic placement of fossils can be difficult, especially for the Cretaceous and Paleogene. Tools to accommodate for this phylogenetic dependency are needed.

Leaf shape variation within lineages

Proteaceae

Proteaceae is an ancient angiosperm family originating in Gondwana over 100 million year ago and possessing 1700 extant species (Weston 2007). The family’s greatest representation is in Australia, followed by South Africa, with smaller numbers across other Gondwanan segments (Cowling and Lamont 1998; Hoot and Douglas 1998; Weston 2007). Within the Proteaceae, there exists an enormous range of leaf sizes and shapes, ranging from a few mm² to 500 cm² and including entire, toothed, broadly lobed, deeply dissected, needle-like, simple or compound leaves. Intriguingly, most of this shape diversity is represented in Australia. The majority of taxa outside Australia possess entire leaves; the exception being four South African genera

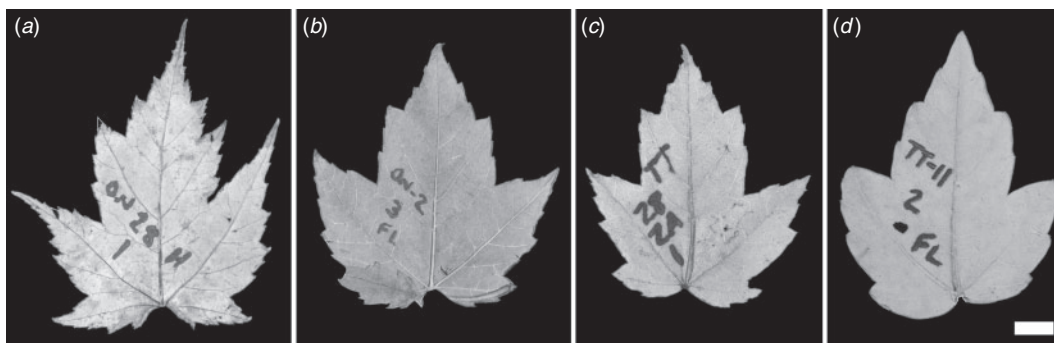


Fig. 6. Representative leaves of *Acer rubrum* showing genetic variation and plasticity in leaf teeth. Leaf derived from cool climate (Ontario) seed stock grown in (a) Rhode Island (cool climate) and (b) Florida (warm climate); leaf derived from warm climate (Florida) seed stock grown in (c) Rhode Island and (d) Florida. Bar (for all leaves) = 1 cm. Reprinted from Royer *et al.* (2009) under Creative Commons Attribution Licence.

that possess dissected (*Serruria* and *Paranomis*) or toothed (*Mimites* and *Leucospermum*) leafed species, all of which occur in a single clade (Sauquet *et al.* 2009).

The reason for the extensive radiation in leaf shape diversity in Australian Proteaceae is unclear. Among South African Proteaceae, leaf size decreases with mean annual temperature and narrow leaves have been shown to overheat less than their wider counterparts (Yates *et al.* 2010). Given that leaf dissection and small size are functionally equivalent with respect to heat loss (see 'Temperature and water' section), it is tempting to suggest that leaf shape variation in Australia represents an adaptive response to increasing hot, dry conditions. Yet, there appears to be no consistent distributional pattern of Proteaceae leaf dimensions in Australia. Many medium or large, un-dissected leaves exist in the hottest habitats; *Grevilleas* with small, simple leaves can be found across most regions including high rainfall areas; and some of the species found in the shaded rainforest understorey have among the most deeply dissected leaves of all. Leaf shape also varies within a single individual. For example, fern-leaf stenocarpus *Stenocarpus davallioides* Foreman & B.Hyland has tripinnatisect juvenile leaves but adult leaves can be pinnate or bipinnate (Foreman and Hyland 1995). Other rainforest species, such as Atherton Oak (*Athertonia diversifolia* (C.T.Whit) L.A.S. Johnson & B.G.Briggs), have very large, lobed intermediate leaves but adult and seedling leaves are small and entire (Weston 1995). Alternatively, leaf lobing within a plant can be random, having no obvious spatial or developmental association, or homogenous across a species. Examining a phylogeny of extant taxa, one finds lobed or dissected leaves alongside entire-leafed species – across most lineages in the group (Weston 2007). Therefore, although suggestions of microclimatic adaptation may be offered at the species level, it seems unlikely that an environmental association with leaf shape could be generalised across Australian Proteaceae. Rather, leaf shape variation in the Proteaceae is likely to reflect the range of ecological solutions to leaf structure/function trade-offs discussed above.

Pelargonium

Similar to the Proteaceae, the genus *Pelargonium* is distributed predominantly in the southern hemisphere, with nearly 80% of its almost 300 species found in South Africa. Although the age of the genus is estimated to be around 30 million years (Bakker *et al.* 2005), the highest rate of species accumulation has occurred in the last 10 million years (Martinez-Cabrera 2010). The combination of climatic and edaphic heterogeneity over short distances and limited gene flow (Latimer *et al.* 2005; Linder 2005) is frequently offered as an explanation for the extraordinary plant species diversity that characterises southern Africa. *Pelargonium*, the third largest genus in the region, is characterised by remarkable variation in growth form that ranges from annuals and geophytes to shrubs. Variation in leaf shape is no less dramatic, ranging from entire to highly dissected (Fig. 1), often within growth forms and taxonomic sections. Parsing of leaf form categorically revealed that the extent of dissection of the blade is highly evolvable within an overall ovate leaf shape outline that is evolutionarily conserved (Jones *et al.* 2009).

In addition to high evolutionary lability of leaf dissection, there is variation among major clades in the evolutionary pattern of major veins. One major clade (Clade C) is dominated by palmate venation whereas another (Clade A) is dominated by pinnate venation, although both types are found in both clades. We note that pinnate venation is significantly associated with increased dissection and reductions in functional leaf size characteristic of the highly diverse 'xerophytic' clade (Clade A2) of the winter rainfall region.

To examine whether leaf shape translates into functional variation, carbon gain and water use efficiency were measured in pairs of species contrasted for leaf shape and replicated across three subclades of Clade A and one subclade of the third major clade, Clade B (Nicotra *et al.* 2008). In each case, the species with the more dissected leaves showed higher rates of carbon gain, but also higher rates of water loss in all treatment conditions. That paper argued that the differences in carbon gain were not direct results of leaf shape *per se*, but rather that the same conditions that result in selection for more dissected leaves also favour the evolution of high photosynthetic rates, high leaf nitrogen content and opportunistic use of water when available. Even in this study, however, there was no correlation between leaf dissection and the climate present in native ranges, suggesting either that climate is selective at a much smaller scale (i.e. the microhabitat), or that for any given set of climate variables, multiple leaf shapes can be adaptive in association with variation in other leaf traits.

Heteroblasty and heterophylly: variation within a single genetic individual

We have so far focussed primarily on cross species variation in leaf shape. As discussed for the Proteaceae genera above ('The Proteaceae' section), however, a single genome can produce a remarkable range of leaf shapes during the lifetime of a plant. There are two broad categories of leaf shape variation that can be expressed by a single individual. This variation can arise either in response to different external environments or to ontogenetic signals (some of which may be influenced by environment as well).

Environmentally induced heterophylly

Environmentally induced heterophylly results when leaf shape responds to environmental cues. The classic case is the dramatically different emergent and submergent leaves in aquatic plants. Although many aquatic plants are also heteroblastic (see below, Sculthorpe 1967), environmentally induced heterophylly is not specific to position or plant age and is reversible, depending largely on the environmental signals (mediated by hormonal signals) perceived by cells of the developing leaf primordium. The developmental age of the leaf at the time of signal perception is critical. Very young primordia (L1–L3) generally respond completely to the novel environment whereas primordia that were slightly older at the time of environmental change show intermediate features as mature leaves. Characteristics of specific cells are related to the position of that cell within the differentiation gradient characteristic of that species at the time of environmental change (e.g. Goliber and Feldman 1990; Bruni *et al.* 1996; Kuwabara and Nagata 2006).

Among terrestrial plants, the 'sun-shade' leaf response is frequently cited as the classic example of environmentally induced heterophylly. Historically, differences in leaf form within a canopy have been assumed to be responses to altered light environments (e.g. larger and less-lobed leaves are produced in shade). However, these different leaf shapes also confer distinct hydraulic advantages (see 'Temperature and water' section). Whether in response to light or to hydraulic limitation, 'sun/shade' heterophylly is accompanied by similar and well-known anatomical differences across a wide range of species (i.e. sun leaves are generally smaller and thicker with smaller cells than shade leaves; Taiz and Zeiger 2002). However, when these anatomical differences become determined developmentally (Dengler 1980; Yano and Terashima 2004) has been less commonly studied. The presence of intermediate anatomical characteristics in leaves transferred among light environments shortly after expansion ceased suggests that leaves of some species have the capacity to respond anatomically to altered light levels until very late in development (Oguchi *et al.* 2005). In contrast, recent studies suggest that systemic light and humidity signals sent from older to younger leaves allow younger leaves to develop phenotypes suited for their anticipated environments (Lake *et al.* 2001; Yano and Terashima 2001; Thomas *et al.* 2004). Exactly when final leaf shape is determined in 'sun/shade' leaves remains an open question for most species.

Heteroblasty describes ontogenetically dependent changes in leaf features, most notably shape

In 1900, Goebel originally distinguished differences in 'juvenile form' from 'adult form' on the basis of dramatic differences in leaf shape and associated traits (Balfour 1969). As an example, he

described the shift from compound leaves to phyllodes in *Acacia* as heteroblastic and reserved the term homoblastic for the less dramatic variation exhibited by *Casurina*. It is now generally accepted that heteroblasty refers to the full suite of features that change along the shoot during development (Jones 1999).

Heteroblasty results from the interaction of developmental programs expressed at both the leaf and the shoot- or whole-plant level (Kerstetter and Poethig 1998; Tsukaya *et al.* 2000; Usami *et al.* 2009; Wu *et al.* 2009; Chuck and O'Connor 2010). Heteroblasty is generally not reversible unless reiterative growth occurs (i.e. following damage). Morphological differences characteristic of heteroblasty such as differences in lobing are usually expressed relatively early in development, shortly after leaf initiation (Jones 1995).

The sequence of heteroblastic leaf shapes produced along the shoot can be accelerated or delayed in response to different growth environments or architectural manipulations (Ashby 1948; Forster and Bonser 2009; Jaya *et al.* 2010). Furthermore, both heteroblasty and plasticity in heteroblasty have been shown to be under strong genetic control (Anderson 1989; Climent *et al.* 2006). Forster *et al.* (2011) suggested that both heteroblasty and plasticity in heteroblasty are likely to occur in response to 'which leaf type confers the greatest benefit for a given light level.' Several studies over the last decade have pointed towards functional consequences of heteroblasty, generally with reference to single environmental variables (e.g. Hansen 1986; Gamage and Jesson 2007; Kubien *et al.* 2007). It is likely that heteroblasty influences multiple leaf functions because suites of leaf traits change with heteroblasty (Jones 2001). Indeed, this conclusion was supported in a recent study that examined differences in more than a hundred leaf traits in *koa* (*Acacia koa* A.Gray; Pasquet-Kok *et al.* 2010). Heteroblasty has also been proposed as an adaptation to herbivory (Karban and

Table 1. Promising directions for cross-disciplinary investigation of leaf shape

No.	Direction
1	A multi-species 'evo-devo' approach similar to that by Illing <i>et al.</i> (2009) is likely to be informative with regard to many enigmatic features regarding the evolution of diverse leaf forms and more generally provides an exciting system for examining the convergence and divergence in the evolution of function
2	A phylogenetic analysis of leaf shape examining correlated evolution with other traits such as venation, size, leaf surface characteristics and branch and architectural measures would enable a thorough investigation of the situations in which leaf shape diversification arises
3	Further research into both the genetic and developmental processes underlying lobes and teeth, and the ecological correlates of each would provide an excellent opportunity to link across wide environmental scales
4	Cross species studies, ideally in the field, of the relative importance of leaf shape among other factors in determining leaf temperature and water status are necessary to test the conclusion put forward here: that the functional significance of leaf shape lies more often in water relations than directly in leaf thermoregulation
5	Studies of the scale of environmental change that selects for ontogenetic response in leaf shape in heteroblastic species from diverse environments will shed light on links between genes, development and ecology
6	Detailed quantifications of the extent to which leaf shape differs within canopies, particularly of dioecious species where selection on reproductive function can lead to dimorphic conditions, will help link leaf shape to whole plant processes
7	Studies at the scale of transects across the leaf surface that simultaneously consider hydraulic resistance, temperature and light will reveal the extent of localised hydraulic and thermal stresses within leaf surfaces that might act as selective forces on leaf shape <i>per se</i> . Imaging approaches incorporating measures of 3-dimensional shape, temperature and fluorescence provide exciting opportunities for examining spatial heterogeneity in temperature and physiological processes
8	Since patterns of leaf venation affect both hydraulic supply and capacity for photosynthate transport, studies that simultaneously examine both will reveal the extent to which interactions among these fundamental processes either co-determine leaf shape or establish trade-offs ultimately reflected in leaf shape
9	Finally, studies examining the fitness values of different leaf shapes, or plasticity in leaf shapes, under a range of environmental conditions are needed to better identify which aspects of leaf shape are most likely to be adaptive

Thaler 1999; Brennan *et al.* 2001; Fadzly *et al.* 2009) and it has been associated with differences in frost resistance (Darrow *et al.* 2001). It is worth noting that studies of the functional significance of heteroblasty have focussed on relatively few plant lineages. Given that heteroblasty occurs widely among the angiosperms, as well as in some ferns and gymnosperms (e.g. Wagner 1952; Mueller 1982; Pryer and Hearn 2009), the next questions may focus on the cost of heteroblasty and its fitness consequences: what scale of environmental change selects for ontogenetic response in leaf shape?

Synthesis and next steps

Whether considered at community, lineage or single genome scales, leaf shape is a trait that is highly labile and responsive to a range of biotic and abiotic factors. Although some leaf traits can be explained in terms of particularly strong trade-offs between two or more factors that result in clear scaling relationships (e.g. leaf mass-to-area traits or leaf mass-to-stem mass), leaf shape, in contrast, does not emerge from the above consideration as a factor capable of explaining ecological differentiation between species. Rather, leaf shape emerges as a trait for which there are many quite varied functional trade-offs.

As stated above, these trade-offs for successful functionality of leaves may generate ecological opportunities; leaf shape is perhaps best viewed not as a single major axis, but rather as an option that fine tunes the leaf to its conditions over both short and evolutionary time spans. Numerous elements of this review support this conclusion. Over evolutionary history the extant range of variation in leaf shape has been explored by many lineages, although only the angiosperms have explored this range with the added element of massive increases in vein densities. Likewise, our growing understanding of genetic controls on leaf shape reveal that it is a complex trait determined by many different genes at several different developmental stages. Although some genes have conserved effects on leaf morphology, including shape, the exact nature of their effects can vary widely. Likewise, the function to which a given element of leaf shape 'is put' can vary. For example, closer investigation of the internal architecture and developmental pathways associated with different leaf shapes suggests that leaf teeth and leaf lobes often have distinctly different functional foundations, and may play different functions in different environments.

Despite a myriad of functional roles, the one recurrent theme in our review is that leaf shape is affected by and strongly influences leaf water relations. From a functional perspective, the role of leaf shape in leaf thermoregulation has perhaps received the most attention in previous considerations of the functional significance of leaf shape. The relationship between leaf size and temperature being physically determined by basic energy balance principles, this was a logical starting point of enquiry. But, as we have shown, the connection between leaf shape (and size) and leaf temperatures under field conditions is not well supported empirically. Rather, leaf shape variation, as distinct from size, more often will reflect water supply trade-offs. High vein densities support high photosynthetic rates (Brodribb *et al.* 2010) and dissected leaves maintain a greater proportion of leaf tissue closer to main veins. Leaf temperature plays a role

in this story, as higher leaf temperatures lead to greater evaporative water loss, but many factors other than leaf shape influence leaf temperature (e.g. leaf thickness, orientation and reflectivity and branch and plant architecture). Finally, it is also worth noting that although there appears to be an obvious link between shape and water supply, there is a concomitant interaction between water supply and phloem function that remains relatively unexplored.

Thus, our review suggests several angles for further research (Table 1). In particular we advocate those approaches that enable investigation across the scales examined here (gene, development, function and evolutionary ecology) and that focus in particular on the links among leaf shape, development and water relations.

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