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Why is Leaf Shape so Variable?

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ABSTRACT

The genetic pathways which control leaf shape have been revealed through work in a range of model systems. We are now beginning to understand how plants produce leaves of different shapes. However, *why* leaves have different shapes is not so well-studied. Leaf shape is extremely variable between species. Shape also varies within species, within populations and as a plastic or developmentally programmed response, within individual plants, suggesting there is an adaptive role for leaf shape. Theoretical studies and modelling have suggested several roles that leaf shape could play due to its effects on affect light capture, water balance and temperature regulation. Clear trends in leaf shape variation are seen along environmental clines but the few studies that have been done on the adaptive role of leaf shape have produced equivocal results. Selection on leaf shape is weak and variable though there is some support for adaptive effects of leaf dissection. Other important factors could be microclimate, correlations with phyllotaxy or vascular patterning, and biotic interactions. Cooperation between ecologists, physiologists, anatomists, geneticists is required to determine the interactions between these factors. This will be aided by the application of large-scale sequencing, and techniques such as PCA and QTL to dissect the genetics involved.

Keywords: adaptation, evolution, genetics, leaf development, leaf shape, phyllotaxy

Abbreviations: **A_{max}**, maximum photosynthetic activity; ***ARP***, *AS1*, *RS2*, *phan*-like transcription factor; **K_{leaf}**, resistance of leaf to water flow; ***KNOX***, *Knotted*-like transcription factor; **N_{mass}**, nitrogen per g of leaf; **P_{mass}**, phosphorus per g of leaf; **R_{mass}**, dark respiration rate per g of leaf; **SAM**, shoot apical meristem; **SLA**, specific leaf area: ***UNI***, *UNIFOLIATA* transcription factor

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INTRODUCTION

Leaves expose a surface to the environment to generate energy. This surface must harvest light and CO_2 with minimal loss of water. The leaves of seed plants have evolved to do this successfully in the full range of climates from shaded, wet tropical forest understorey to exposed arctic tundras. In some extreme environments most plants have similar leaves – for example the tiny, robust leaves of many alpine plants, but in most habitats a range of leaf shapes is seen. **Fig. 1** shows the variation in leaf form seen in a 0.5 m^2 patch of recently disturbed ground. In this open and relatively uniform environment species with a huge range of leaf forms are thriving.

Variation in leaf shape is due to variation in the outgrowth of the leaf lamina. The leaf arises as a determinate group of cells on the flanks of the indeterminately dividing shoot apical meristem (SAM). The leaf initially grows out as a rounded peg. Within this peg the expression patterns of transcription factors define regions where lamina outgrowth (and/or lateral recruitment of cells from the meristem flanks) will occur. In most monocots additional cells are

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Invited Review

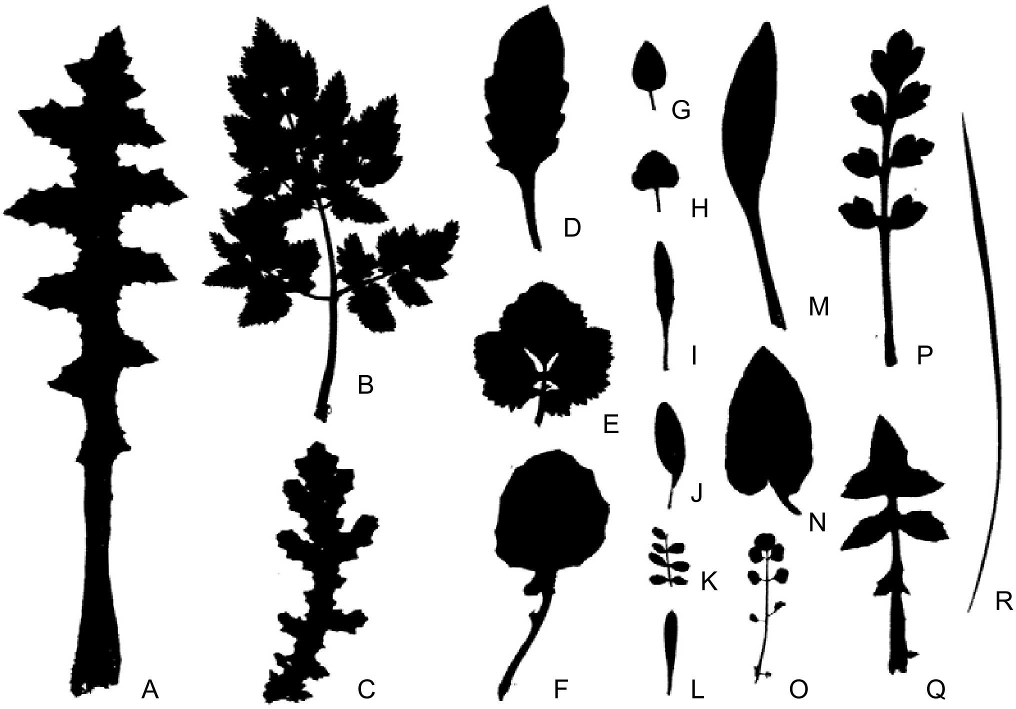

Fig. 1 The variation in leaf shape seen in 50 cm^2 of open disturbed ground. (**A**) *Sonchus oleraceus*, (**B**) *Anthriscus slyvestris*, (**C**) *Senecio vulgaris*, (**D**) *Arabidopsis thaliana*, (**E**) *Geum urbanum*, (**F**) *Lapsana communis*, (**G**) *Anagallis arvensis*, (**H**) *Veronica filiformis*, (**I**) *Leontodon hispidus*, (**J**) *Myosotis arvensis*, (**K**) *Vicia sativa*, (**L**) *Galium aparine*, (**M**) *Silene latifolia*, (**N**) *Rumex obtusifolius*, (**O**) *Cardamine hirsuta*, (**P**) *Papaver rhoeas*, (**Q**) *Taraxacum officinalis*, (**R**) *Poa annua*. Location of survey - North Berwick, Eastern Scotland (56°04'N02°42'W).

recruited to the primordium from the flanks of the meristem, forming a leaf which wraps around the stem. In most other angiosperms lamina outgrowth does not occur in the very proximal regions of the leaf primordia, resulting in a petiole (Steeves and Sussex 1989).

Lamina outgrowth can be more or less equal along the leaf creating a strap-like or obovate leaf (**Fig. 1D, 1I, 1R**); greater in distal regions, or proximal regions, creating a spatulate (**Fig. 1L**) or deltoid leaf (**Fig. 1H**), respectively. If lamina outgrowth is greater in discrete regions along the leaf, lobes are created (**Fig 1A, 1C, 1P, 1Q**). Limitation of outgrowth to discrete regions results in compound leaves made up of individual leaflets with petiole-like rachis between them and individual petiolules supporting each leaflet (**Fig. 1E, 1O**) or multiply compound leaves with primary leaflets divided into secondary leaflets separated by rachilla (**Fig. 1B**). Compound leaves can be pinnate (leaflets arranged in rows along the leaf) (**Fig. 1E, 1O**) or palmate, leaflets radiating from a single point (Horse chestnut, *Aesculus hippocastranum*) Outgrowth of the lamina all around the primordia results in a peltate leaf like that of nasturtium (*Tropaeolum majus*). In some species the lamina may be modified later in development by programmed cell death creating holes or leaf dissection (palms (Jouannic *et al.* 2007), the 'Swiss cheeese plant' *Monstera obliqua* (Gunawardena *et al.* 2005). The terminology of leaf form is rich due to its extensive use in systematics and paleobotany (Ellis *et al.* 2009).

Most of the leaf shape variation in any one environment is due to differences between species, but some comes from variation within species and even within plants. Leaves of any one species usually vary somewhat in size, shape and anatomy. Common garden experiments show that much of this variation is a plastic response to the environment termed heterophylly (Winn 1999). An extreme heterophylly is seen in the aquatic water crowfoots (*Ranunculus* subenus Batrachium) which make entire leaves in the air (**Fig. 2A**) and highly divided leaves in the water (**Fig. 2B**). Mulberry (*Morus pendula*) makes leaves with deeper lobes in the sun (**Fig. 2D**) than in the shade (**Fig. 2C**). Leaf shape also varies with the age of the plant (heteroblasty). Many plants show subtle differences in their leaf shape as they age (*Arabidopsis*), but some shape changes are much stronger: (*Hedra helix*) (Rogle and Hackett 1975), *Acacia implexa* (Forster and Bonner 2008) and *Eucalyptus globulus* (James and Bell 2001). Genetically programmed variation in shape in different circumstances implies that leaf shape has a function but what that function is, is not clear.

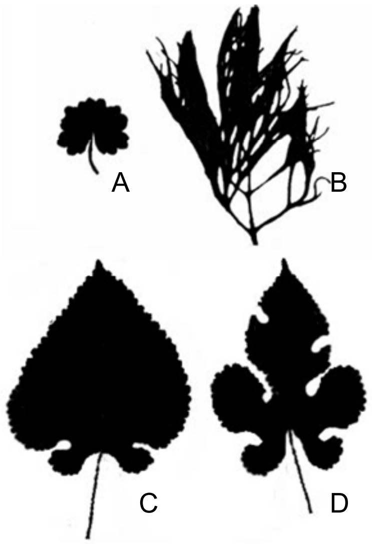

Fig. 2 Plasticity of leaf form in different environments – heterophylly. (**A**) *Ranunculus aquatilis* arial leaf, (**B**) *Ranunculus aquatilis* submerged leaf, (**C**) *Morus pendula* shade leaf, (**D**) *Morus pendula* sun leaf.

The function of leaves is to photosynthesize. To maximise photosynthesis the leaf must be exposed to the sun, but it must not overheat or receive too much light which could cause photoinhibition. The surface exposed to the sun must be well supplied with water for physiological processes, for cooling, and for support, but the leaf must be thin and permeable enough to allow CO_2 to freely diffuse in and reach the photosynthetic tissues of the leaf. The leaf must also be robust enough to function in a variable and hostile environment. The many factors that affect leaf function suggest that many adaptive peaks for leaf shape could exist in any one habitat (Vogel 2009). In this review we will summarize the constraints some of these factors impose on leaf shape and describe what is known about the genetic changes behind variation in leaf shape between and within species. We will discuss experiments which have measured the strength of selection on leaf shape and consider whether leaf shape is adaptive.

LEAF ECONOMICS

Analysis of very large databases has been used to look for patterns and correlations in leaf functional traits. Glopnet (global plant traits network) used data from 2,548 species at 175 sites covering arctic tundra, tropical rainforest, hot and cold deserts, boreal forests and grasslands (Wright *et al.* 2004). They measured specific leaf area (SLA), maximum photosynthetic assimilation rate (A_{max}), leaf nitrogen (N_{mass}), leaf phosphorous (P_{mass}), dark respiration rate (R_{mass}) and leaf lifespan. The results they describe show how leaves can adopt a range of strategies to adapt to an environment.

These traits are, of course interrelated. In the huge number of species and sites examined PCA revealed that a single Principle Component explained 74% of the total variance in these six traits. They found large scale relationships between high A_{max} and high N_{mass} (the more protein investment in the leaf, the higher the photosynthetic activity), long leaf lifespans and high leaf mass per area (long-lived leaves are more robust), and high A_{max} and short life spans (leaves with a high ratio of surface area: mass and rich in photosynthetic enzymes are vulnerable. They also tend to drive the production of more leaves and eventually shade themselves). The solutions to trade-offs between the different environmental constraints appear to limit leaf form resulting in a spectrum of viable leaf forms. The spectrum runs from high activity, high nutrients, short lifetime, low dry mass investment (fast and cheap), to low activity, low nutrients, high dry mass investment (expensive and long lived), though there is considerable variation around this trend. Some types of leaves are never seen, such as expensive but short lived (high SLA, N_{mass}, and P_{mass}, low life span), or cheap but with high activity (low SLA, N_{mass}, and P_{mass} with high A_{max} and R_{mass}).

Surprisingly the spectrum was only weakly correlated to climate, though the relationship was significant. The weak correlation was due to the very high variation in traits within sites. Only an empty environment is uniform in the opportunities it offers to plants, and once one plant is established it creates new niches (such as shaded, sheltered and drier). Most of the variation in leaf function is probably attributable to variation within an environment as plants adapt different strategies in the battle for light, water and nutrients. This very fine-scale variation is where we should look for the adaptive value of leaf shape.

LIGHT

Sun and shade leaves

Light is a key resource for plants, they vary in their tolerance of shade and in their ability to respond to it. The differences between leaves produced by plants growing in the shade and those growing in the sun has been noted many years ago (Hanson 1917). Shade leaves tend to be larger, thinner and less lobed than sun leaves (**Fig. 2C, 2D**). However, there are differences in the shade leaves produced by plants that are tolerant or intolerant of shade. Shade leaves of shade tolerant plants tend to be smaller, and thicker than the shade leaves of plants intolerant of shade (Reich *et al.* 2003). Leaves produced by plants that live entirely in the shade tend to have low leaf mass per area, be long lived, are often less tolerant of damage by photo inhibition but better defended against herbivores (Coley 1988; Lovelock *et al.* 1994; Veneklaas and Poorter 1998; Poorter and Rozendaal 2008).

The form of leaf adopted as a solution to a particular environment can depend whether the plant is aiming to outgrow and out compete neighbours or flourish in their shade. Some plants have a higher demand for light and even in the sun have large leaves which function to shade out competitors as well as to gather light (Coley 1988; Lovelock *et al.* 1994; Veneklaas and Poorter 1998; Poorter and Rozendaal 2008). Leaves of plants which are pioneer species are often very large, giving them the energy to grow quickly upwards. Once in the full sunlight they begin to produce smaller leaves (Reich *et al.* 2003).

Tropical trees tend to have leaves that suit the requirements of their juvenile forms, often a shady understorey, rather than the niche in which they spend most of their lives as adult plants (Poorter 2007). This may explain some of the variation seen between leaf forms of plants in the same adult niche, and presumably is due to the stronger selection on leaf form at the juvenile stage as young plants struggle to overtop each other. In general, understorey herbs are more variable than trees in leaf form and physiology in both tropics and temperate forests. This could be due to microclimatic effects, as herbs live on a finer scale than trees. Alternatively the moist, light-limited environments could provide few stresses for the plants and allow leaf form to vary neutrally (Parkhurst and Loucks 1972).

Foraging for light

Leaf size and shape are closely related to phyllotaxy (the arrangement of leaves along the stem) and petiole length as these are key factors in the generation of the leaf-mosaic and, together with internode length, determine how the plant forages for light (Bragg and Westoby 2002; Niinemets and Sack 2006). This aspect of plant form has been examined through models of plant growth such as Y-plant and L-systems (Pearcy and Yang 1996; Niklas 1999; Prusinkiewicz and Rolland-Lagan 2006). Modeling light interception by shoots or whole plants has demonstrated a range of optimal solutions for different light environments. For example, larger leaves contribute to plant height, shading out competitors and are cheaper than woody branches and twigs with many small leaves (Givnish 1986; Westoby and Wright 2003). However, larger leaves tend to self shade and need long petioles to avoid this (Pearcy *et al.* 2005). They also require greater investment in support structures within the leaf, up to 70% of biomass, and this cost may place an upper bound on leaf size (Givnish 1986). Large leaves require a disproportionate increase in biomass investment as support must increase with the cube of leaf length and the wind drag is directly proportional to leaf area (Niklas 1999). Support tissues are low in nitrogen and variation in leaf size may explain some of the variation in peak photosynthetic activity (A_{max}) for a given nitrogen level (N_{mass}) (Brites and Valladares 2005).

Efficiency of light interception increases with investment in support. Leaf shape is one way of optimising structural support for the largest possible surface area. Leaves close packed along branches are often triangular in outline as this gives densest packing without self-shading (Givnish 1986). Self shading can be further reduced by asymmetric leaves with lobes on one side such as *Begonia* (Beal 1871). Leaves exposed to only unidirectional light are often peltate (leaves with the petiole attached in the center). This type of structure is the most efficient way of extending a leaf perpendicular to the petiole and is common in lianas, vines, aquatics of the water surface and plants of the forest floor (Givnish and Vermeij 1976). A similar solution is the cordate, or strongly heart-shaped leaf form seen in *Begonia*s (Burt 1985) and *Viola*s (Givnish 1986), and many vines (Givnish and Vermeij 1976; Goodwillie *et al.* 2004).

A role for leaf lobing in increasing canopy light interception is suggested by modeling (Niklas 1989). Computer simulations showed that lobing was not associated with increased light interception but if the distal leaves were lobed and the proximal leaves entire the light interception of the whole canopy was maximized. This pattern is similar to the distribution of lobed leaves seen in *Sassafras* (de Souza and Kincaid 1991).

Efficient interception of light may be the reason for some of the greatest asymmetry of leaf shapes – anisophyllous leaves. In this case leaves develop different shapes depending on which side of the shoot they are on. This results in the shoot having a clear dorsal and ventral side. Anisophyllous leaves have evolved many times and occur fairly frequently in the families with many tropical understorey plants (Acanthaceae, Gesneriaceae, Melastonataceae and Urticaceae). In some cases the effect is intrinsic to a species either throughout its life or in a juvenile phase, but in other cases it is a plastic response and the developmental changes can occur fairly late in leaf development (Dengler 1999). The flat lying, close packed leaf mosaic that is formed maximizes light capture in dimly lit understories by reducing self shading (Givnish 1986).

TEMPERATURE

Insulating

Leaves can generate and maintain a fair amount of heat. Across 50′ of latitude, from subtropical to boreal, the leaf temperature in the tree canopy is 21.4+/-2.2°C over the long term, based on O_2 isotope measurements (Helliker and Richter 2008). This clearly shows that leaves can both accumulate heat and dissipate it.

Leaves can prevent freezing and maintain metabolism in cold environments by conserving metabolic and incident heat though insulation such as long wooly hairs (*Salix lanata*) or packs of older leaves (*Dendrosenecio kilimanjari*). The shape of the leaf can also affect temperature regulation. This is due to the effect of shape on the boundary layer – the still air created around an object due to surface friction. The depth of the boundary layer increases with the square root of the distance from the edge of the leaf. A thick boundary layer will slow evaporation of water and convection of heat from the leaf or branch, but also slow the diffusion of CO_2 into the leaf. Thick boundary layers are found in large entire leaves, thin boundary layers in small leaves or leaves with dissected laminas. The orientation of the leaf relative to the prevailing wind has a strong effect on the thickness of a boundary layer (Schuepp 1993). Narrow or small packable leaves which can give a small, close mosaic (e.g. bunches of pine needles) can maintain temperature by clustering which increases the thickness of the boundary layer.

Cooling

For most leaves overheating is a far greater risk than freezing. Exposed to the light and with no form of cooling a leaf's temperature can rise by over 1°C/second (Vogel 2009). Leaves can maintain a temperature cooler than ambient by evaporative cooling and by reduction of heat absorbance. Reduction of absorbance seems to be more important as leaves do not necessarily transpire more in hotter environments (Nicotra *et al.* 2008). Reduction of absorbance can be generated by reflection of light by hairs or waxes, or by changing the leaf angle so the leaf is not so exposed to the sun. In the dry woodland of Western Australia species with more upright leaves are found in higher light (Bragg and Westoby 2002).

Another way of preventing the leaf from heating up is by reducing the surface area, opting for more, smaller leaves or a highly dissected leaf. This works by increasing heat dissipation due to a smaller boundary layer. Models show that heat is lost more easily from irregularly shaped leaves, and that entire leaves are very sensitive to orientation but lobed leaves much less so (Vogel 1970). Producing lobed leaves rather than entire ones would therefore remove the constraint on leaf orientation, requiring less investment in support structures.

Correlative studies show that compound leaved species are found in environments with high light, and less commonly in the shade (Stowe and Brown 1981). Intraspecific studies have also seen correlations seen between dissected leaves and sunnier habitats (Andersson and Shaw 1994), and warmer climates (Gurevitch 1988, 1992a, 1992b). *Viola septemloba* is a perennial violet of the south east US which produces cordate and lobed leaves on one plant. During winter an average of 15% of the leaves are lobed, at midsummer the proportion rises to 73%. Lobed leaves average 2.2°C cooler than cordate (Winn 1999). Variation in dissection within plant also has a measurable effect on cooling. *Sassafras albidium* produces fewer lobed leaves in the shade and in the middle of branches and more lobed leaves in open environments and at the proximal and distal ends of branches. The lobed leaves cool faster and are more photosynthetically active (De Soyza and Kincaid 1991). It has even been suggested that the leaf dissection produced by herbivore activity could be an advantage to plants in the height of summer (Vogel 2009).

Dissected leaves are associated with high photosynthetic activity in *Pelargonium*, *Ranunculus repens* and in cotton (Lynn and Waldren 2002; Stiller *et al.* 2004; Nicotra *et al.* 2008). However, in none of these cases was it clear that this affect was due to cooling and thinner boundary layers as the *Pelargonium* measurements were taken in a wind tunnel to remove boundary layers, *R. repens* is an aquatic plant, and in cotton leaf dissection was strongly related to variation in water use efficiency which was thought to have a greater effect on the results. Gurevitch (1988, 1992) showed that the more dissected ecotype of *Achillea mille-folium* maintained a lower temperature than the less dissected form but had lower photosynthetic rates. The difference in photosynthetic rates is seen at both the warm temperatures native to the dissected form and at the cooler temperatures native to the less dissected form, and the less dissected form had higher photosynthetic rates at the higher temperatures. This suggests that leaf dissection is not always an adaptation to cool leaves to a temperature at which photosynthesis is most productive.

WATER

Supply and support

The hydraulics of a leaf are related to shape. Peltate leaves with radiate veins are the optimum supply system solution for any given area, though they are not usually the optimum light interception shape. Ovate leaves with parallel veination are similarly efficient for vascularisation if arranged in a rosette (Givnish 1986). As veins increase in cross section their strength increases faster than the cost of building them, so most leaves consolidate support along their longest axis into a single strong midrib (Niklas 1999). A compromise between supply and support results in wedge shaped leaves with a single major vein. In either spiral or planar phyllotaxy wedge shaped leaves give some overlap, rectangular shapes are better for packing. A compromise gives the typical lanceolate leaf with straightish sides and a tapered wedge-shaped tip such as that of the bay laurel (*Laurus nobilis*) (Givnish 1986).

Leaf dissection also affects water relations. Water flows more efficiently through a deeply lobed leaf than through an entire one. Measurements on sun and shade leaves of a range of trees have shown that lobed, sun leaves had a leaf hydraulic conductance (K_{leaf}) of up to 67% higher than that of entire, shade leaves. Lobed leaves may be an adaptation to drought, allowing the leaf to be easily and evenly supplied with water when it is in low abundance (reviewed in Sack and Holbrook 2006).

The distance water must travel from the vein through the mesophyll to the stomata affects K_{leaf} and A_{max} over a huge range of habitats, functional groups and phylogenetic ranges (Yang and Tyree 1994; Sack and Holbrook 2006; Brodribb *et al*. 2007). Differences in leaf anatomy may explain how plants with very different leaf shape thrive in the same environment. Plants in water stressed conditions may develop lobed leaves or they could achieve a similar increase in K_{leaf} by decreasing the distance between their veins and stomata.

Analysis of correlations between K_{leaf} and both leaf shape and anatomy in a wide range of species is needed to disentangle their effects as leaf shape and vascular patterning are developmentally coupled (Dengler and Kang 2001). The vascular system provides the leaf with water but also provides support for the extended lamina. Vascular patterns may form in response to physical stresses created by the patterns of cell division in the expanding lamina (Laguna *et al*. 2008). The physical stresses show where the leaf needs support but also where cell expansion, which requires water, is occurring. Venation patterns are usually fully optimised neither for support nor for hydraulic supply but represent a compromise between two ideals. Constraints on the compromises possible between support and hydraulics may be behind the constraints on larger scale leaf traits identified by Wright *et al*. (2004) (Shipley *et al*. 2006).

Shedding water

The long, tapering tips of many tropical forest plants are a classic example of a leaf adaptation. They are postulated to speed the drying of the leaf and help prevent epiphytes settling there. Water on the leaf can block stomata and water drops can focus light sufficiently to cause damage to the leaf. Experiments have shown that leaves with drip tips do shed water faster and leave less water on the leaf, but drip tips have no effect on the accumulation of epiphytes on the leaf (Ivey and de Silva 2001; Lücking and Bernecker-Lücking 2005; Burd 2007). Drip tips do, however affect fungus-load. Leaves without drip tips were found to have 1.7 times more fungal growth (Ivey and de Silva 2001). This could have enough effect on leaf health to justify the small costs of extending the lamina a little to create drip tips.

Serrations can be seen as a mild case of compoundness – they help break up boundary layers and dissipate heat, but they also may be correlated with managing water flow through the leaf. A survey of a subtropical Australian flora identified water availability as a key factor in the abundance of serrated species and the proportion of serrated leaves in a habitat (Royer *et al*. 2009). The functional basis of this correlation may be due to guttation.

Guttation is the production of drops of water from hydathodes often found at the end of serrations. Field *et al*. (2005) show that production of these water drops prevents flooding of the mesophyll by high root water pressure. CO_2 travels through water 20 times less well than through air so water in the mesophyll airspaces limits photosynthesis. Guttation can occur without serration but the serrations help the drops fall. The ability to shed extra water may allow serrated-leaved plants to use root pressure to help drive fast leaf expansion.

Bailey and Sinnott (1916) first noted the prevalence of serrated leaves in temperate, but not tropical forests. The proportion of serrated leaves in a habitat is strongly negatively correlated with annual mean temperature. This correlation is so strong that leaf margin analysis is used to study ancient climates (Royer *et al*. 2005), although the prevalence of serrations in waterside plants can bias estimates (Burnham *et al*. 2001). The functional basis for this correlation is unknown, but could be related to seasonality. The serrations are the first parts of the leaf to expand fully from the bud. The teeth have high rates of photosynthesis and they accumulate photosynthate early in spring (Baker-Brosh and Peet 1997; Royer and Wilf 2006). This may be enough to give serrated leaved plants the competitive edge in early spring.

WIND

High winds are an occasional hazard for most plants and a regular hazard for some. As surfaces held out to the environment leaves generate drag which can tear them, pull them off, or exert sufficient force to bring down branches or trees. Leaves have four options for reducing drag: they can curl into cones, leaf clusters can curl into cones, pinnately compound leaves can form cylinders or leaves can stack against each other along the branch (Vogel 2009).

To be able to curl into a cone a simple leaf requires a stiff petiole longer than 2 cm and lobes of lamina at the proximal end of the blade. This may be another explanation for the lobed leaves seen in the *Sassafras albidum* growing in open areas (de Soyza and Kincaid 1991). Serrated margins and short, flexible petioles are common in leaves that form cones as clusters rather than individually (birch, *Betula* spp.). The lowest drag coefficients are seen in pinnately compound leaves which form cylinders in the wind. This suggests compound leaves are a good option for emergent trees and plants in windy areas (Vogel 1989, 2009). Tropical palms, which can withstand hurricanes, all have compound leaves.

Another explanation for compound leaves is that they act as collections of many small leaves, as ‘cheap and quick’ branches (Givnish 1978). Support for this comes from their frequency in seasonally arid habitats and disturbed areas (such as ash (*Fraxinus excelsior*), or elder (*Sambucus nigra*) in woodland light gaps). However, recent experiments have shown pinnate leaves to require more investment in support tissues than entire leaves (Niinemets and Sack 2006; Niinemets *et al*. 2007), suggesting they are not a 'cheap' option. A third explanation is that they are competitively advantageous in crowded and dry environments when height competition favours large leaves, but gas exchange conditions favour small (Givnish 1979).

THE BIOTIC ENVIRONMENT

Overtopping

Plants growing on a tangled bank is a classic image in biology (Darwin 1856). There is intense competition between plants to overtop each other and gain access to the most light (Beyschlag *et al*. 1990). Rosette herbs cannot use branches to overtop neighbours and a long petiole can become unstable. Pinnately compound or highly lobed leaves allow the leaf to expand in the ‘cheap’ regions of rachis or narrowly laminate blade raising up the leaflets or lobes above neighbouring plants (Semchenko and Zobel 2007). In dandelions (*Taraxacum* spp.) leaf lobing responds to CO_2 concentrations (Thomas and Bazzaz 1996). At higher levels of CO_2 leaves become more dissected. This could be due to sensitivity to the respiration of neighbouring plants but comparisons with the effects seen on *Plantago* and *Rumex* suggest that this effect is likely to be due to changes in the carbohydrate metabolism resulting in the adoption of a more adult, or more ‘sun-leaf’ leaf form, rather than an adaptive response. This is an example of feedback between metabolism and leaf form which may explain some of the plasticity in leaf form (Raines and Paul 2006).

Herbivores

A huge proportion of animal life depends on leaves for food. The selective pressure of continual attack by herbivores could drive variation in leaf shape. There are obvious cases of leaves defending against herbivores by developing as spines as in Cactaceae, or with spiny edges as in holly (*Ilex*), but there are also more subtle strategies. Compound leaves can withstand grazing better than entire as the cut edges are often smaller. The basal meristems of grass leaves are also an adaptation to grazing. Unfortunately for gardeners, continual lawn mowing does not hinder the growth of replacement leaf from the basal meristem. Narrow leaves deter pests by making it difficult to manouvre in comparison to broad leaves (Kareiva and Sahakian 1990; Brown *et al.* 1991; Jones and Lawton 1991). Extreme cases of leaf shapes deleterious to insects are the carnivorous plants which have modified their leaves to act as traps (Ellison and Gotelli 2001).

Experiments show that some herbivores have preferences for specific leaf shapes, and that some leaf forms are more susceptible to damage than others. Flea beetles (*Phyllotreta*) quickly leave the curly, bumpy leaves of kale, but remain longer on the smooth leaves of cabbage (Vaughn and Hay 1993). Lobed leaves of the shepherd's purse (*Capsella bursa-pastoris*) suffer greater damage from the flea beetle than the unlobed, but the vine weevil (*Otiorhynchus*) prefers unlobed leaves (Rivero-Lynch *et al.* 1996). Similar variability in herbivore preferences is seen in *Ipomoea* (Elmore 1986; Campitelli *et al.* 2008).

Insect resistance in the okra leaf form of cotton has been the subject of several studies. Okra leaf is a semi-dominant mutation that acts in all cell layers of the leaf to promote lobe growth and decrease lamina expansion, resulting in narrow, long lobes (Dolan and Poethig 1998). This seems to deter boll weevil and white fly and the loss of leaf surface does not adversely affect the yield, possibly due to reduction in self-shading, though studies show large leaf X environment effects (Andries *et al.* 1969; Heitholt 1998; Chu *et al.* 1999).

Leaf shape could also affect plant's susceptibility to the fungal and bacterial disease. The size of fungal and bacterial populations on leaves has been correlated with leaf position, plant architecture and height in the canopy. This could be due to the effect of boundary layers on the accumulation of inhibitory volatile organic compounds around leaves (Whipps *et al.* 2008). If boundary effects are important then dissected leaves should have a lower fugal and bacterial load than entire leaves.

Diversity in leaf shape between species in a single habitat could be important in avoiding herbivore attack. Some herbivores develop a search image of their food plants. Species which have a very different leaf shape to other components of a herbivore's diet will be less likely to be attacked. Such selective pressure could drive the evolution of diverse leaf shapes in a community (Rausher 1978: Prokopy and Owens 1983).

Leaves can deceive potential predators as well as deter or avoid them. Australian mistletoes mimic the foliage of their host trees, and mimicry is most prevalent in the most nutritious mistletoes (Barlow and Wiens 1977). Cryptic leaf form is also seen in the white deadnettle (*Lamium album*), which is often found growing along side the very similar true nettle (*Urtica dioica*), and therefore avoids predation (Wheeler 2004). Holes and lobes in the leaves may suggest the leaf has a resident herbivore and deter more arriving (Niemelä and Tuomi 1987) but see Rivero-Lynch 1996), or make the leaves difficult for herbivores to see (Givnish 1990). The passionflowers as a group have some of the most sophisticated leaf-defenses. Many *Passiflora* have leaves that closely resemble those of common non-host plants. *Passiflora actinia* vines have yellow dots on their leaves, petioles and stipules. These resemble the eggs of *Heliconius* butterflies which avoid laying eggs where there is apparently a clutch already (Gilbert 1982).

Biotic interactions are potentially an important force in the evolution of leaf shapes but they are much harder to study than the ecophysiology of different leaf shapes because of problems working with plant pests in the greenhouse or growth room (maintaining the animals, preventing escape, getting the levels right) and doing field surveys (the time involved, identification of predators, variation between sites). The selective force is also likely to be very sensitive to the other plants in the community, their morphological variation, and genetic variation in the full range of plant pests attacking them. However, this is one aspect of leaf shape which is of great interest to plant breeders and may attract the attention and funding required to determine patterns.

THE GENETIC BACKGROUND

The genetic behaviour of leaf shape

Leaf shape can be plastic but much of the variation seen between species, population and families is due to genetics (Clausen *et al.* 1947). In most cases leaf shape does not segregate as a single Mendelian trait or behave in an additive way, with F1 hybrids having a shape intermediate between the two parents. Heterosis, F1 phenotypes which exceed, or resemble neither of the parents, is common (Melville 1960; Gottlieb 1986). However, there are simpler situations, **Table 1** lists several examples of leaf traits which vary between population or species and are controlled by only a few loci.

Leaf shape is a very complicated phenomenon and genetic analysis of its variation may have been constrained by the difficulty in quantifying it (McLellan and Endler 1998). The traits listed in **Table 1** are generally easy to score traits with simple present/absence states, predominantly compound or lobed versus entire leaves. It is perhaps, not surprising that the few cases where the genes responsible for variation between species in leaf form have been cloned are also cases of compound leaves (Hay *et al.* 2007; Kimura *et al.* 2008). Other aspects of leaf form such as the ratio between length and breadth, the angle formed by the tip of the lamina, the variation in lamina growth along the axis of the leaf, are quantitative and highly interconnected traits.

QTL analysis is a powerful tool to dissect the genetic architecture of these traits (Mauricio 2001). QTL analysis of leaf traits in a range of inter- and intraspecific crosses has identified numbers and strengths of the loci involved e.g. (Wu *et al.* 1997; Jiang *et al.* 2000; Langlade *et al.* 2005). Comparisons are difficult because of the differences in the measurements made but in general large numbers of QTL are identified for each trait. In most cases parents contained loci with positive and negative effects on each trait. This indicates that although in some cases some aspects of leaf shape may be under the control of 1 or 2 loci, the more general trend is that variation between species is due to variation at many loci. The evolution of leaf shape may require co-ordinated change in many genes, or there may be many different changes that can lead to the same effect on shape. Positive and negative QTLs in each parent suggest that selection on leaf shape has been variable or there have been episodes of genetic drift.

Mutants in leaf form rarely affect single aspects such as length or width (though there are exceptions (Tsuge 1996)), usually they are highly pleiotropic, affecting many aspects of leaf form. It is to be expected that the loci controlling natural variation will also be pleiotropic. Genes controlling variation in leaf shape may also regulate variation in shape of other organs such as petals. Selective pressures on petal shape could constrain or drive variation in leaf form. However, QTL analysis of leaf and flower traits in *Arabidopsis* shows few QTL affect both types of organ (Juenger *et al.* 2005). As *Arabidopsis* is a selfing species this result is not necessarily transferable to an outbreeding species where selection on petal form will be much stronger.

QTL results suggest that a key problem in genetic analysis of leaf shape is not pleiotropy but the large numbers of loci regulating each leaf trait. One of the reasons for this difficulty is that what we see as individual leaf traits may be composed of several independent aspects of development. Phylogenetic analysis shows that different aspects of leaf shape evolve independently in *Pelargonium* (Jones *et al.* 2009). This makes genetic analysis difficult as the correct analysis can only be achieved if the correct traits are measured.

Leaf shape analysis has become easier using computer programs which automatically describe leaf shape parameters (Langlade *et al.* 2005; Bylesjo *et al.* 2008; Weight *et*

Table 1 Leaf traits whose variance between or within natural populations or species is controlled by few loci.

Trait	Dominance	Number of Loci	Species	Variation level	Reference
Lobed leaves	Dominant over entire leaves	1	*Lactuca sariola*	Intraspecific	Durst 1929
Lobed leaves	Dominant over entire leaves	2	*Lactuca sativa*	Intraspecific	Durst 1929
Lobed leaves	Dominent over entire leaves	1	*Carthamus flavescens X C. tinctorius*	Intraspecific	Imrie and Knowely 1970
Lobed leaves	Dominant over entire leaves	1	*Lactua graminifolia X L. canadensis*	Intraspecific	Whitaker 1944
3 leaflets	Semidominent to 5-7 leaflets	Few	*Potentilla glandulosa*	Intraspecific	Clausen and Hiesy 1958
Highly dissected leaves	Semidominanet to less dissected leaves	1	*Solanum cheesemaniae X S. galapagenese*	Intraspecific	Kimura *et al*. 2008
Lobed leaves	Dominant over entire leaves	2	*Capsella bursa pastorilis*	Intraspecifc	Shull 1909
Three-lobed leaves	Dominant over entire leaves		*Ipomoea bederacea*	Interspecific	Elmore 1986
Deeply lobed leaves	Dominant over weakly lobed leaves	1	*Crepis tectorum*	Interspecific	Andersson 1999
Peltately compound leaves	Dominant over entire leaves	1	*Vitis spp.*	Interspecific	Peter and Prins 2008
Shallow serrations	Domiant to deep serrations	1	*Urtica pilifera X U. dioica*	Interspecific	Correns 1930
Rounded lobes	Dominant over acute lobes	1	*Tropaeolum majus X Tropaeolum peltophorum*	Interspecific	Whaley and Whaley 1942
Entire, orbicular	Dominant over 5-lobed	1	*Tropaeolum majus X Tropaeolum peltophorum*	Interspecific	Whaley and Whaley 1942

al. 2008) and this should contribute to a renewed focus on the genetics of leaf shape in the coming years. All 3 leaf analysis programs use the co-ordinates of landmarks placed around the leaf margin to produce a data matrix describing its shape. These co-ordinates can then be used for morphometric analysis (Zelditch *et al*. 2004). Large amounts of data are produced by this method and the patterns in it are best analysed using multivariate analysis such as Principle Componant Analysis (PCA) which provides a means of objectively determining the main axes of variation in a population. It has been cleverly combined with QTL analysis by Langlade *et al*. (2005) to determine the number and strength of loci responsible for the difference between leaf form in two closely related *Antirhinnum* species. They show that three PCA describe most of the variation and these PCA traits are controlled by 15 different QTLs. Most QTL affect principally one PCA each but there are several QTLS for each PCA. This could represent a small number of developmental pathways (the different PCAs) each regulated by a large number of loci (the QTLs).

There are difficulties with these approaches. QTL analysis requires the production of genetic maps and extensive genotyping, which is an expensive and time consuming business, though becoming cheaper every month with the development of next generation sequencing technologies. QTLs are also notoriously difficult to repeat and almost impossible to clone except for in model systems, or if a candidate gene approach works (Salvi and Tuberosa 2005). Combination of QTL analysis with analysis of transcript levels is a technology intense approach that may prove the easiest way to identify the genes involved in species-level differences (Hansen *et al*. 2008; Street *et al*. 2008).

We are currently studying the genetics of species level differences in *Begonia*. This is a very specious (1,500+) genus distributed pan-tropically with parallel radiation in South America and South East Asia. Peltate leaves have evolved at least eight times in this genus and compound leaf forms at least three times (Forrest 2000). These ‘replicate evolutionary events’ make this a good system to study the genetic background behind morphological evolution (Neal *et al*. 2007).

Initial analysis of the genetics of peltateness and compoundness in a single Central American section (Section Gireoudia, 64 spp.) shows that neither is genetically simple. Non-complementation of non-dominant traits in F1 interspecific hybrids suggests that peltate leaves and compound leaves were produced by changes at the same locus each time they evolved (5 times independently for peltate leaves, twice for compound leaves). The presence of modifiers from non-peltate species which promote peltate leaf form in backcross populations indicates the ease with which peltateness could evolve in this group and may explain the phylogenetic lability of the trait. Unlike many of the examples listed in **Table 1**. Compoundness in this group is not a genetically simple trait, suggesting more than change at a single gene is responsible for its evolution. We are currently using association mapping and QTL analysis to determine the role of key developmental gene families in the generation of this variation.

The genes controlling leaf shape variation between species

The genetic pathways controlling leaf shape have been the focus of research in model organisms for many years. Exhaustive mutant screens and genetic and molecular studies have built up a clear picture of the key components and some of their interactions, which are reviewed in several recent papers (Kidner and Timmermans 2007; Barkoulas *et al*. 2008; Micol 2009). Expression studies and functional analysis using forward and reverse genetics have identified a small number of gene families which are associated with variation in leaf shape.

KNOX genes are required for meristem function. The first sign of leaf primordia initiation is the down regulation of *KNOX* expression. In entire leaves expression of *KNOX* genes is kept off in the leaf primordia. In a wide range of vascular plants with compound leaves *KNOX* is initially down regulated but latter expression returns to the leaf primordia (Barathan *et al*. 2000). Functional evidence for the importance of KNOX expression in compound leaf primordia comes from transgenic studies. Ectopic expression of *KNOX* in the primordia of simple leaved species results in lobed and divided leaves (Sinha *et al*. 1993; Lincoln *et al*. 1994; Frugis *et al*. 2001; Muller *et al*. 2006), and in compound leaved species over expression of *KNOX* in the leaves increases the degree of compoundness (Chen *et al*. 1997).

Evidence that variation in *KNOX* expression in the leaf primordia is responsible for natural variation in leaf form is provided by experiments with *Cardamine hirsuta.* This species is closely related to *Arabidopsis thaliana*, which it resembles apart from its compound leaves. As expected for a compound leaved species, *C. hirsuta* expresses *KNOX* in its leaf primordia, whereas *Arabidopsis* does not. Down regulation of *KNOX* activity in *C. hirsuta* converted the compound leaves into simple leaves. The differences in *KNOX* expression patterns between the two species are driven by variation between the species in the gene’s promoters (Hay and Tsiantis 2006). One key region of the *KNOX* promoter is the K-box which controls repression of *KNOX* in many simple leaved species. This region may be related to down regulation of *KNOX* by AS1, RS2, PHAN-like (*ARP)* genes (Uchida *et al.* 2007).

ARP genes are required for maintenance of downregulation of *KNOX* genes in the leaf primordia (Timmermans *et al.* 1999; Tsiantis *et al.* 1999; Byrne *et al.* 2000). Analysis of their expression patterns in a range of species suggests a correlation with regions of lamina outgrowth (Waites and Hudson 1995; Waites *et al.* 1998; Kim *et al.* 2003b; Golz *et al.* 2004; Tsukaya 2005). Mutant and transgenic phenotypes of plants with altered *ARP* activity support a role for *ARP* genes in regulation of the position of lamina outgrowth in a wide range of species (Kim *et al.* 2003b) but a correlation between natural variation in leaf shape and changes at the *ARP* locus has not yet been described.

A second example of the changes in *KNOX* genes resulting in species-level differences in leaf form is in the native tomatoes of the Galapagos Islands (Kimura *et al.* 2008). A single base pair deletion in the promoter of the *KNOX*-like gene *PETROSELINUM* (*PTS*) in the highly dissected *Solanum galapagense* upregulates expression in leaves in comparison to the expression levels in the less dissected sister species *S. cheesmaniae. PTS* is required to promote interaction between the transcriptional regulators *BIPINNATA* (*BIP*) and LeT6 (a class1 *KNOX* protein) and nuclear localisation of the complex (Kimura *et al.* 2008).

In pea (*Pisum sativum*) *UNIFOLIATA* (*UNI*), an ortholog of the floral regulators *FLORICAULA* (*FLO*) from *Antirrhinum majus* and *LEAFY* (*LFY*) from *Arabidopsis thaliana* is required for compound leaf formation (Hofer *et al.* 1997; Gourlay *et al.* 2000; Hofer *et al.* 2001; Champagne *et al.* 2007). It is striking that throughout seed plants *KNOX* expression in leaves is associated with compound leaves but in at least one lineage a completely different genetic pathway has been adopted. Compound leaves have evolved many times in different lineages and very few of these lineages have been molecularly studied. As more species are examined in detail other instances of divergent methods of compound leaf formation may emerge or the legumes may remain a solitary example which proves that there is no single ontogenic route to compound leaves.

In both legumes and other angiosperms genes of the *NO APICAL MERISTEM/ CUP-SHAPED COTYLEDONS3* (*NAM/CUC3*) family are required for proper expression of *KNOX/UFO* like genes during compound leaf formation. *NAM/CUC3* genes are involved in defining the boundaries between organs (Weir *et al.* 2004; Hibara *et al.* 2006; Blein *et al.* 2008). A feed forward regulatory loop between *NAM/ CUC3* genes and *KNOX/UNI* genes controls leaflet formation in compound leaves of *Solanam lycopersicon*, *Cardamine hirsuta* and *Pisum sativum* (Blein *et al.* 2008). Regulation by *miR164* refines the expression pattern and controls placement of leaflets and degree of lobe or serration outgrowth in a range of species (Nikovics *et al.* 2006; Blein *et al.* 2008; Berger *et al.* 2009). Variation in expression patterns of *NAM/CUC3* or *miR164* could be behind the evolution of compound leaves in cases where there is no variation in *KNOX* genes.

YABBY genes are also involved in leaf polarity and are required for lamina outgrowth (Bowman 2000; Golz *et al.* 2004). *YABBYs* are expressed on the abaxial side of eudicot leaves but on the adaxial side of monocot leaves (Juarez *et al.* 2004). This shows that key leaf developmental genes can vary their expression patterns in angiosperms with similar leaf forms. This reversal of expression domain maintains the distinction between YABBY and no-YABBY at the point of lamina outgrowth. Support for a role in regulating the position of lamina outgrowth is provided by their expression pattern in nasturtium (*Tropaeolum majus*). The development of the peltate leaves of this species is prefigured by expression of the *YABBY* gene *TmFIL* all around the base of the leaf primordia (Gleissberg *et al.* 2005). Unfortunately all the closest relatives of *T. majus* are also peltate so it has not yet been possible to demonstrate whether changes at a *YABBY* locus are responsible for the leaf shape in this genus.

SELECTIVE PRESSURE ON LEAF SHAPE

Leaf shape affects photosynthesis, water balance, temperature control, and interactions with other organisms. Variation in leaf shape is also correlated with environmental factors. In some cases it appears to be a genetically simple trait – increasing the chances of leaf shape variation going to fixation within a species. However, the observation of variation, and showing that the regulation of leaf shape can be genetically simple is not enough to demonstrate that leaf shape is necessarily adaptive or that it has a strong enough selective effect to drive speciation. What's needed are measures in the field, or in natural conditions of the effect of leaf form on total lifetime fitness, which is usually measured by survival, dry biomass, and fertility (numbers and/ or weight of seed). A few studies have included leaf shape in their analysis of fitness effects.

Butterfly weed (*Asclepias tuberosa*) exists in two intergraded morphs in the US. The eastern form has an obovate leaf with a cuneate base (the lamina grades into the petiole), whereas the western morph has a rounder, ovate lead with a cordate base (the lamina forms two lobes where it joins the petiole) (Woodson 1962; Wyatt and Antonovics 1981). Ovate leaves with cordate bases are found in drier regions. The leaf shape differences are heritable (though not completely so) and a shift towards the western form was observed between 1946 and 1960. Repetition of the studies during the 1970s established that the western form had slight higher fitness but this was not enough to fully explain the shift in morph frequency, suggesting it may be been due to random fluctuations such as founder effects (Wyatt and Antonovics 1981).

A second study used the variation in leaf shape between wild and domesticated chicory (*Chicorium intybus*). Wild chicory has deep lobes along the whole leaf, whereas the cultivated form has none. The two forms hybridise along roadsides. A large population of hybrids was scored for survival, leaf phenotype, flowering, seed production, and biomass production and genotyped over two years. No clear difference in the range of leaf morphologies was seen over the two years, and there was no correlation between fitness and leaf morphology in either year (Sorensen *et al.* 2007).

Nagy (1997) studied ecotypes of *Gillia capitata*. A lobed leaf ecotype is found on the coast, and a more entire leaf type is typical of inland ecotypes. Transplant experiments have shown that the native ecotypes each outcompete the other on their native ground, though hybrids are fertile (Grant 1950; Nagy 1997). F2 hybrids were grown in each environment scored for phenotypes and fitness (number of inflorescences at senescence) and their offspring then grown up in greenhouses to score for the effects of selection. Results showed that selection favored the less lobed landward ecotype leaves at both coastal and inland sites, and that selection was relatively strong, particularly at the inland site. As this selection pressure appears to contradict the adaptation to native habitats seen in previous experiments, Nagy concludes that the apparent selection on leaf shape is due to correlations (either genetic or physiological) with other traits.

A fourth study of the fitness effects of leaf shape comes from a three year demographic study of *Crepis tectorum* ssp. pumilla growing on a Baltic island (Andersson and Shaw 1994). Here, the authors showed that selection favored deeply lobed leaves (though early in the spring they had a deleterious effect). Leaf dissection was strongly selected for, having a greater effect on fecundity than germination date, flowering date, plant height and the number of branches. This effect was thought to be due to the effect of leaf dissection of heat dissipation.

The latest analysis of fitness and leaf shape comes from a study in *Ipomoea hederacea*, the morning glory – a weed of arable crops in the US that is polymorphic for lobed and entire leaf forms. Analysis of fitness (measured by survival and seed number and weight) showed that the selection effect of dissection in morning glory varies by year and by

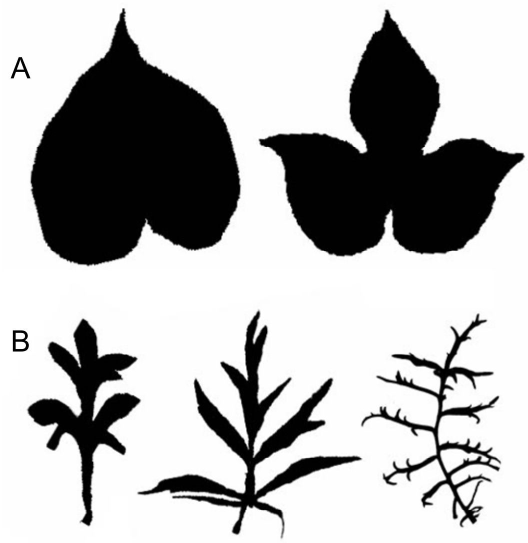

Fig. 3 Variation in leaf shape within species. (**A**) Lobed and unlobed leaves of *Ipomoea hederacea* from the same population, (**B**) Variation in dissection of *Gilia capitata* leaves from different populations.

site. However, they also show that the heterozygotes have a selective advantage in most of the circumstances. Since the dissection phenotype is mostly dominant this suggests that linked loci could be producing the fitness effects seen, rather than leaf shape itself (Bright *et al.* 2008).

Out of 5 studies, 2 found no effect of leaf shape, 2 found an effect but concluded it was due to correlations with other traits and a single one found a strong effect of leaf dissection. Dissection is the only aspect of leaf shape for which an adaptive case can be made. It has also been shown to affect leaf function and to vary along environmental clines (see above). Evolutionary studies have shown that leaf dissection and leaf size are the most labile traits in the very diverse genus *Pelargonium* (Jones *et al.* 2009). This could be due to either selection or drift. Correlations with photosynthetic function suggest selection (Nicotra *et al.* 2008), but it would be interesting to see the genetics of leaf shape in this genus. Frequent gain and loss of a genetically complex trait would be further evidence of selection.

Although leaf shape in *Pelargonium* is very diverse, many genera or families have similar leaf shapes. Fabaceae all have compound leaves, most Rosaceae have serrated leaves with prominent stipules, the leaves of the milkweed family, Apocynaceae are never compound and all have pinnate venation. These instances of phylogenetic conservatism could be due to similar niches occupied by all the members of a family or to fixation of the trait though loss of the pathways that could modify it.

Is there any evidence that leaf shape variation can result in speciation? There are numerous groups of sister species that vary widely in leaf-shape but little in floral form or plant habit (e.g. *Tecoma*, *Begonia*, *Pelargonium*, *Tropaeolum* and see **Figs. 3** and **4**), and there are examples of population-level variation in leaf form associated with environmental clines (Gregor 1938; Clausen *et al.* 1947; Gurevitch 1992b). It is also clear that the genetic control of leaf shape can be very simple – controlled at one or two loci (**Table 1**). This would allow for quick fixation of a leaf-shape trait under selection. However, though current evidence is slight, selection on leaf shape appears to be variable. This could be sufficient to maintain polymorphisms (as in *Capsella* for hundreds of generations in populations all over the world) but not enough to result in the loss of fitness in hybrids that is required to start the two morphs along the road to speciation.

Leaf shape difference between species could evolve by drift and once fixed they may be important enough to allow species to persist in sympatry. Sterck *et al.* (2006) showed that correlations between variation in light level and leaf traits (SLA and A_{max}), which are affected by leaf shape, may explain how tree species co-exist. However, there is no evidence of strong enough selection on leaf shape form to give speciation in sympatry.

CONCLUSION

Large-scale surveys of genetic and phenotypic variation are becoming more common as genotyping becomes cheaper and computer power is better able to cope with the large amounts of data involved. This type of study (such as QTLs on natural populations) may give further examples of selection acting on loci regulating leaf form. Lab-based analysis will help define which aspects of leaf form can be treated as independent variables, and loci identified as under selection can become the focus of developmental studies.

Disentangling leaf shape from the anatomical and physiological changes associated with it and from linked loci is difficult and would require the use of near isogenic lines. Such lines are available for *Arabidopsis*, which unfortunately shows only subtle changes in leaf shape. *Solanum* is a system in which the genetic resources are excellent and there is a wide variation in leaf form, which is genetically well characterised, both in mutants and in natural populations (Moyle 2008), but a single genus will not be sufficient for the identification of patterns. Ideally, genetic and molecular resources would be developed for a number of genera encompassing a range of habitats and life histories, allowing comparisons and the generation of robust conclu-

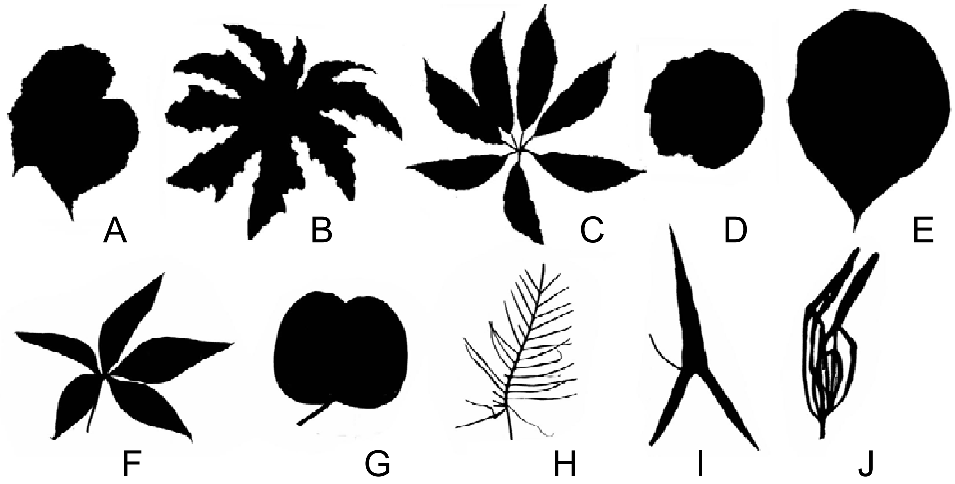

Fig. 4 Variation in leaf shape between closely related species. (**A**) *Begonia urophylla*, (**B**) *B. heracleifolia*, (**C**) *B. carolineifolia*, (**D**) *B. conchifolia*, (**E**) *B. nelumbiifolia*, (**F**) *Ipomoa palmata*, (**G**) *I. pes-caprae*, (**H**) *I. quamoclit*, (**I**) *I. sagittata*, (**J**) *I. plummerae*.

sions. We are developing genetic resources for the analysis of variation in *Begonia* and many other groups are bringing the resources of molecular genetic analysis to a wide range of plant species, which should provide the data required to discover patterns in the generation of natural variation (Abzhanov *et al.* 2008).

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Appendix: Ecophysiology and genetics terms used in this review.

SLA	Specific Leaf Area; dry mass of leaf per unit of light-intercepting area, which represents the plant's investment of resources in each square cm of leaf which can photosynthesize.
A_{max}	Maximum photosynthetic assimilation rate under high light, ample soil moisture and ambient CO_2. This is usually measured by monitoring the rate of uptake of CO_2 from a sealed chamber containing the leaf (or part of a leaf) using a photosynthetic infra red gas analyser.
R_{mass}	Dark respiration rate; metabolic rate, mostly protein turnover and phloem loading. This is usually measured by O_2 consumption in the dark
N_{mass}	Leaf nitrogen; a measure of the protein invested in the leaf.
P_{mass}	Leaf phosphorus; a measure of the ATP, lipid and nucleic acid in the leaf and resources the plant has drawn from the soil.
K_{leaf}	Leaf hydraulic conductance; a measure of how efficiently water is transported through the leaf
KNOX	Homeobox transcription factors required for the maintenance of indeterminate cell division.
ARP	Asymmetric leaves1 (AS1), Rough Sheath2(RS2), Phantastica (PHAN) -like family of MYB transcription factors required to maintain down regulation of *KNOX* genes and for leaf polarity
QTL	Quantitative Trait Locus; a genomic location strongly associated with variation in a trait. Usually indicates the position of a gene controlling the trait.
PCA	Principal component analysis; analysis of a data covariance matrix to identify the eigenvectors and eigenvalues which best describe the variation in data. Analysis begins with identifying the axis which encompasses most of the variation in the data and moves towards successively minor effects.