Hydraulic conductance and the maintenance of water balance in flowers

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Abstract

The production and maintenance of flowers is critical for successful reproduction in the angiosperms, yet we know little about how flowers remain hydrated under desiccating conditions. Here we quantify the variability in floral hydraulic conductance (K_{flower}) for a phylogenetically diverse set of 17 species from 10 angiosperm families and relate the variability in K_{flower} to traits associated with the liquid phase transport of water into and through flowers and traits associated with the vapor phase diffusion of water vapor from floral surfaces. We found that K_{flower} was coordinated with traits associated with water supply (vein length per area, VLA) and loss (minimum epidermal conductance, g_{min}), particularly among the magnoliids. Among the monocots and eudicots, K_{flower} was largely insensitive to variation in VLA, suggesting that the low VLA of these species may require them to rely on other mechanisms of maintaining turgor than continuous supply of water by the xylem. K_{flower} varied considerably among species, and its reduction among the monocots and eudicots may have relaxed physiological constraints on flowers and allowed floral morphological traits to be more efficiently molded by pollinator selection in these clades.

Key words: angiosperms, flower, hydraulic conductance, vein density, water balance

Introduction

The primary function of flowers in most angiosperms is to promote sexual reproduction by either attracting animal pollinators or promoting pollen dispersal by wind or water. Animal pollinators have long been thought to be one of the most important agents of selection on flowers (Sprengel 1793; Darwin 1888), and numerous studies have shown that among narrowly defined groups, pollinator selection can influence a variety of morphological traits, such as color, size, and shape (Galen 2000; Bradshaw & Schemske 2003; Whittall & Hodges 2007; Hopkins & Rausher 2012). However, flowers are subject to the same biophysical constraints of resource supply and demand as other plant structures. These non-pollinator agents of selection, though rarely studied, can also exert selective pressures on floral traits, often in opposition to the preferences of pollinators (Strauss & Whittall, 2006). For example, pollinators generally prefer larger, more showy flowers, but larger flowers have higher water requirements that can exact physiological costs on the rest of the plant (Galen et al. 1999; Lambrecht & Dawson 2007; Lambrecht 2013). Flowers of many species are often located in the hottest, driest parts of the plant canopy, which can influence the amount of water they require throughout the day (Roddy & Dawson 2012). Knowing how flowers remain turgid and attractive to pollinators is fundamental to understanding their structure, function, and evolution.

Compared to other structures, such as leaves and stems (Feild et al. 2009; Pittermann 2010), the hydraulic architecture of flowers has received little attention. Presuming that water influx and efflux must be in balance over some time scale, the mass balance of flower water can be defined as:

$$\Delta W = J - E \qquad \text{(eqn 1)}$$

where W is the water content, and J and E the rates of water supply and loss, respectively. If mass balance of water is to be maintained, then $\Delta W = 0$, and J must equal E. Various factors can influence these rates of water loss from and water supply to flowers. Water can be transpired from flowers via stomata or evaporate through the cuticle from epidermal cells. The relative contributions of these two pathways to the total transpirational flux are unknown and may vary among species. In some taxa, flowers are unable to regulate epidermal conductances to water vapor (Hew et al. 1980; Teixido & Valladares 2014), while in other species stomata remain capable of opening and closing (Azad et al. 2007; Feild et al. 2009). Whether or not transpiration is regulated by stomata, maintaining high rates of floral transpiration would require that flowers have constant, ample supplies of water. The most efficient way of providing large amounts of water to flowers

would be via the xylem, the tissue predominantly used for transporting water throughout the plant. Flowers of some early-diverging, 'basal' angiosperms and magnoliids (the genera *Illicium* (Schisandraceae) and *Magnolia* (Magnoliaceae)) have been shown to have water potentials more negative than the stem, indicating that they are hydraulically connected to the stem xylem (Feild et al. 2009; Feild et al. 2009). In contrast, some eudicot flowers and petals tend to have higher, less negative water potentials than subtending bracts and leaves (Trolinder et al. 1993; Chapotin et al. 2003). These 'reverse' water potential gradients imply that for flowers to remain hydrated, water must be imported against an apoplastic water potential gradient in the xylem. This could be performed 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; Windt et al. 2009). Relying on phloem-delivered water instead of xylem-delivered water would mean that J in eqn 1 would probably be lower, and in order to keep $\Delta W = 0$, then E must also be lower. In other words, to maintain a constant water content, J and E must be coordinated such that reductions in water supply associated with a possible switch to phloem-hydration would be accompanied by reductions in water loss.

The dichotomy between xylem- and possibly phloem-hydrated flowers leads to the prediction that there may be substantial variation in the xylem hydraulic conductance of flowers (K_{flower}). Indeed, the xylem in some taxa may not be continuous from pedicel to petal, and the point of discontinuity could be a source of high resistance to apoplastic water flow (Lersten & Wemple 1966). If flowers are hydrated by the phloem, then we would expect the xylem pathway to be occluded or otherwise nonfunctional to prevent backflow of water to the stem, which would result in a lowered K_{flower}. Low values of K_{flower} could be associated with lower rates of water loss (E), which would mitigate the need for maintaining a high hydraulic supply capacity (J). Thus, coordinated changes in water supply and water loss should correlate with changes in K_{flower}, and the phloem may be capable of supplying enough water to flowers to maintain water balance if E is sufficiently low. Regardless of whether the phloem is involved in supplying significant amounts of water to flowers, there may nonetheless be significant variation in xylem hydraulic conductance among flowers which may correspond to other traits associated with water supply and loss.

By comparison, leaf hydraulic conductance (K_{leaf}) is highly variable among species and influenced by both environmental conditions and anatomical traits (Sack et al. 2003; Brodribb & Jordan 2011). Maintaining water balance within thresholds that prevent significant dehydration

requires coordination between traits associated with water supply and traits associated with water loss. In leaves, a few important traits have been shown to be critical in maintaining water balance (Boyce et al. 2009; Brodribb et al. 2013). In terms of water supply, vein length per area (*VLA*; or vein density) is one of the major traits controlling leaf hydraulic conductance (Sack & Frole 2006; Brodribb et al. 2007; Brodribb & Jordan 2011). Leaves with higher *VLA* can deliver liquid water closer to the sites of evaporation in the leaf to prevent desiccation of the photosynthetic tissues during CO_2 exchange with the atmosphere. By increasing the supply of water closer to the sites of evaporation, leaves can maintain higher stomatal conductances and photosynthetic rates. Furthermore, *VLA* has been shown to be a critical determinant of leaf hydraulic supply capacity across diverse land plant lineages and increased dramatically during angiosperm evolution (Brodribb et al. 2007; Boyce et al. 2009; Brodribb & Feild 2010; Feild et al. 2011; Feild et al. 2011). In terms of water loss from leaves, maximum stomatal conductance is tightly coordinated with stomatal size and density, all of which are coordinated with hydraulic conductance of the leaf lamina (Sack et al. 2003; Brodribb et al. 2013). However, flowers generally have few stomata, which would mean that cuticular leakiness may have a larger effect on flower water loss than stomata.

In the present study, we measured whole flower hydraulic conductance (K_{flower}) for 17 species, including one previously published species, from 10 angiosperm families that included the magnoliids, monocots, and the eudicots. We sought to determine the variability of K_{flower} among species and the anatomical and physiological traits associated with this variation. In addition to *VLA* and stomatal traits, we also measured the Huber ratio (ratio of xylem cross-sectional area to evaporative surface area) and the minimum epidermal conductance to water vapor under non-transpiring conditions (g_{min}). We made four predictions about the hydraulic efficiency and maintenance of water balance in flowers. First, we predicted that there would be positive correlations between water supply traits and water loss traits. Positive correlations between traits in these two suites would suggest that these traits are involved in synchronizing the liquid phase transport of water into flowers and the regulation of vapor phase water losses from flowers. Further, such positive correlations would support the hypothesis that the constraints of maintaining water balance have required coordinated changes in water supply and water loss. Second, we predicted that there would be positive correlations among traits within the two suites of traits. As a result, we predicted that K_{flower} would correlate with both water supply and water loss traits. Coordination between K_{flower} and water balance traits would provide strong support that these traits are mechanistically related to the hydraulic capacity of flowers.

126
127
128 **Materials and Methods**
129 *Plant material*
130 We collected flowering shoots from around the University of California, Berkeley, campus
131 and from the University of California Botanic Garden during the springs of 2013 and 2014. All
132 plants had been kept well-watered. We chose a phylogenetically diverse set of species that varied by
133 almost two orders of magnitude in floral display size (Table 1). These species also varied
134 morphologically, from flowers with undifferentiated perianths to those with a fully differentiated
135 calyx and corolla and from those with free petals to those with sympetalous connation.
136 Additionally, we included inflorescences of *Cornus florida* (Cornaceae), which have small,
137 inconspicuous flowers but large, white bracts as their showy organs. Although the showy floral
138 structures of this set of species are not homologous, they all serve the same function, namely
139 pollinator attraction. For each species, we measured K_{flower} on at least three flowers, and most species
140 had low variance among individual flowers. Sample sizes for each species are shown in Table 1.
141
142 *Measurements of hydraulic conductance*
143 We used a low pressure flow meter to measure hydraulic conductance of whole flowers and
144 developing flower buds (Kolb et al. 1996). This method has been shown to be insensitive to
145 variation in irradiance and measures the capacity for water transport into the leaf or flower (Sack et
146 al. 2002). We chose this method rather than the evaporative flux method because the evaporative
147 flux method depends on maximizing boundary layer conductance. Because of the morphological
148 complexities of flowers (i.e. unlike leaves, flowers are rarely planar), we were not confident we could
149 maximize the boundary layer conductance to obtain realistic maximum values of K_{flower}. However,
150 the low pressure flow meter has the potential to clear any xylem occlusion. We tested this on a
151 subset of species by (1) comparing flow rates when increasing the vacuum pressure to flow rates
152 measured when decreasing the vacuum pressure and (2) by repeatedly measuring the same flower.
153 We found no differences between flow rates measured while increasing the vacuum or while
154 decreasing the vacuum and no significant increase in K_{flower} with subsequent measurements (data not
155 shown).
156 Flowering shoots were excised early in the morning (before 9:00 am) when stem water
157 potentials of plants growing in this area are generally higher than -0.25 MPa. Cut shoots were

158 immediately recut under distilled water at least one node apical to the first cut and transported back
159 to the lab in a covered bucket to minimize water loss. Shoots were kept in water in the covered
160 bucket for at least one hour during transport and after returning to the lab before any flowers were
161 excised, allowing for relaxation of xylem tension. We only measured the most recently opened
162 flowers on each plant, based on each flower's development relative to other flowers on the plant.
163 Once in the lab, individual flowers were excised underwater at the pedicel base and connected to
164 hard-walled tubing that led back to an electronic balance (Sartorius CPA225, Sartorius, Goettingen,
165 Germany), on which sat a vial of dilute electrolyte solution (10 mM KCl, filtered to 0.2 um and
166 degassed the morning of measurements). Flowers were placed in a cylindrical acrylic chamber that
167 was attached to a vacuum pump and lined with wet paper towels. Flow rates of KCl solution into
168 the leaf or flower from the balance were measured every 10-60 seconds depending on the absolute
169 flow rate under 5-6 different pressures ranging from 15 to 60 kPa below ambient. At each pressure,
170 flow rates were allowed to stabilize for 3-20 minutes and until the coefficient of variation of the last
171 ten readings was, ideally, less than 5% and the instantaneous measurements converged on the
172 average of the last ten measurements. In practice, low absolute flow rates meant that stable averages
173 could be reached but the coefficient of variation often remained above 5%. To determine K_{flower}, we
174 linearly regressed the flow rates versus pressure and removed, at most, one outlying point from the
175 regression. Immediately after measurements, we scanned the flowers to determine the one-sided
176 projected surface area of all perianth parts, which we used to normalize hydraulic conductance to
177 calculate K_{flower} in units of mmol s^{-1} m^{-2} MPa^{-1}. For comparison of measurements made using the low
178 pressure flow meter to measurements made using other methods, values of K_{flower} for *Magnolia*
179 *grandiflora* reported by Feild et al. (2009b) using a different method were approximately equivalent to
180 values produced using our method for congeneric species, and maximum measurements of K_{flower} in
181 the field based on Darcy's Law for *Calycanthus occidentalis* approached those measured using the low
182 pressure flow meter (data not shown).
183 To compare our measured values of K_{flower} to values of K_{leaf}, we compiled data from a variety
184 of papers that reported K_{leaf} for both temperate (Nardini & Salleo 2000; Sack et al. 2002; Nardini et
185 al. 2005; Gullo et al. 2005; Scoffoni et al. 2008) and tropical (Sack & Frole 2006) plants.
186
187 *Trait measurements*
188 The Huber ratio is the ratio of the xylem cross-sectional area to the evaporative surface area.
189 In the laboratory, pedicels of the flowers measured for K_{flower} were sliced underwater using a sharp

razor blade. 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 5-40x 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). We did not measure the area of individual xylem conduits, but instead quantified the amount of cross-sectional area that was occupied by xylem.

For flowers that had differentiated perianths, we made trait measurements only on the corolla because the petals comprised the largest evaporative surface area. 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 multiple 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 5-20x 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 Image J (version 1.44o; Rasband 2012).

The minimum epidermal conductance, g_{min}, is the area-normalized conductance to water vapor in the dark, after stomata have been allowed to close (Kerstiens 1996). We measured g_{min} on individual petals or tepals by sealing the cut edges with a thick layer of petroleum jelly and placing the structures in a dark box into which was placed 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 minutes, 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.

We used two methods to measure stomatal traits, stomatal density and guard cell length. First, we cleared sections 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 5-40x. We imaged 5-20 fields of view to determine stomatal densities, depending on the abundance of stomata. We also made stomatal impressions using dental putty (Coltene Whaledent President Light Body). Nail varnish impressions of the dental putty were then imaged 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. 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. (2003a).

We lacked some trait data for some species but, because of the paucity of K_{flower} measurements, we have chosen to include these species in the present analyses when possible. Data for *Magnolia grandiflora* were taken from Feild (Feild et al. 2009) and so lacked many of the traits we measured. There are no anatomical or structural traits for *Magnolia doltsopa*, so mean K_{flower} for this species occurs only in Table 1.

Statistical analyses

For correlations between traits and between traits and K_{flower}, we tested whether there was a significant correlation between variables using the conservative non-parametric Spearman rank correlation. We compared linear, logarithmic, power, and quadratic fits and chose the model with the lowest root mean square error (RMSE). Because calculating R^2 values from nonlinear fits is statistically inappropriate, instead we report the Spearman correlation coefficient, r_s. Clade-specific correlations were similarly examined, and correlations within the magnoliids were analyzed using linear regressions with their respective R^2 values reported. All analyses were performed in R (; v. 3.1.1; R Core Team 2012).

Results

K_{flower} varied widely among the species we studied from a mean of 1.30 mmol s^{-1} m^{-2} MPa^{-1} for *Cornus florida* inflorescences to 18.79 mmol s^{-1} m^{-2} MPa^{-1} for *Calycanthus occidentalis* flowers. *C. florida* inflorescences, whose showy organs are bracts, had the lowest K_{flower} of any species measured. Interestingly, the two magnoliid genera spanned most of the variation in K_{flower} of all species measured, and K_{flower} of these two genera were marginally significantly different (averages of 13.84 and 3.18 mmol s^{-1} m^{-2} MPa^{-1} for *Calycanthus* and *Magnolia*, respectively; P = 0.05). 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 *Cornus florida* inflorescences to 3.81 mmol s^{-1} m^{-2} MPa^{-1} for *Paeonia suffruticosa*.

If the abundance of xylem conduits were a major limitation to K_{flower}, then species with more abundant xylem, whether as veins in petals and tepals or as conduit cross-sectional area in pedicels, should have higher K_{flower}. In general, xylem traits related to water supply were significantly and

positively correlated with K_{flower} (Figure 1). There was a significant relationship between K_{flower} and VLA (r_s = 0.61, df = 13, P = 0.015), which was best fit by a quadratic relationship (RMSE = 1.53, df = 12). However, within clades only the magnoliids showed a significant effect of VLA on K_{flower} (R^2 = 0.93, F = 63.88, df = 4, P = 0.001). There was no significant relationship between K_{flower} and the Huber ratio (P = 0.84), nor between VLA and Huber ratio (P = 0.85; Figure 3).

Water loss traits were also predicted to correlate with K_{flower}. Consistent with this hypothesis, there was a significant relationship between K_{flower} and g_{min} (r_s = 0.52, df = 12, P = 0.05), which was best fit by a quadratic function (RMSE = 1.22, df = 11; Figure 2a). This relationship was driven predominantly by the large variation in g_{min} and K_{flower} among the Calycanthaceae. No relationships between stomatal traits and K_{flower} were significant, although species with higher stomatal densities (and thus also SPI) tended also to have higher K_{flower} (Figure 2b-d). It should be noted that leaves have much higher stomatal densities than measured here on flowers; angiosperm leaves range from 100 to 500 stomata per mm^2 of lamina surface area (Sack et al. 2003), while flowers in the present study had no more than 15 stomata per mm^2. The range of guard cell lengths we measured on flowers (12-32 um was consistent with guard cell size recorded for leaves (Sack et al. 2003).

These water loss traits were also predicted to correlate with each other. Stomata represent an open path for liquid water to evaporate from the flower intercellular spaces into the atmosphere, so higher abundances or larger stomata should increase the rate at which flowers lose water, particularly if stomata do not close completely (Hew et al. 1980). However, there was no significant relationship between g_{min} and stomatal traits (Figure 4a).

Because of the need to maintain water balance, water supply traits and water loss traits were predicted to correlate positively with each other. Indeed, the strongest correlation was between g_{min} and VLA (r_s = 0.60, df = 10, P < 0.05), which was best described by a quadratic function (RMSE = 6.96, df = 9; Figure 5a). The strength of this correlation, combined with the strength of correlation between each of these traits and K_{flower}, strengthens the importance of these two water balance traits in influencing floral hydraulic architecture. Within clades, the correlation between g_{min} and VLA was significant only for the magnoliids (R^2 = 0.88, F = 30.82, df = 3, P = 0.012). No other correlations between water supply and water loss traits were significant (Figure 5b-d).

The hydraulic conductance of flowers is almost as variable as that of leaves (Figure 6). However, most of this variation was due to variation among the magnoliids (the genera *Magnolia* and *Calycanthus* in the current study), which had values of hydraulic conductance even higher than some leaves. Average K_{flower} of the eudicots was slightly lower than that of the monocots, which was lower

than that of the magnoliids. Thus, most flowers, particularly of the monocots and eudicots, had conductances in the low ends of the distributions for leaves and for stems. Only three species in this combined dataset have had hydraulic conductance values measured for both leaves and flowers, and for all of them K_{leaf} was higher than K_{flower} (*Calycanthus floridus* leaves and flowers averaged 13.44 (Nardini & Salleo 2000) and 10.38 mmol m^{-2} s^{-1} MPa^{-1}, respectively, *Magnolia soulangiana* leaves and flowers averaged 7.87 (Nardini & Salleo 2000)and 3.51 mmol m^{-2} s^{-1} MPa^{-1}, respectively, and *Magnolia grandiflora* leaves and flowers averaged 5.86 and 2.62 mmol m^{-2} s^{-1} MPa^{-1} (Feild et al. 2009), respectively).

Discussion

Maintaining water balance in desiccating environments requires coordination between water supply and water loss. As leaves or flowers transpire water, their water contents and water potentials decline, which drives the movement of water along the soil-plant-atmosphere continuum. In order to maintain turgor and physiological functioning, the capacity to transport liquid phase water should be tightly coordinated with the rates at which water is lost to the atmosphere. In leaves, differences between microenvironments, for example between sun and shade, induce coordinated changes in traits associated with liquid phase and vapor phase conductances to water, lending strong support to the idea that the need to maintain water balance is a critical determinant of leaf structure (Brodribb & Jordan 2011). Although they are much more ephemeral than leaves, flowers must remain turgid to attract pollinators and, as a result, may also need to maintain water balance throughout anthesis. How they do this has been a point of controversy, with some suggesting that water is delivered primarily by the phloem against the xylem water potential gradient (Trolinder et al. 1993; Chapotin et al. 2003) and others showing that water is imported by the xylem (Feild et al. 2009; Feild et al. 2009).

In the present study, we took a different approach to examining variability in the hydraulic capacity of recently opened flowers from a phylogenetically diverse set of species. Our results showed that K_{flower} is linked to anatomical and physiological traits associated with water supply and loss. K_{flower} increased significantly with increasing VLA (Figure 1), suggesting that the xylem is involved in the delivery of liquid water to petals and tepals. K_{flower} also increased with increasing g_{min}, suggesting that the capacity for water transport through flowers is coordinated with rates of water loss from them (Figure 2). The coordination between liquid phase water supply and vapor phase

water loss has been characterized for leaves (Brodribb & Jordan 2011), and the present study is, to our knowledge, the first to demonstrate such coordination for flowers (Figure 5). Our results further imply that the monocots and eudicots have minimized the hydraulic costs of flowers by coordinated changes in water balance traits and K_{flower} (Figure 5). Despite being highly variable, hydraulic conductances of flowers were generally lower than hydraulic conductances for leaves (Figure 6). From the inflorescences of *Cornus florida* to the undifferentiated perianths of monocots, there was remarkable convergence of K_{flower} across widely divergent morphologies and sizes among the monocots and eudicots.

Regulating water loss from flowers

Rates of water loss from flowers can be affected by physiological processes, as well as morphology and anatomy. More complex, three-dimensional morphologies with overlapping parts would thicken boundary layers and reduce the gross transpiration flux. Our method for measuring K_{flower} should be immune to boundary layer effects and allow us to elucidate the relationship between structure and function. Although the abundance of stomata both within individual flowers and among species is highly variable, floral stomata, unlike leaf stomata, may be limited in their ability to curtail water loss from flowers (Hew et al. 1980; Teixido & Valladares 2014; Feild et al. 2009). Although there were no significant correlations between stomatal traits and either K_{flower} or g_{min}, the abundance and positioning of floral stomata, like foliar stomata (Sack et al. 2003), may nonetheless influence water loss at least over microscopic spatial scales. Compared to leaves, lower stomatal densities on flowers increase the relative importance of the cuticular pathway for water loss, particularly among monocot and eudicot flowers, which had few, if any, stomata (Figure 4). As a result, g_{min} was a strong predictor of K_{flower} among species (Figure 2). If it is a general pattern that stomata on monocot and eudicot flowers do not close passively in response to environmental conditions or actively in response to hormonal signaling (Hew et al. 1980; Teixido & Valladares 2014), then floral stomata, when they are present, may represent an open path for uncontrolled water loss. While angiosperm leaves can actively close their stomata to prevent desiccation, floral stomata, even when they do close, seem incapable of significantly curtailing water loss (Hew et al. 1980; Brodribb & McAdam 2011; McAdam & Brodribb 2012; Teixido & Valladares 2014). Stomata provide an open pathway for water inside the flower to evaporate into the atmosphere, and the near absence of stomata from many flowers may be an efficient way to prevent transpiration from tissues incapable of assimilating CO_2. Thus, the role of stomata in regulating water loss from most flowers

may be not in their capability to close to prevent transpiration but rather in their near absence from many flowers, which forces transpirational losses to be cuticular. In leaves, this cuticular pathway is usually thought to be highly resistant to water loss.

Supplying water to flowers

For both flowers and fruits, there is controversy over the relative roles of the xylem and the phloem in delivering water. The water content of reproductive organs results from a balance between water supply by vascular tissues (xylem and phloem) and water losses to transpiration and possible backflow to the stem. There has been substantially more work aimed at understanding these dynamics in fruits. In various agricultural species, water flow to developing fruits early in development is considered to come predominantly via the xylem, but this contribution diminishes and is surpassed by the phloem contribution later in fruit development (Ho et al. 1987; Lang 1990; Greenspan et al. 1994). However, more recent studies have called into question the idea that the xylem becomes non-functional and that water import later in development is due solely to the phloem (Choat et al. 2009; Windt et al. 2009; Clearwater et al. 2012; Clearwater et al. 2013). Instead, water may be delivered by both xylem and phloem even if the xylem flow declines or fluctuates throughout development.

With comparatively less work done on flowers, the controversy over xylem- and phloem-delivery of water still remains unsolved. While the present study is incapable of determining whether the xylem or the phloem is the predominant source of water to open flowers, our results do suggest a role for the xylem, at least among some species. Among the magnoliids, increasing VLA had a significant effect on K_{flower}, while it had little effect on K_{flower} of the moncots and eudicots. (Figure 1). Increasing K_{flower} associated with increasing VLA strongly suggests that xylem is functional, consistent with other studies measuring water potential gradients (Feild et al. 2009; Feild et al. 2009). Interestingly, K_{flower} was insensitive to changes in VLA below a threshold of about 4 mm mm^{-2} (Figure 1a). The species with VLA below this threshold were composed predominantly of the monocots and eudicots. In a broader analysis of floral VLA variation, the vast majority of flowers had VLA below 4 mm mm^{-2}, although species with floral VLA above 4 mm mm^{-2} appear in groups across the angiosperm phylogeny (Roddy et al. 2013). Coincidentally, *Magnolia* species all had VLA around this threshold 4 mm mm^{-2}, and inner whorl and outer whorl tepals of *M. grandiflora* vary both in the direction of their water potential gradients and in VLA; inner whorl tepals had an average VLA of 3.26 mm mm^{-2} and water potential gradients indicative of phloem-hydration and outer

whorl tepals had a VLA of 5.18 mm mm^{-2} and water potential gradients indicative of xylem hydration (Feild et al. 2009). These results for different *M. grandiflora* tepals within the same flower further suggest that a threshold VLA of 4 mm mm^{-2} may be important in determining how flowers are hydrated.

The insensitivity of K_{flower} to VLA variation among these low VLA species has two possible implications. First, veins in these species may not conduct water during anthesis, and the predominant source of water for the flower may be delivered by the phloem in response to carbon demand. However, estimates of the water flux that could be delivered by the phloem given estimates of the carbon demand of flowers suggest that the phloem alone cannot supply enough water to meet transpiration (Chapotin et al. 2003). Second, the near absence of stomata on flowers with low VLA and the low g_{min} of these flowers may mean that the resistance in the hydraulic pathway between the vein and the epidermis may be so high that the flux rate of water through the veins may be spatially and temporally decoupled from rates of transpiration. VLA, relative to the distance between vein and stoma has been shown to be optimized for transpiration in leaves (Brodribb et al. 2007; Noblin et al. 2008; Zwieniecki & Boyce 2014). The low VLA in many flowers suggests that they may not be similarly optimized for supplying water for transpiration and that flowers may not rely on continuous delivery of water by the xylem. Instead, flowers may rely on water imported before anthesis and stored in their hydraulic capacitors. Flowers tend to have higher hydraulic capacitance than leaves (Chapotin et al. 2003), which may functionally isolate them from diurnal water potential variation in the rest of the plant (Trolinder et al. 1993). Further, water stored in hydraulic capacitors may buffer flow rates through veins from rates of transpiration, which may explain why sap flow rates to flowers and inflorescences can be little affected by environmental conditions (Higuchi & Sakuratani 2005; Roddy & Dawson 2012). Given the right conditions, water may flow from flowers back to stems, as has been shown for developing mango fruits (Higuchi & Sakuratani 2006).

There may be a strong phylogenetic component to the tradeoffs among the strategies described here. Our results suggest that variation in how flowers maintain water balance may be associated with coordinated shifts in anatomical and physiological traits. On one end of the spectrum would be species that maintain a high K_{flower} with high VLA, g_{min}, and stomatal densities. On the other end of the spectrum would be species that have low K_{flower} due to low VLA, g_{min}, and few or no stomata. The coordination of traits associated with water supply and water loss supports our hypothesis that the maintenance of water balance is fundamental to the functioning of flowers

and highlights, for the first time, that there may be variation in the hydraulic capacity of flowers. Despite the coordination of K_{flower} with structural and physiological traits, precisely how flowers remain turgid in desiccating microenvironments and the mechanisms of water import to flowers remain unknown. Better characterization of the coordinated shifts in traits associated with evolutionary transitions between the major angiosperm lineages could better elucidate how these strategies may have evolved.

Caveats about comparative physiology of non-homologous floral structures

Measuring floral physiology at the broad phylogenetic scales used in the current study requires comparing non-homologous structures. For example, the showy organs of flowers in our dataset included undifferentiated tepals of the magnoliids and monocots, bracts of *Cornus florida*, and petals of the eudicots (Table 1). Measuring and calculating K_{flower} and other traits required normalizing flux measurements by an evaporative surface area. In the present study, we chose to normalize by the showy display organs that would be attractive to pollinators responding to visual cues. For these flowers, the showy organs also represent the majority of their evaporative surfaces areas. Yet, other floral organs whose surface areas we did not measure could also contribute to transpirational water losses. While comparing non-homologous structures is not ideal, these structures nonetheless perform similar pollinator functions, which, we believe, warrants their comparison. We are not ruling out the possibility that there may, in fact, be differences between flowers associated with differences in perianth differentiation; indeed, our results suggest that among clades differing in perianth differentiation there is significant variation in hydraulic architecture. Future work could characterize more precisely how floral whorls may differ in hydraulic architecture and function.

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 molded by pollinator selection. Reducing the physiological costs of flowers would relax constraints on floral form. The diverse monocot and eudicot clades have traits consistent with reduced hydraulic capacity compared to the magnoliids. Reductions in both water loss and water supply among the monocot and eudicot clades may have relaxed physiological constraints on floral morphology and allowed greater lability of morphological traits. While

maintaining water balance may nonetheless be important to flowers, there may be substantial variation among species in how they remain turgid 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 separate 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 the possible mechanisms for maintaining water balance will be an important advancement in our understanding of floral physiology and its interaction with pollinator selection over evolutionary timescales.

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590 Figure legends

591

592 Figure 1. Coordination between whole flower hydraulic conductance (K_{flower}) and water supply traits:
593 (a) vein length per area (VLA; or vein density) and (b) the ratio of xylem cross-sectional area to
594 evaporative surface area (Huber ratio). Dashed lines indicate curve fits for all species, and solid lines
595 indicate linear fits for the magnoliids only. All points include error bars, some of which may be
596 smaller than the points themselves.

597

598 Figure 2. Coordination between K_{flower} and water loss traits: (a) minimum epidermal conductance
599 (g_{min}), (b) stomatal density, (c) stomatal length, (d) stomatal pore area index (SPI). Dashed lines
600 indicate curve fits for all species, and solid lines indicate linear fits for the magnoliids only. Point
601 symbols are the same as in Figure 1. All points include error bars, some of which may be smaller
602 than the points themselves.

603

604 Figure 3. Lack of correlation among the water supply traits, VLA and Huber ratio.

605

606 Figure 4. Correlations between g_{min} and (a) stomatal pore area index (SPI) and (b) stomatal density.
607 Statistically significant curve fits are shown. Points symbols are the same as in Figure 1.

608

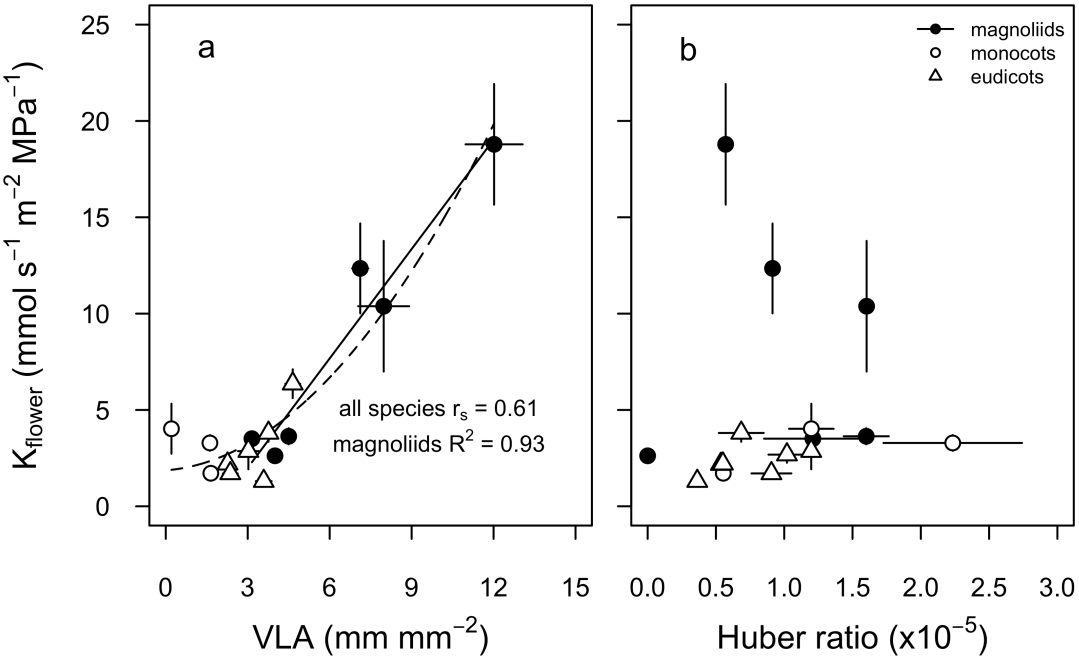
609 Figure 5. Coordination between water supply traits (VLA and Huber ratio) and water loss traits (g_{min}
610 and SPI). Statistically significant relationships are shown with curve fits and correlation coefficients.
611 Dashed lines indicate curve fits for all species, and solid lines indicate linear fits for the magnoliids
612 only. Point symbols are the same as in Figure 1. All points include error bars, some of which may
613 be smaller than the points themselves.

614

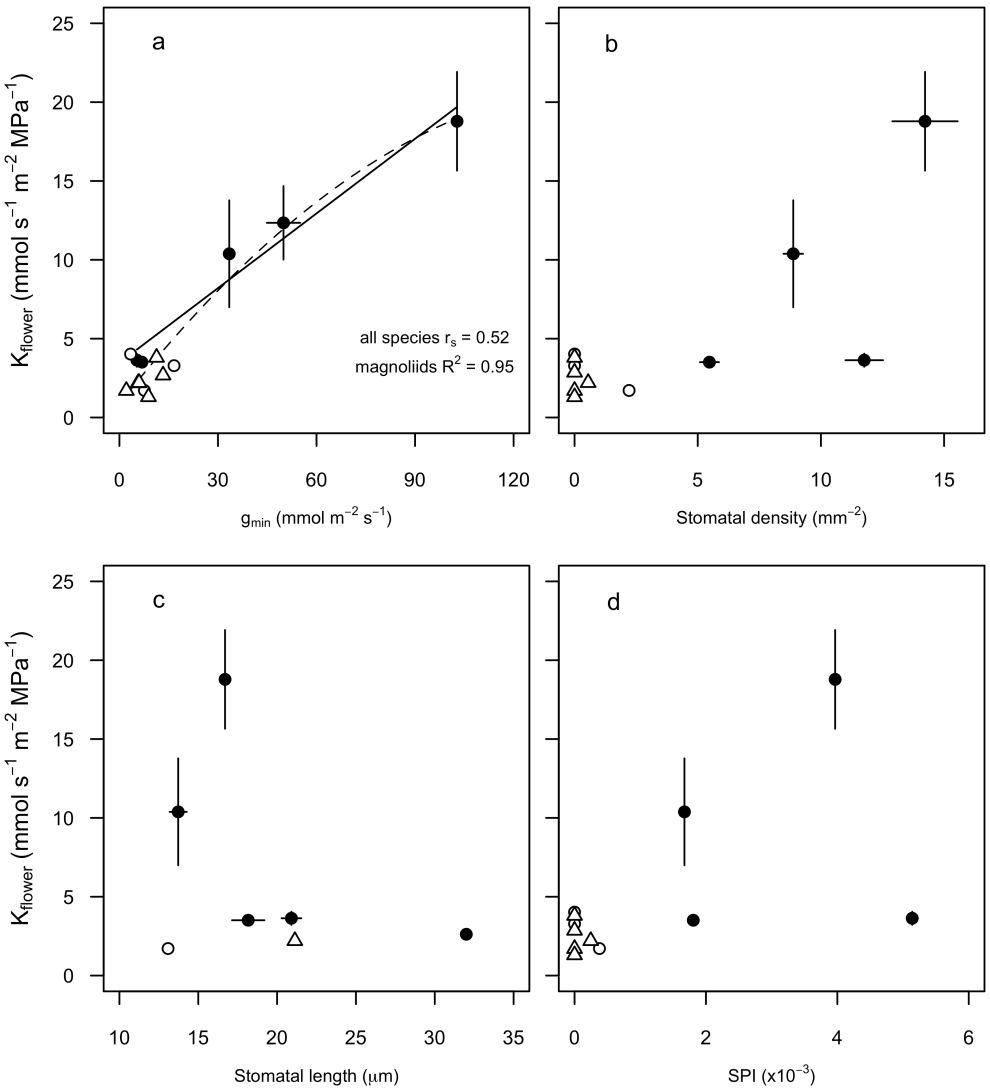
615 Figure 6. Variation among plant structures in hydraulic conductance normalized by the single-sided
616 evaporative surface area (i.e. total leaf or flower area). Groupings were chosen to highlight the
617 major differences within structures. Values below the bars indicate the number of species sampled
618 in each group. Data for flowers are from the current study and Feild et al.{%Feild 2009}; for
619 tropical leaves are from Sack and Frole{%Sack 2006}; for temperate leaves from Nardini and
620 Salleo{%Nardini 2000}, Sack et al.{%Sack 2002}, Nardini et al.{Nardini 2005}, Lo Gullo et
621 al.{%Gullo 2005}, Scoffoni et al.{%Scoffoni 2008}, Simonin et al.{%Simonin 2012}.

622

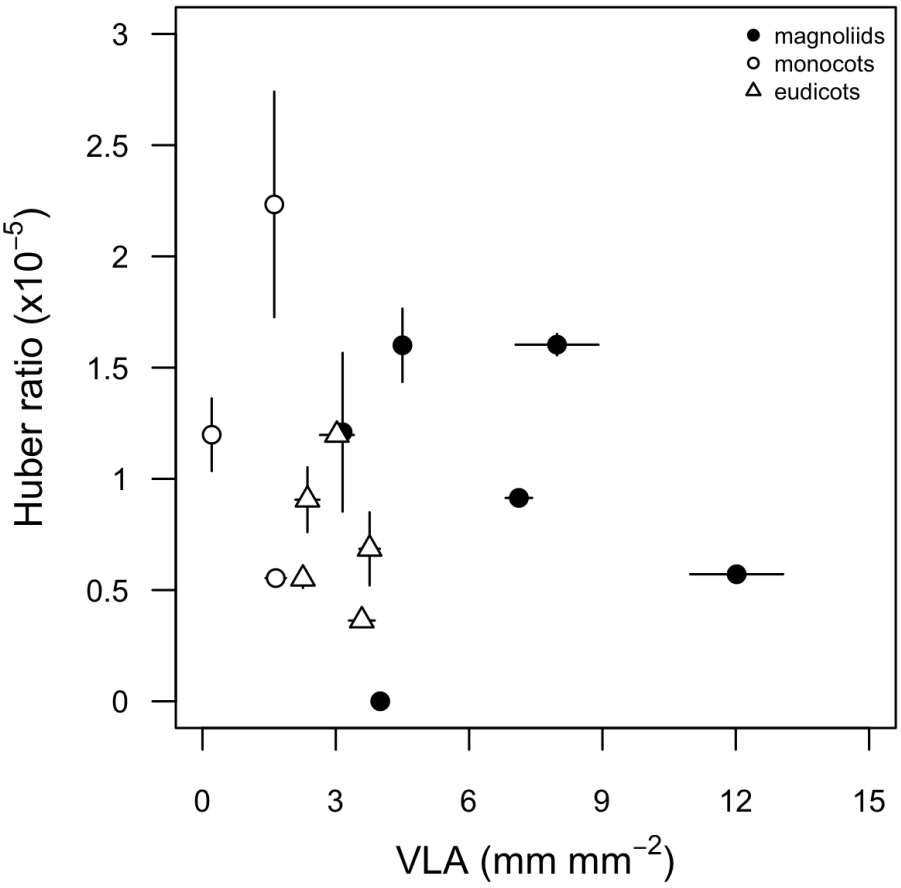
623 Figure 1



624 Figure 2



625 Figure 3



626 Figure 4
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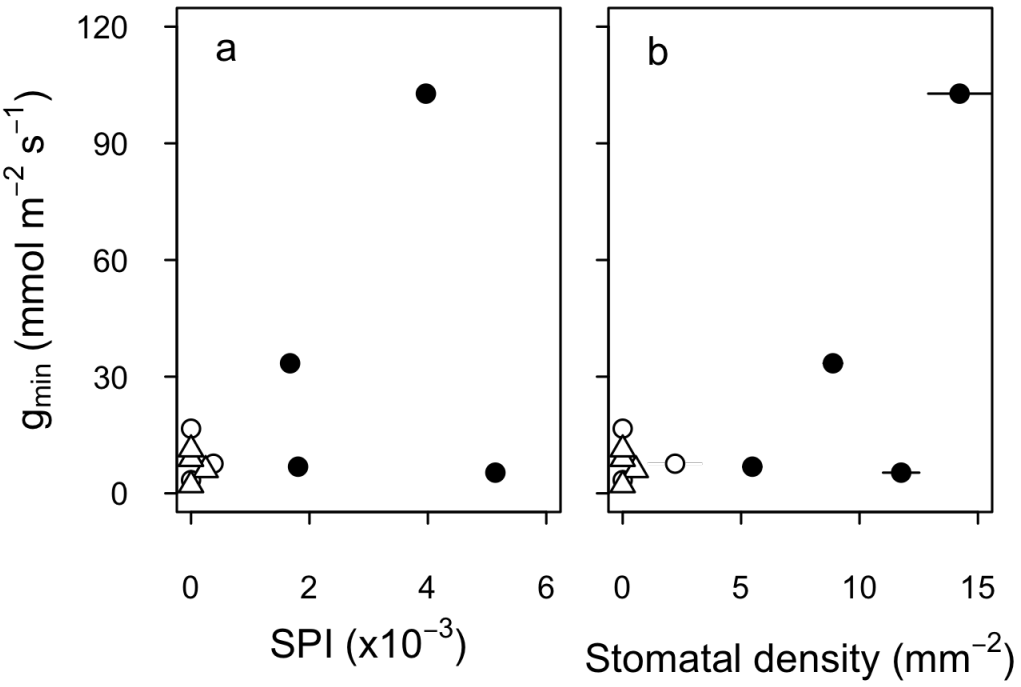
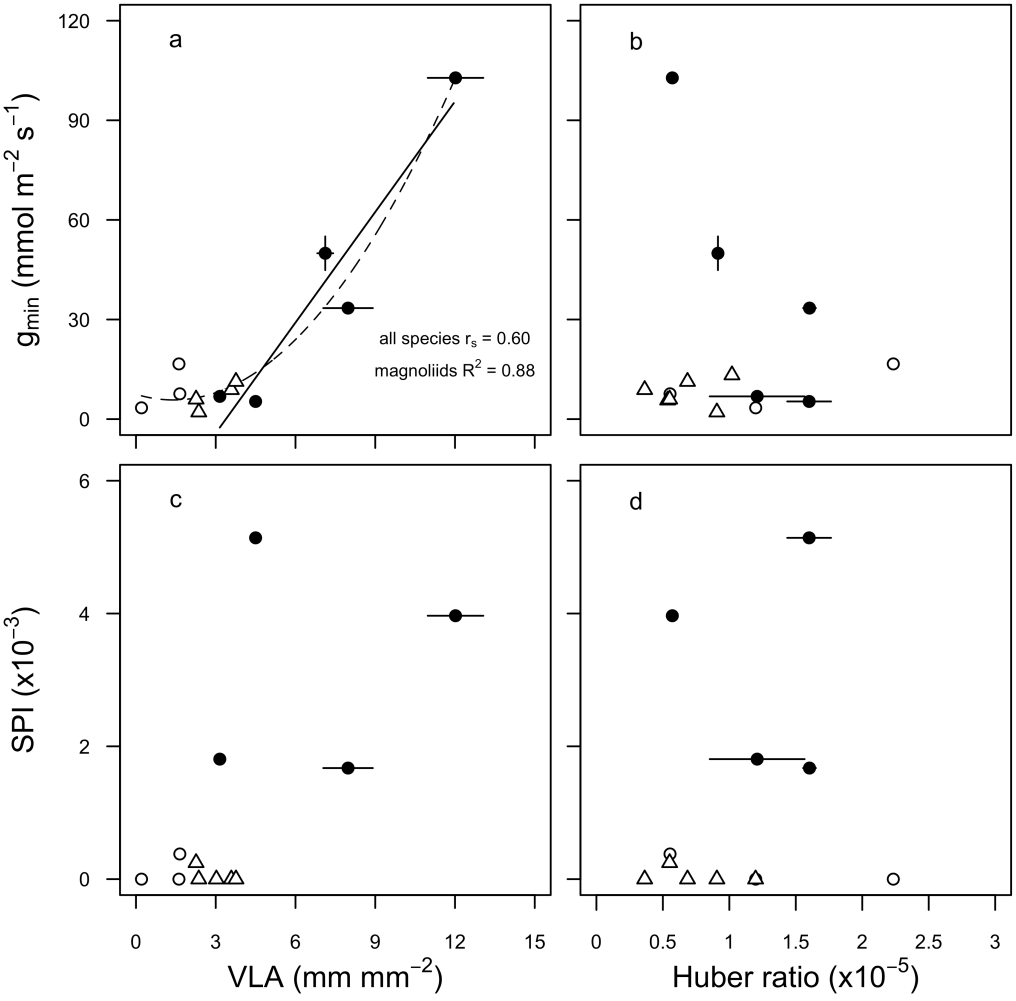


629 Figure 5



630 Figure 6
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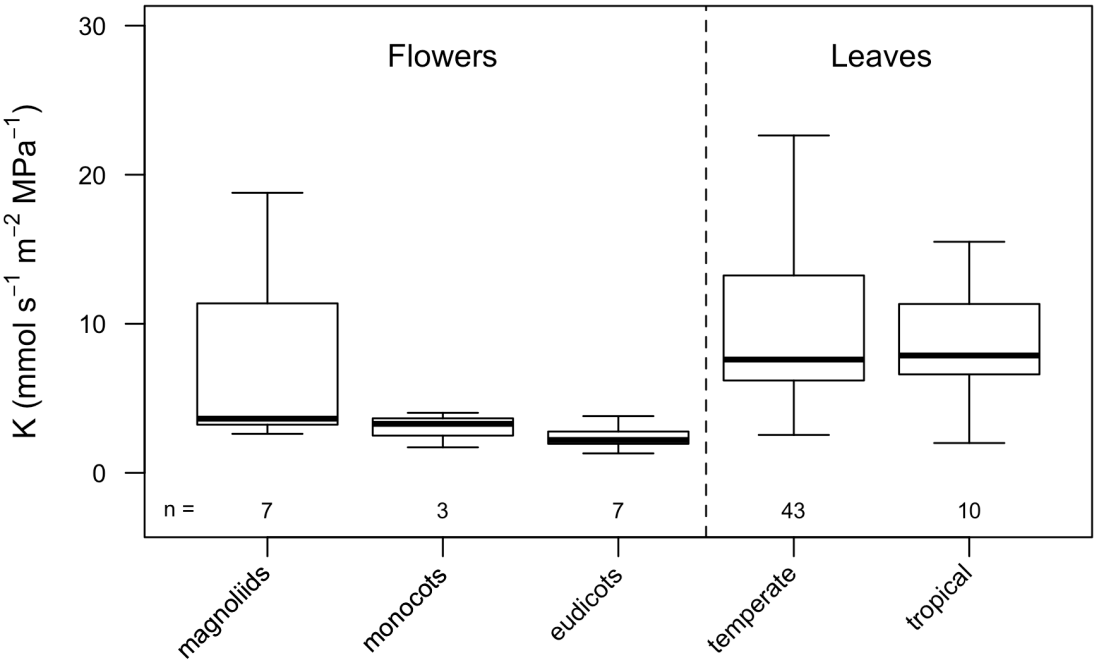

633 Table 1. List of species used in the current study and some structural and morphological traits.
634

Species	Family	Area (x10^{-3} m^2)	Mass per area (x10^{-3} g cm^{-2})	Perianth differentiation	Corolla fusion	n	k_{flower} (mmol m^{-2} s^{-1} MPa^{-1})
Agapanthus africanus	Amaryllidaceae	1.07	2.63	monochlamydeous	fused	4	4.02
Amphilophium buccinatorium	Bignoniaceae	6.95	7.73	dichlamydeous	fused	4	2.68
Calycanthus chinensis	Calycanthaceae	9.08	4.86	graded tepals (monochlamydeous)	unfused	7	12.34
Calycanthus floridus	Calycanthaceae	2.53	5.68	graded tepals (monochlamydeous)	unfused	4	10.38
Calycanthus occidentalis	Calycanthaceae	4.91	15.42	graded tepals (monochlamydeous)	unfused	5	18.79
Camellia yunnanensis	Theaceae	4.92	NA	dichlamydeous	unfused	5	2.86
Cornus florida	Cornaceae	4.76	1.87	dichlamydeous	unfused	3	1.31
Hemerocallis sp.	Xanthorrhoeaceae	13.43	4.65	monochlamydeous	unfused	10	3.29
Iris douglasiana	Iridaceae	5.30	1.88	monochlamydeous	unfused	5	1.71
Magnolia doltsopa	Magnoliaceae	22.69	NA	monochlamydeous	unfused	4	2.95
Magnolia grandiflora	Magnoliaceae	45.0	5.3	monochlamydeous	unfused	NA	2.62
Magnolia soulangiana	Magnoliaceae	24.44	2.58	monochlamydeous	unfused	13	3.50
Magnolia stellata	Magnoliaceae	11.07	2.00	monochlamydeous	unfused	6	3.63
Paeonia suffruticosa	Paeoniaceae	80.45	3.57	dichlamydeous	unfused	4	3.81

Species	Family	Area ($x10^{-3}$ m^2)	Mass per area ($x10^{-3}$ g cm^{-2})	Perianth differentiation	Corolla fusion	n	k_{flower} (mmol m^{-2} s^{-1} MPa^{-1})
Rhododendron johnstoneanum	Ericaceae	4.52	2.20	dichlamydeous	fused	4	2.21
Rhododendron loderi	Ericaceae	16.08	1.96	dichlamydeous	fused	4	2.19
Rhododendron protistum	Ericaceae	8.24	2.92	dichlamydeous	fused	5	1.71

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