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Original Article

Hydraulic conductance and the maintenance of water balance in flowers

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ABSTRACT

Flowers face desiccating conditions, yet little is known about their ability to transport water. We quantified variability in floral hydraulic conductance (K_{flower}) for 20 species from 10 families and related it to traits hypothesized to be associated with liquid and vapour phase water transport. Basal angiosperm flowers had trait values associated with higher water and carbon costs than monocot and eudicot flowers. K_{flower} was coordinated with water supply (vein length per area, *VLA*) and loss (minimum epidermal conductance, g_{min}) traits among the magnoliids, but was insensitive to variation in these traits among the monocots and eudicots. Phylogenetic independent contrast (PIC) correlations revealed that few traits had undergone coordinated evolution. However, *VLA* and the desiccation time (T_{des}), the quotient of water content and g_{min}, had significant trait and PIC correlations. The near absence of stomata from monocot and eudicot flowers may have been critical in minimizing water loss rates among these clades. Early divergent, basal angiosperm flowers maintain higher K_{flower} because of traits associated with high rates water loss and water supply, while monocot and eudicot flowers employ a more conservative strategy of limiting water loss and may rely on stored water to maintain turgor and delay desiccation.

Key-words: Angiosperms; flower; hydraulic conductance; vein density; water balance.

INTRODUCTION

Flowers are often considered the hallmark of angiosperm evolution. Their appearance enabled the evolution of more intimate, specialized interactions with animal pollinators than had previously been possible (Crepet and Niklas 2009; Darwin 1888; Fenster *et al.* 2004; Kölreuter 1761; Sprengel 1793). Despite the influence of flowers on plant-pollinator networks (Memmott and Waser 2002), the generation and maintenance of biodiversity (Dodd *et al.* 1999; Stebbins 1970), and ecosystem services (Costanza *et al.* 1997), the fundamental relationships between floral structure and physiological function have been relatively ignored, even though non-pollinator agents of selection, such as physiological costs, often oppose the effects of pollinators (Galen 1999; Galen *et al.* 1999; Lambrecht 2013; Strauss and Whittall 2006).

Like leaves, flowers are terminal structures often located in the hottest, driest parts of the plant canopy, but flowers and leaves have experienced different selective forces. Leaves are a critical component in the plant hydraulic pathway and have a large influence on the terrestrial water cycle (Hetherington and Woodward 2003). Because carbon assimilation is mechanistically linked to plant transpiration (Brodribb and Feild 2000; Brodribb *et al.* 2007; Sack and Holbrook 2006), moving water from the roots to the leaves requires coordination in the structural traits governing water flow through each component in this pathway to prevent declines in water content that could irreversibly prohibit hydraulic function (e.g. cavitation; Drake *et al.* 2015; Skelton *et al.* 2015). Angiosperm leaves are particularly capable of efficiently transporting water because of high leaf vein densities (vein length per area, *VLA*) that move water closer to the sites of evaporation (Brodribb *et al.* 2007; Brodribb *et al.* 2010; Buckley 2015; Feild and Brodribb 2013), minimize changes in leaf water content (Noblin *et al.* 2008; Zwieniecki and Boyce 2014) and, ultimately, increase growth rates (Berendse and Scheffer 2009). The fundamental constraint of maintaining water balance by matching liquid water supply to vapour loss determines the range of possible leaf designs, from the organization of cells within leaves to the shapes and sizes of leaves themselves (John *et al.* 2013; Li *et al.* 2013; Sack *et al.* 2008; Sack *et al.* 2012).

Unlike leaves, flowers are relatively ephemeral and assimilate little carbon (but see Galen *et al.* 1993 for an important exception) although they still may transpire significant amounts of water (Roddy and Dawson 2012; Teixido and Valladares 2014). Instead, they promote pollen dispersal with animal- and wind-pollinated flowers differentiating along a spectrum of carbon and water investment. Because flowers often face desiccating conditions that would lead to wilting and prevent successful pollination, they must maintain water balance and turgor throughout anthesis to attract pollinators. At least part of this hydraulic pathway may need to remain functional after pollination to facilitate proper fruit and seed development. Understanding how flowers do this is fundamentally important to understanding non-pollinator agents of selection and, by extension, floral evolution.

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Even basic information about flower water relations, such as the mechanisms used to deliver water, are not clear. Basal angiosperm flowers from the ANA grade (*Amborella*, Nymphaeales, Austrobaileyales) and magnoliids seem to rely predominantly on continuous delivery of water by the xylem (Feild *et al.* 2009a, 2009b). Such a strategy could be costly because it would require a vascular system capable of meeting potentially large transpiration demands and risky because it would require flower water potential to decline diurnally with stem water potential. In contrast, some eudicot flowers and petals tend to have higher, less negative water potentials than subtending bracts and leaves (Chapotin *et al.* 2003; Trolinder *et al.* 1993). These 'reverse' water potential gradients imply that for these flowers to remain hydrated, water must be imported against an apoplastic water potential gradient in the xylem. These authors have concluded that flowers are hydrated predominantly by the phloem, which is primarily responsible for the transport of photosynthates throughout the plant. In contrast to the xylem, the phloem has much higher hydraulic resistance and lower water flux rates because phloem transport occurs symplastically (Münch 1930; Nobel 1983; Savage *et al.* 2016; Windt *et al.* 2009). However, implicating the phloem as the sole source of floral water is not necessary to explain the results from these studies; flowers can be xylem-hydrated and still maintain higher water potentials than leaves so long as they are more negative than the stem xylem (e.g. Feild *et al.* 2009a, 2009b), or they could rely on large amounts of water imported early in development (e.g. during bud expansion) and stored locally (i.e. hydraulic capacitance; Chapotin *et al.* 2003) that could be depleted throughout anthesis. Regardless of whether flowers have high hydraulic capacitance or rely on water delivered by the phloem, maintaining a higher water status in flowers could result in water being drawn back into the stem during the day, as has been shown to occur in fleshy fruits (Higuchi and Sakuratani 2006). The supposed dichotomy between xylem-hydration and phloem-hydration in flowers is likely not a dichotomy after all but rather a spectrum between more or less contributions from the phloem. In fruits, there has been a similar debate about the contribution of the phloem (Greenspan *et al.* 1994; Ho *et al.* 1987; Lang 1990), and the most recent evidence suggests that both the xylem and the phloem supply water, but the relative contributions of the two pathways may vary during development (Choat *et al.* 2009; Clearwater *et al.* 2012; Clearwater *et al.* 2013; Windt *et al.* 2009).

Regardless of the pathways of water entry into flowers, prior evidence suggests that there may be substantial variation in the hydraulic structure–function relationships of flowers and that much of this variation may be explained by phylogenetic history. In the present study, we quantified whole flower hydraulic conductance (K_{flower}) for 20 species from 10 families across the angiosperm phylogeny as a first attempt at characterizing interspecific variation in hydraulic capacity. K_{flower} has previously been measured on only one species, *Magnolia grandiflora* (Feild *et al.* 2009b). Using leaf structure–function relationships as a starting point, we hypothesized that K_{flower} would be mechanistically linked to xylem traits that may control water supply, such as *VLA* and the Huber ratio, and also to stomatal and epidermal traits of tepals and petals that may regulate water loss rates, such as stomatal density and size and epidermal conductance to water vapour. Positive correlations between K_{flower} and other hydraulic traits would suggest that these traits are important in determining the efficiency of water flow through flowers. We further predicted that positive correlations between K_{flower} and hydraulic traits, particularly xylem traits, should exist for magnoliid flowers because flowers of two of these genera, *Magnolia* and *Calycanthus*, have been shown to maintain a hydraulic connection to the stem xylem throughout anthesis (Feild *et al.* 2009b; Roddy *et al.* in prep.). If flowers from the eudicots, and possibly also the monocots, rely more heavily on a different source of water during anthesis (e.g. either delivery by the phloem or depletion of stored water), then they should not exhibit positive correlations between xylem traits and K_{flower}. Given that much of the variation in traits may be due to differences among clades, we used correlations of phylogenetic independent contrasts (PICs) to test whether pairs of traits co-evolved (Felsenstein 1985). Co-evolution of traits would further imply a functional link.

MATERIALS AND METHODS

Plant material

We collected flowering shoots from around the University of California, Berkeley campus and from the University of California Botanic Garden during the springs of 2013 and 2014, and from the Marsh Botanical Garden, New Haven, CT, in the spring of 2015. All plants had been kept well-watered. We chose a phylogenetically diverse set of species that varied by almost two orders of magnitude in floral display size (Table 1). These species also varied morphologically, from flowers with undifferentiated perianths to those with a fully differentiated calyx and corolla and from those with free petals to those with sympetalous connation. Additionally, we included inflorescences of *Cornus florida* (Cornaceae), which have small, inconspicuous flowers but large, white bracts as their showy organs. Although the showy floral structures of this set of species are not homologous, they have a convergent function of attracting pollinators. For each species, we measured K_{flower} on at least three flowers, and most species had low variance among individual flowers. Sample sizes for each species are shown in Table 1.

Measurements of hydraulic conductance

We measured hydraulic conductance of whole, recently opened flowers using a low pressure flow metre (LPFM) under low-light, laboratory conditions (Kolb *et al.* 1996). We chose this method rather than the evaporative flux method because the evaporative flux method depends on maximizing boundary layer conductance. Because of the morphological complexities of flowers (i.e. unlike leaves, flowers are rarely planar), we were not confident we could maximize the boundary layer conductance to obtain realistic maximum values of K_{flower}. However, the LPFM has the potential to clear any xylem occlusion, which we tested on a subset of species by (1) comparing

Table 1. List of species, their morphological traits, and the number (n) of flowers measured for K_{flower}

Species	Family	Area ($\times 10^{-3}$ m^2)	Mass per area ($\times 10^{-3}$ g cm^{-2})	Perianth differentiation	Corolla fusion	n	K_{flower} (mmol m^{-2} s^{-1} MPa^{-1})
Austrobaileyales							
Illicium floridanum	Illiciaceae	2.70 ± 0.15	3.88 ± 0.10	Graded tepals (monochlamydeous)	Unfused	13	NA
Illicium lanceolatum	Illiciaceae	0.96 ± 0.06	9.04 ± 0.66	Graded tepals (monochlamydeous)	Unfused	15	NA
Illicium mexicanum	Illiciaceae	2.10 ± 0.28	4.22 ± 0.24	Graded tepals (monochlamydeous)	Unfused	5	NA
Magnoliids							
Calycanthus chinensis	Calycanthaceae	9.08 ± 0.37	4.86 ± 0.26	Graded tepals (monochlamydeous)	Unfused	7	12.35 ± 2.33
Calycanthus floridus	Calycanthaceae	2.53 ± 0.06	5.68 ± 0.11	Graded tepals (monochlamydeous)	Unfused	4	10.38 ± 3.39
Calycanthus occidentalis	Calycanthaceae	4.91 ± 0.32	15.42 ± 0.19	Graded tepals (monochlamydeous)	Unfused	7	18.79 ± 3.13
Liriodendron tulipifera	Magnoliaceae	11.69 ± 0.50	6.57 ± 0.02	Monochlamydeous	Unfused	4	2.38 ± 0.31
Magnolia ashei	Magnoliaceae	39.31 ± 1.78	NA	monochlamydeous	Unfused	4	7.60 ± 2.03
Magnolia doltsopa	Magnoliaceae	22.69 ± 1.97	NA	Monochlamydeous	Unfused	4	2.95 ± 0.37
Magnolia grandiflora	Magnoliaceae	45.0	5.3	Monochlamydeous	Unfused	NA	2.62
Magnolia soulangiana	Magnoliaceae	24.44 ± 1.43	2.58 ± 0.05	Monochlamydeous	Unfused	13	3.51 ± 0.17
Magnolia stellata	Magnoliaceae	11.07 ± 0.68	2.00 ± 0.10	Monochlamydeous	Unfused	6	3.63 ± 0.42
Monocots							
Agapanthus africanus	Amaryllidaceae	1.07 ± 0.02	2.63 ± 0.11	Monochlamydeous	Fused	4	4.02 ± 1.30
Hemerocallis sp.	Xanthorrhoeaceae	13.43 ± 0.62	4.65 ± 0.26	Monochlamydeous	Unfused	10	3.29 ± 0.41
Iris douglasiana	Iridaceae	5.30 ± 0.13	1.88 ± 0.10	Monochlamydeous	Unfused	5	1.71 ± 0.35
Eudicots							
Amphilophium buccinatoria	Bignoniaceae	6.95 ± 0.16	7.73 ± 0.19	Dichlamydeous	Fused	4	2.68 ± 0.42
Camellia yunnanensis	Theaceae	4.92 ± 0.37	NA	Dichlamydeous	Unfused	5	2.86 ± 0.94
Cornus florida	Cornaceae	4.76 ± 0.37	1.87 ± 0.09	Dichlamydeous	Unfused	3	1.31 ± 0.21
Paeonia suffruticosa	Paeoniaceae	80.45 ± 9.32	3.57 ± 0.10	Dichlamydeous	Unfused	4	3.81 ± 0.46
Rhododendron johnstoneanum	Ericaceae	4.52 ± 0.32	2.20 ± 0.05	Dichlamydeous	Fused	4	2.21 ± 0.21
Rhododendron loderi	Ericaceae	16.08 ± 0.33	1.96 ± 0.03	Dichlamydeous	Fused	4	2.19 ± 0.22
Rhododendron protistum	Ericaceae	8.24 ± 0.39	2.92 ± 0.04	Dichlamydeous	Fused	5	1.71 ± 0.22
Rhododendron sp.	Ericaceae	2.60 ± 0.04	2.30 ± 0.03	Dichlamydeous	Fused	6	3.30 ± 0.35

flow rates when increasing the vacuum pressure to flow rates measured when decreasing the vacuum pressure and (2) by repeatedly measuring the same flower. We found no differences between flow rates measured while increasing the vacuum or while decreasing the vacuum and no significant increase in K_{flower} with subsequent measurements (data not shown).

Flowering shoots were excised early in the morning (before 9:00 h) when stem water potentials of plants growing in this area are generally higher than −0.25 MPa. Cut shoots were immediately recut under distilled water at least one node apical to the first cut and transported back to the lab in a covered bucket to minimize water loss. Shoots were kept in water in the covered bucket for at least 1 h during transport and after returning to the lab before any flowers were excised, allowing for relaxation of xylem tension. We only measured the most recently opened flowers on each plant, based on each flower's development relative to other flowers on the shoot. Once in the lab, individual flowers were excised underwater at the pedicel base and connected to hard-walled tubing. Pedicels were glued into short lengths of tubing of various diameters using cyanoacrylate glue, and this tubing was, in turn, connected with a compression fitting to hard-walled tubing that led back to an electronic balance with resolution to 0.1 mg (Sartorius CPA225 or Practum 224-1S, Sartorius, Goettingen, Germany), on which sat a vial of dilute electrolyte solution (10 mM KCl, filtered to 0.2 um and degassed the morning of measurements). Flowers were placed in a cylindrical acrylic chamber that was attached to a vacuum pump and lined with wet paper towels. Flow rates of KCl solution into the flower from the balance were measured every 10–60 s depending on the absolute flow rate under 5–6 different pressures ranging from 15 to 60 kPa below ambient. At each pressure, flow rates were allowed to stabilize for 3–20 min and until the coefficient of variation of the last 10 readings was less than 5% and the instantaneous measurements converged on the average of the last 10 measurements. In practice, low absolute flow rates meant that stable averages could be reached but the coefficient of variation often remained above 5%. The hydraulic conductance was taken as the slope of the linear regression between flow rate and pressure, and we removed, at most, one outlying point from the regression (Figure S1). Flowers of most species did not have any points removed, and regressions used to calculate K_{flower} had R^2 values above 0.85. Points were removed if the relationship between pressure and flow rate was non-linear. Often, the point at the highest pressure was responsible for causing non-linearity, and this point was removed, which brought R^2 values above 0.85. Immediately after measurements, we scanned the flowers to determine the projected surface area of all perianth parts, which we used to normalize hydraulic conductance to calculate K_{flower} in units of mmol s^{-1} m^{-2} MPa^{-1}. Only two species, *Amphilophium buccinatoria* and *Paeonia suffruticosa*, had a persistent, green calyx, which comprised 7.6% and 5.5%, respectively, of the total evaporative surface area. For comparison, values of K_{flower} for *M. grandiflora* reported by Feild *et al.* (2009b) using a different method were approximately equivalent to values produced using our method for congeneric species, and maximum measurements of K_{flower} in the field based on transpiration rate and the water potential gradient between stem and flower for *Calycanthus occidentalis* were equivalent to those presented here using the low pressure flow metre (data not shown). We excluded K_{flower} data for *Illicium* flowers because flowers of these species had open paths for water transport that, we believe, produced erroneously high values of K_{flower}. Almost immediately after applying the vacuum, water seeped out of the centre of the flowers or formed rapidly growing droplets on the tepal surfaces. This behaviour was unique to *Illicium* flowers and justified excluding them from subsequent analyses involving K_{flower}.

Trait measurements

We measured two xylem traits predicted to be associated with water supply, the Huber ratio and *VLA*. The Huber ratio is the ratio of the xylem cross-sectional area in the pedicel to the evaporative surface area. Immediately after measuring K_{flower}, pedicels were freehand-sectioned underwater. The sections were placed in distilled H_2O, while floral structures (tepals, petals, sepals) were individually removed and scanned on a flatbed scanner. The pedicel cross-sections were quickly stained with phloroglucinol and imaged at 5–40× under a compound microscope outfitted with a digital camera. We measured the xylem cross-sectional area and the surface area of flowers using IMAGEJ (version 1.44o; Rasband 2012). Sampling for vein density was identical to Roddy *et al.* (2013) and briefly summarized here. To account for the high variability in vein density within a petal, we excised multiple 1 cm^2 pieces from petals of at least four flowers. These sections were placed in 2% NaOH for clearing. Sections were rinsed briefly in distilled H_2O and then placed in 95% ethanol. Once in ethanol, samples were briefly stained with Safranin O and imaged at 5–20× magnification under a compound microscope outfitted with a digital camera. One or two images per section from each of five to twelve sections per species were captured, and vein densities were measured using IMAGEJ (version 1.44o; Rasband 2012).

We also measured traits predicted to influence rates of water loss. These included stomatal traits [stomatal density, guard cell length, and stomatal pore area index (SPI)] and the minimum epidermal conductance to water vapour, g_{min}. The minimum epidermal conductance is the area-normalized conductance to water vapour, measured in the dark after stomata have been allowed to close (Kerstiens 1996). We measured g_{min} on individual floral structures by sealing the cut edges with a thick layer of petroleum jelly and placing them in a dark box into which was placed with a fan, and a temperature and relative humidity sensor. For connate flowers, we measured the entire tubular structure and sealed the cut base with petroleum jelly. Structures sat on a mesh screen while the fan pulled air across the flowers inside the container. Every 5 to 20 min, the container was briefly opened and the structure weighed on a balance with a resolution of 0.1 mg. A regression of the linear part of this resulting curve was used to calculate g_{min}. After approximately 10 measurements, each structure was scanned to measure its area and then placed in a drying oven for later dry mass measurement. Using the temperature, humidity, mass and area measurements, we calculated g_{min} and the desiccation

time (T_{des}), which we define as the time required for the structure to fully desiccate and is calculated as

$$T_{des} = \frac{H_2O_{area}}{g_{min}} \quad (1)$$

where H_2O_{area} is the fresh water content per area. The fresh water content was defined as the difference between the hydrated mass measured immediately after excision during measurements of g_{min} and the final dry mass, divided by the projected surface area. T_{des} therefore has units of time. A conservative strategy of limiting water loss would be associated with a long T_{des}. In statistical analyses, T_{des} was log-transformed to improve normality. We also calculated the floral dry mass per area (*FMA*) from the area and dry mass measurements needed for measuring g_{min}.

We used two methods to measure stomatal traits. First, we cleared tepal and petal sections (and, in the case of *C. florida*, sections of showy bracts) in 2% NaOH, rinsed them briefly in distilled H_2O, and transferred them into 95% EtOH. Images of the epidermis were made using a compound microscope at 5–40×. We imaged 5–20 fields of view to determine stomatal densities, depending on the abundance of stomata. Second, we also made stomatal impressions using dental putty (Coltene Whaledent President light body). In our experience, nail varnish applied directly to petals is difficult to remove while maintaining a good impression. Instead, we made nail varnish impressions of the hardened dental putty negatives and imaged the nail varnish impressions with a compound microscope. Guard cell length was determined by measuring the maximum length of at least 10 guard cells for each species with stomata. All of these measurements were made on abaxial and adaxial surfaces and averaged to calculate stomatal density on a per unit area basis. The stomatal pore area index (*SPI*) was calculated as the product of stomatal density and the square of average guard cell length, according to Sack *et al.* (2003).

We lacked trait data for some species but, because of the paucity of these measurements on flowers, we have chosen to include these species in the present analyses when possible. Data for *M. grandiflora* were taken from Feild (Feild *et al.* 2009b) and so lacked many of the traits we measured. Because of the limited number of flowers available for *Magnolia doltsopa*, only K_{flower} was measured on this species, which appears exclusively in Table 1 and Fig. 1.

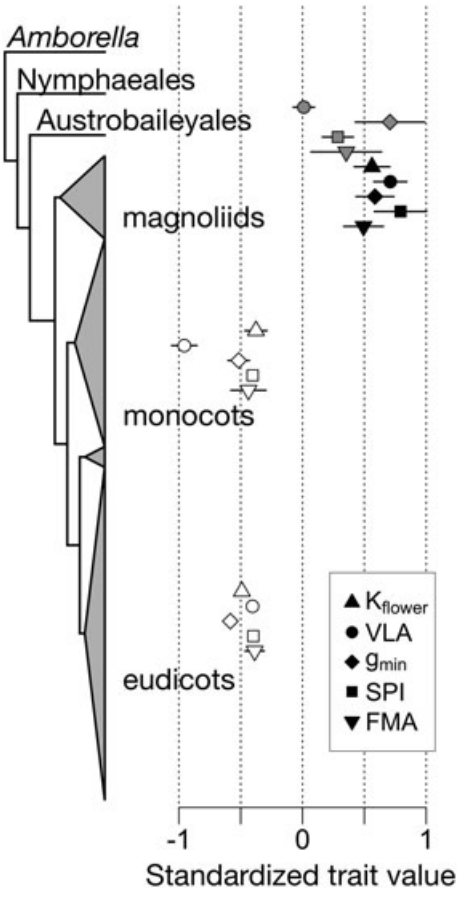

Figure 1. Hydraulic and structural trait variation among major angiosperm lineages. Data were scaled and normalized by their means so that all traits are on the same scale. Points represent mean and standard error for each lineage based on the species listed in Table 1. Positive standardized trait values signify high trait values and negative standardized trait values signify low trait values. Colours and vertical positioning differentiate trait values for each clade (grey for the Austrobaileyales, black for magnoliids and white for monocots and eudicots).

Statistical and phylogenetic analyses

All analyses were performed in R (v. 3.1.1; R Core Team 2012). For correlations between traits, we used the conservative non-parametric Spearman rank correlation and report the correlation coefficient (r_s). Additionally, for certain relationships we fit linear regressions when appropriate and report R^2 values only for these linear fits. To test for correlated trait evolution, we generated a phylogeny for the species in our dataset using PHYLOMATIC (v. 3; Webb and Donoghue 2005) with branch lengths proportional to diversity, based on the method of Grafen (1989). Phylogenetic independent contrasts (PICs; Felsenstein 1985) were calculated using the 'pic' function in the R package *picante* (Kembel *et al.* 2010).

Because much of the variation in traits was due to large trait variation among the magnoliids, particularly the genus *Calycanthus*, we calculated trait and PIC correlations on different subsets of species. Many basal angiosperms share common ecophysiological traits (Feild *et al.* 2009a), and so we examined correlations only among these species and separately among only the monocots and eudicots. Similarly, we examined trait correlations among the entire dataset with and without the genus *Calycanthus* to determine the extent to which significant correlations were due to this one genus.

RESULTS

There was large variation among species in all traits, due mainly to differences among major clades. Basal angiosperm flowers, which include the magnoliids and *Illicium*, tended to have traits associated with higher costs in terms of both water

and carbon than monocot and eudicot flowers (Fig. 1). There was little variation in trait values among species in the monocots and eudicots, suggesting that these species share a physiological strategy of having low area-normalized trait values despite varying almost 75-fold in flower size in our dataset (from *Agapanthus africanus* at 10.76 cm^2 to *Paeonia suffruticosa* at 804.53 cm^2).

K_{flower} varied widely among the species we studied from a mean of 1.30 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *C. florida* inflorescences to 18.79 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Calycanthus occidentalis* flowers (Fig. 2a). *C. florida* inflorescences had the lowest K_{flower} of any species measured, despite its showy organs being bracts. Interestingly, the three magnoliid genera spanned most of the variation in K_{flower} of all species measured, ranging from 2.38 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Liriodendron tulipifera* to 18.79 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Calycanthus occidentalis*. The monocots varied from 1.71 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Iris douglasiana* to 4.03 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Agapanthus africanus*, while the eudicots ranged from 1.30 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *C. florida* inflorescences to 3.81 $mmol\,s^{-1}\,m^{-2}\,MPa^{-1}$ for *Paeonia suffruticosa*.

Other traits similarly varied approximately two orders of magnitude (Figs 1 & 2). *VLA* exhibited a similar range to that seen by Roddy *et al.* (2013), ranging from 0.21 $mm\,mm^{-2}$ for *Agapanthus africanus* to 12.02 for *Calycanthus occidentalis*. Within clades, *VLA* ranged for the basal angiosperms (magnoliids and *Illicium*) from 2.96 $mm\,mm^{-2}$ for *Liriodendron tulipifera* to 12.02 $mm\,mm^{-2}$ for *Calycanthus occidentalis*, for the monocots from 0.21 $mm\,mm^{-2}$ for *Agapanthus africanus* to 1.65 $mm\,mm^{-2}$ for *Iris douglasiana*, and for the eudicots from 1.12 $mm\,mm^{-2}$ for *Rhododendron* sp. to 3.76 $mm\,mm^{-2}$ for *Paeonia suffruticosa*. The Huber ratio ranged from 3.63×10^{-6} for *C. florida* to 2.69×10^{-5} for *Illicium mexicanum*, while g_{min} values ranged from 2.10 $mmol\,m^{-2}\,s^{-1}$ for *Rhododendron protistum* to 102.78 $mmol\,m^{-2}\,s^{-1}$ for *Calycanthus occidentalis* tepals. Stomatal traits were similarly variable, with many species lacking floral stomata entirely, as has been reported previously (Lipayeva 1989). Among the monocots only *Iris douglasiana* had stomata (2.21 mm^{-2} and 13.08 μm in length), and among the eudicots only *Rhododendron johnstoneanum* had stomata (0.55 mm^{-2} and 21.13 μm in length). Interestingly, we did not find stomata on flowers of the other *Rhododendron* species. However, the basal angiosperms had more abundant stomata on their tepals. *Illicium lanceolatum* had the least abundant stomata (4.87 mm^{-2} and 26.63 μm in length), while *Calycanthus occidentalis* had the most abundant stomata (14.23 mm^{-2} and 16.70 μm in length).

There were surprisingly few significant correlations among traits in our dataset (Table 2). We were particularly interested in which traits were coordinated with K_{flower}. Interestingly, only xylem traits, *VLA* and Huber ratio, were significantly correlated with K_{flower}, although PIC correlations between K_{flower} and g_{min} were also significant. However, with the exception of the correlation with the Huber ratio, K_{flower} was not correlated with anything once *Calycanthus* was excluded. *VLA* correlated with traits associated with water loss: g_{min}, stomatal density, *SPI*, and T_{des} (Fig. 3, Table 2). However, without *Calycanthus* *VLA* did not correlate with any other trait, but PIC correlations between *VLA* and T_{des} and between *VLA* and K_{flower} were significant even without *Calycanthus*.

Because most of the variation in our dataset was driven by the basal angiosperms (particularly the genus *Calycanthus*), which share many other ecophysiological traits (Feild *et al.* 2009a), we examined trait correlations and co-evolution just among these lineages. The relationship between K_{flower} and *VLA* was stronger just among the basal angiosperms ($R^2 = 0.86$, df = 6, $P < 0.001$) than among all species ($R^2 = 0.78$, df = 16, $P < 0.001$; Fig. 2), and the PIC correlation among the basal angiosperms was also significant ($r_s = 0.70$, df = 6, $P = 0.04$). The correlation between K_{flower} and g_{min} was stronger among all species ($R^2 = 0.87$, df = 14, $P < 0.001$) than among the basal angiosperms ($R^2 = 0.85$, df = 4, $P < 0.01$), and the PIC correlations among all species ($r_s = 0.55$, df = 13, P = 0.03) and among basal angiosperms were significant ($r_s = 0.88$, df = 6, $P = 0.02$). Similarly, among the basal angiosperms, g_{min} and *VLA* were correlated both for traits ($R^2 = 0.76$, df = 6, $P < 0.01$) and independent contrasts ($r_s = 0.86$, df = 6, $P < 0.01$). However, while *VLA* and T_{des} were correlated among all species both for traits ($R^2 = 0.50$, df = 15, $P < 0.01$;

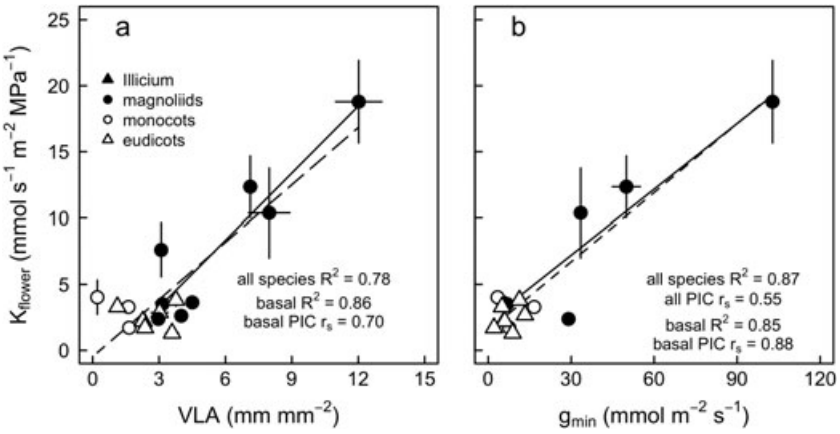

Figure 2. Coordination between K_{flower} and (a) vein length per area and (b) g_{min}. Solid lines represent linear fits only through the basal angiosperm and magnoliid points, and dashed lines represent linear fits through all species.

Table 2. Trait and phylogenetic independent contrast correlations

	K_{flower}	Area	*VLA*	Huber ratio	g_{min}	Stomatal density	Stomatal length	*SPI*	log(T_{des})	*FMA*
(a) All species										
K_{flower}	–	−0.01	0.47*	0.54*	0.42	0.3	−0.27	0.4	−0.29	0.57*
Area	0	–	0.08	−0.3	−0.14	0.14	−0.26	−0.14	0.18	−0.16
VLA	0.24	0.19	–		0.67**	0.54*	0.08	0.47*	−0.64**	0.43
Huber ratio	0.1	−0.21	0.06	–	0.32	0.07	0.14	0.38	−0.07	0.42
g_{min}	0.55*	0	0.71**	0.26	–	0.56*	0.04	0.53*	−0.9***	0.69**
Stomatal density	0.02	0.2	0.16	−0.46	0.3	–	0.08	0.92***	−0.45	0.42
Stomatal length	0.04	0.39	0.17	−0.28	0.22	0.25	–	0.45	−0.05	0.11
SPI	0.03	−0.09	0.17	0.15	0.49*	0.78***	0.48	–	−0.44	0.39
log(T_{des})	−0.10	−0.04	−0.46*	0.06	−0.66**	−0.45	−0.10	−0.54*	–	−0.43
FMA	0.49	−0.09	0.35	−0.05	0.43	0.09	0.2	−0.11	0.01	–
(b) All species except *Calycanthus*										
K_{flower}	–	0.26	0.09	0.66*	−0.08	0.14	−0.05	0.24	0.22	0.37
Area	0.13	–	0.3	−0.28	−0.09	0.19	−0.42	−0.12	0.19	−0.13
VLA	−0.53*	0.41	–	−0.02	0.42	0.45	0.54	0.38	−0.44	0.09
Huber ratio	0.34	−0.06	0.25	–	0.42	0.16	0.17	0.41	−0.16	0.6*
g_{min}	0.01	0.15	0.37	0.64*	–	0.42	0.43	0.46	−0.85***	0.6*
Stomatal density	−0.1	0.14	0.02	−0.36	0.17	–	0.19	0.95***	−0.29	0.22
Stomatal length	0.01	0.31	0.21	−0.16	0.24	0.09	–	0.45	−0.45	0.52
SPI	−0.11	−0.21	0.09	0.37	0.53*	0.75***	0.28	–	−0.36	0.29
log(T_{des})	0.20	0.02	−0.51*	−0.15	−0.67**	−0.39	−0.11	−0.59*	–	−0.23
FMA	−0.02	0	−0.22	0.19	0.15	−0.09	0.18	−0.21	0.23	–
(c) Basal angiosperms										
K_{flower}	–	−0.65	0.81*	0.09	0.77	−0.39	−0.71	−0.4	−0.66	0.31
Area	0.13	–	−0.5	−0.52	−0.38	0.62	−0.21	−0.17	0.27	−0.38
VLA	0.70*	−0.18	–	−0.02	0.6	0.02	−0.13	−0.17	−0.52	0.2
Huber ratio	−0.2	−0.47	−0.09	–	−0.12	−0.38	0.1	0.4	0.24	−0.5
g_{min}	0.88*	−0.16	0.86**	0	–	0.24	−0.02	0.19	−0.95***	0.47
Stomatal density	−0.08	0.49	0.36	−0.49	0.46	–	−0.05	0.38	−0.43	0.1
Stomatal length	0.03	−0.11	0.04	−0.2	0.3	0.38	–	0.7*	−0.1	−0.17
SPI	0.09	0.4	0.4	0.27	0.74*	0.71*	0.5	–	−0.43	−0.12
log(T_{des})	−0.39	0.22	−0.49	−0.32	−0.77*	−0.72*	−0.04	−0.86**	–	
FMA	0.73	−0.25	0.44	−0.78*	0.29	0.21	0.23	0.02	0.08	–
(d) Monocots and eudicots										
K_{flower}	–	−0.14	−0.41	0.71*	0.07	−0.03	–	−0.07	0.15	0.55
Area	−0.1	–	0.54	−0.01	0.43	−0.38	–	−0.14	−0.19	0.33
VLA	−0.63*	0.53	–	−0.45	0.3	−0.42	–	−0.39	−0.42	−0.12
Huber ratio	0.14	0.16	0.1	–	0.22	−0.48	–	−0.55	0.38	0.8**
g_{min}	0.2	0.42	0.3	0.35	–	−0.19	–	−0.24	−0.75*	0.32
Stomatal density	0.03	−0.54	−0.55	−0.47	−0.14	–	–	1.00***	0.08	−0.46
Stomatal length	–	–	–	–	–	–	–	–	–	–
SPI	0.12	−0.09	−0.5	−0.53	−0.1	0.97***	–	–	0.15	−0.46
log(T_{des})	0.13	−0.16	−0.34	0.2	−0.63	−0.49	–	−0.54	–	0.24
FMA	0.07	0.43	0.17	0.83**	0.48	−0.46	–	−0.44	0.15	–

Stomatal length is omitted from (d) because so few species had stomata. Trait correlations are in the upper triangle and phylogenetic independent contrast correlations are in the lower triangle. Spearman rank correlation coefficients are shown.
*$P < 0.05$
**$P < 0.01$
***$P < 0.001$

Fig. 3) and contrasts ($r_s = -0.46$, df = 14, $P = 0.05$), neither the trait correlation ($P = 0.07$) nor the contrast correlation ($P = 0.20$) was significant among the basal angiosperms. Although excluding *Calycanthus* from the entire dataset caused the correlation between T_{des} and *VLA* to be insignificant, the independent contrast correlations between T_{des} and *VLA* were significant whether or not *Calycanthus* was included for the entire dataset (Table 2).

DISCUSSION

Aerial plant structures often face hot, dry conditions that can lead to desiccation and impair physiological function. Despite the constraint of maintaining positive water balance in distal structures, there is substantial variation in how different structures and species avoid desiccation. For example, leaf hydraulic architecture has been optimized to efficiently transport large

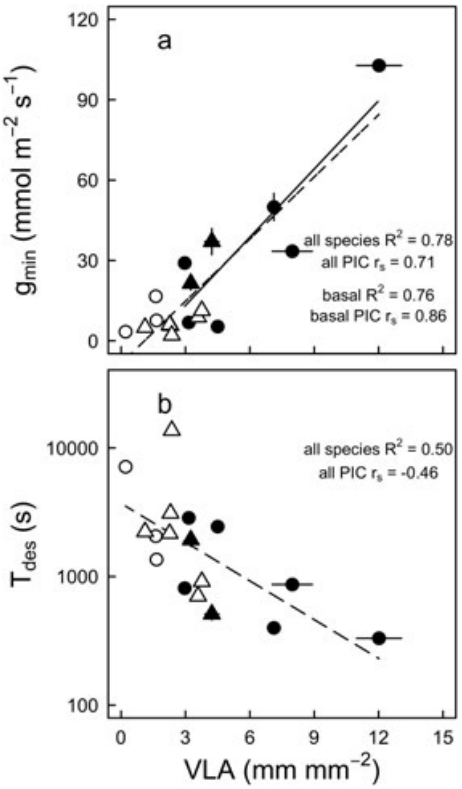

Figure 3. (a) Coordination between vein length per area and g_{min}. (b) Tradeoff between vein length per area and Tdes. Solid lines represent linear fits only through the basal angiosperm and magnoliid points, and dashed lines represent linear fits through all species. Point symbols are the same as in Fig. 2.

fluxes of water for sustained periods of time, although there is substantial variation among species, depending, for example, on leaf lifespan and dry mass investment (Simonin *et al.* 2012). By coordinating rates of water loss with rates of water supply, most leaves are able to avoid large declines in water content that could restrict carbon assimilation and cause irreversible hydraulic failure. Flowers, by contrast, need not assimilate carbon, so maintaining a high capacity for transporting water may not be advantageous. Furthermore, prior evidence has suggested that there may be large differences among major angiosperm clades in how flowers maintain water balance (Chapotin *et al.* 2003; Feild *et al.* 2009b; Feild *et al.* 2009a; Trolinder *et al.* 1993).

Almost all of the hydraulic and structural traits measured in the current study showed significant variation among species with basal angiosperms (*Illicium* and magnoliids) having trait values consistent with higher carbon and water costs (Fig. 1). Basal angiosperm flowers had more abundant stomata, higher g_{min}, higher *VLA*, higher K_{flower}, and also higher *FMA* than flowers of the monocots and eudicots. Basal angiosperm flowers also showed strong coordination between K_{flower}, *VLA*, and g_{min}, suggesting that these traits may be critical in determining floral hydraulic capacity among these lineages (Figs 2 & 3). Monocot and eudicot flowers, by contrast, had lower values of all traits measured except the Huber ratio, and trait values were much less variable among the monocots and eudicots. Monocot and eudicot flowers may have a common physiological trait syndrome despite large variation in other floral characters such as size, shape, and developmental origin. For example, in our dataset, monocot and eudicot flowers varied nearly 75-fold in flower size (Table 1), yet exhibited little variation in area-normalized trait values (Figs 1–3). Indeed, lower physiological and structural costs have likely relaxed the strength of non-pollinator selection on monocot and eudicot flowers and allowed morphological traits important to pollination to vary more widely.

The evolutionary pattern of trait variation (Fig. 1) and prior measurements of water potentials and gas exchange (Chapotin *et al.* 2003; Feild *et al.* 2009b; Feild *et al.* 2009a; Trolinder *et al.* 1993) suggest that there may be fundamental differences among clades in how flowers are built and hydrated. Magnoliid flowers, including both *Magnolia* (Feild *et al.* 2009b) and *Calycanthus* (Roddy et al., in prep.), have high transpiration rates and maintain a functional connection to the stem xylem for water supply. Magnoliid flowers are developmentally similar with undifferentiated perianths composed of dense, tough tepals (high *FMA*; Fig. 1). Many of these characteristics are common to other basal angiosperm lineages as well; trait variation for *Illicium* flowers (Figs 1 & 3) is consistent with their having similarly costly flowers that maintain a hydraulic connection to the stem xylem (Feild *et al.* 2009a). These common characteristics suggest that ANA grade and magnoliid flowers may rely predominantly on xylem delivery of water throughout anthesis to replace transpired water and maintain turgor. In order to maintain high transpiration rates and rely on xylem delivery of water throughout anthesis, basal angiosperm flowers have an efficient water transport system with highly branched veins and a high K_{flower}. Significant trait and PIC correlations among the basal angiosperms suggest that these flowers may maintain water balance by coordinating water supply and loss over relatively short timescales, similar to angiosperm leaves (Figs 2 & 3).

In contrast, monocot and eudicot flowers exhibited little interspecific variation in hydraulic and structural traits and consistently had trait values associated with low fluxes of water and flowers with lower carbon investment. Although there were no significant relationships between stomatal traits and K_{flower} (Table 2), stomatal abundance may nonetheless be important in determining the physiological strategies these flowers use. Stomata provide an open pathway for water inside the flower to evaporate into the atmosphere, and their near absence from many flowers may be an efficient way to prevent transpiration from tissues incapable of assimilating CO_2. Stomatal traits were consistently and positively correlated with g_{min}, suggesting that the near absence of stomata from monocot and eudicot flowers is critical in minimizing water loss. Furthermore, while angiosperm leaves can actively close their stomata to prevent desiccation (Brodribb and McAdam 2011; McAdam and Brodribb 2012), floral stomata may be limited in their ability to close (Hew *et al.* 1980) and, even when they do close, may not significantly curtail water loss (Feild *et al.* 2009b; Teixido and Valladares 2014). Indeed, even under conditions that would induce stomatal closure,

g_{min} was lower among the monocots and eudicots than among basal angiosperm flowers (Figs 1 & 2a). Among the monocots and eudicots, stomata may not play an important role in actively regulating water loss from flowers. Rather, water loss through the cuticle may have more strongly influenced the evolution of traits responsible for supplying water and maintaining turgor.

Reduced transpiration rates resulting from low stomatal abundance and low g_{min} would have cascading consequences on other hydraulic and structural traits. Without the need to supply high fluxes of water to meet transpirational demands, constraints on the floral vascular system would be relaxed, allowing flowers to have a less ramified, less carbon intensive hydraulic system than those used by leaves, which have much longer functional lifespans (Roddy *et al.* 2013). Lower rates of water supply and loss would lengthen the turnover time of flower water, meaning that stored water (i.e. hydraulic capacitance) may be critical for maintaining turgor (Chapotin *et al.* 2003) and delaying desiccation (Fig. 3b). Stored water may buffer changes in water supply rates from changes in water loss rates, which has been shown to occur in leafy shoots (Hunt and Nobel 1987) and which may explain why environmental conditions can have little impact on sap flow rates to flowers and inflorescences (Higuchi and Sakuratani 2005; Roddy and Dawson 2012). Because rates of water supply can be low, the phloem contribution of water might be a relatively larger fraction of the total water needed by the flower. In reality, flowers probably rely on a combination of water imported via the xylem and phloem and on stored water to maintain turgor throughout anthesis. The relative contributions of these sources, however, may be highly variable among species and throughout floral development.

CONCLUSIONS

Flowers are one of the key innovations of the angiosperms and are incredibly diverse morphologically, yet the physiological costs of flowers can limit the extent to which floral morphology can be moulded by pollinator selection. Flowers of the diverse monocot and eudicot clades have traits consistent with reduced rates of both water supply and water loss compared with basal angiosperms. This suggests that there may be substantial variation among these major clades in how flowers maintain turgor and prevent desiccation. One end of this continuum may be defined by maintaining a high hydraulic conductance to continuously supply water via the xylem, while the other end of the continuum may be defined by the reduction of water loss rates to delay desiccation. Reduced hydraulic conductance and greater reliance on stored water may physiologically buffer flowers from diurnal variability in the water status of other plant structures. Better understanding the mechanisms and timing of water transport to flowers and the tradeoffs between these mechanisms will be an important advancement in our understanding of floral physiology and its interaction with pollinator selection over evolutionary timescales. The present study establishes a comparative framework for characterizing the hydraulic mechanisms and strategies used by flowers.

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