Hydraulic conductance and the maintenance of water balance in flowers

Adam B. Roddy[1,2] and Todd E. Dawson[1]

[1]Department of Integrative Biology, University of California, Berkeley, CA, USA

[2]Present address: School of Forestry & Environmental Studies, Yale University, New Haven, CT, USA

Author of contact:

Adam B. Roddy

email: adam.roddy@yale.edu

phone: +1 510.224.4432

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

62 would be via the xylem, the tissue predominantly used for transporting water throughout the plant.
63 Flowers of some early-diverging, 'basal' angiosperms and magnoliids (the genera *Illicium*
64 (Schisandraceae) and *Magnolia* (Magnoliaceae)) have been shown to have water potentials more
65 negative than the stem, indicating that they are hydraulically connected to the stem xylem (Feild et al.
66 2009; Feild et al. 2009). In contrast, some eudicot flowers and petals tend to have higher, less
67 negative water potentials than subtending bracts and leaves (Trolinder et al. 1993; Chapotin et al.
68 2003). These 'reverse' water potential gradients imply that for flowers to remain hydrated, water
69 must be imported against an apoplastic water potential gradient in the xylem. This could be
70 performed by the phloem, which is primarily responsible for the transport of photosynthates
71 throughout the plant. In contrast to the xylem, the phloem has much higher hydraulic resistance
72 and lower water flux rates because phloem transport occurs symplastically (Münch 1930; Nobel
73 1983; Windt et al. 2009). Relying on phloem-delivered water instead of xylem-delivered water would
74 mean that J in eqn 1 would probably be lower, and in order to keep $\Delta W = 0$, then E must also be
75 lower. In other words, to maintain a constant water content, J and E must be coordinated such that
76 reductions in water supply associated with a possible switch to phloem-hydration would be
77 accompanied by reductions in water loss.
78 The dichotomy between xylem- and possibly phloem-hydrated flowers leads to the
79 prediction that there may be substantial variation in the xylem hydraulic conductance of flowers
80 (K_{flower}). Indeed, the xylem in some taxa may not be continuous from pedicel to petal, and the point
81 of discontinuity could be a source of high resistance to apoplastic water flow (Lersten & Wemple
82 1966). If flowers are hydrated by the phloem, then we would expect the xylem pathway to be
83 occluded or otherwise nonfunctional to prevent backflow of water to the stem, which would result
84 in a lowered K_{flower}. Low values of K_{flower} could be associated with lower rates of water loss (E), which
85 would mitigate the need for maintaining a high hydraulic supply capacity (J). Thus, coordinated
86 changes in water supply and water loss should correlate with changes in K_{flower}, and the phloem may
87 be capable of supplying enough water to flowers to maintain water balance if E is sufficiently low.
88 Regardless of whether the phloem is involved in supplying significant amounts of water to flowers,
89 there may nonetheless be significant variation in xylem hydraulic conductance among flowers which
90 may correspond to other traits associated with water supply and loss.
91 By comparison, leaf hydraulic conductance (K_{leaf}) is highly variable among species and
92 influenced by both environmental conditions and anatomical traits (Sack et al. 2003; Brodribb &
93 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

222 each species with stomata. The stomatal pore area index (SPI) was calculated as the product of
223 stomatal density and the square of average guard cell length, according to Sack et al. (2003a).
224 We lacked some trait data for some species but, because of the paucity of K_{flower}
225 measurements, we have chosen to include these species in the present analyses when possible. Data
226 for *Magnolia grandiflora* were taken from Feild (Feild et al. 2009) and so lacked many of the traits we
227 measured. There are no anatomical or structural traits for *Magnolia doltsopa*, so mean K_{flower} for this
228 species occurs only in Table 1.
229
230 *Statistical analyses*
231 For correlations between traits and between traits and K_{flower}, we tested whether there was a
232 significant correlation between variables using the conservative non-parametric Spearman rank
233 correlation. We compared linear, logarithmic, power, and quadratic fits and chose the model with
234 the lowest root mean square error (RMSE). Because calculating R^2 values from nonlinear fits is
235 statistically inappropriate, instead we report the Spearman correlation coefficient, r_s. Clade-specific
236 correlations were similarly examined, and correlations within the magnoliids were analyzed using
237 linear regressions with their respective R^2 values reported. All analyses were performed in R (; v.
238 3.1.1; R Core Team 2012).
239
240
241 **Results**
242 K_{flower} varied widely among the species we studied from a mean of 1.30 mmol s^{-1} m^{-2} MPa^{-1} for
243 *Cornus florida* inflorescences to 18.79 mmol s^{-1} m^{-2} MPa^{-1} for *Calycanthus occidentalis* flowers. *C. florida*
244 inflorescences, whose showy organs are bracts, had the lowest K_{flower} of any species measured.
245 Interestingly, the two magnoliid genera spanned most of the variation in K_{flower} of all species
246 measured, and K_{flower} of these two genera were marginally significantly different (averages of 13.84
247 and 3.18 mmol s^{-1} m^{-2} MPa^{-1} for *Calycanthus* and *Magnolia*, respectively; P = 0.05). The monocots
248 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*
249 *africanus*, while the eudicots ranged from 1.30 mmol s^{-1} m^{-2} MPa^{-1} for *Cornus florida* inflorescences to
250 3.81 mmol s^{-1} m^{-2} MPa^{-1} for *Paeonia suffruticosa*.
251 If the abundance of xylem conduits were a major limitation to K_{flower}, then species with more
252 abundant xylem, whether as veins in petals and tepals or as conduit cross-sectional area in pedicels,
253 should have higher K_{flower}. In general, xylem traits related to water supply were significantly and

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

259 Water loss traits were also predicted to correlate with K_{flower}. Consistent with this hypothesis,
260 there was a significant relationship between K_{flower} and g_{min} (r_s = 0.52, df = 12, P = 0.05), which was
261 best fit by a quadratic function (RMSE = 1.22, df = 11; Figure 2a). This relationship was driven
262 predominantly by the large variation in g_{min} and K_{flower} among the Calycanthaceae. No relationships
263 between stomatal traits and K_{flower} were significant, although species with higher stomatal densities
264 (and thus also SPI) tended also to have higher K_{flower} (Figure 2b-d). It should be noted that leaves
265 have much higher stomatal densities than measured here on flowers; angiosperm leaves range from
266 100 to 500 stomata per mm^2 of lamina surface area (Sack et al. 2003), while flowers in the present
267 study had no more than 15 stomata per mm^2. The range of guard cell lengths we measured on
268 flowers (12-32 um was consistent with guard cell size recorded for leaves (Sack et al. 2003).

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

274 Because of the need to maintain water balance, water supply traits and water loss traits were
275 predicted to correlate positively with each other. Indeed, the strongest correlation was between g_{min}
276 and VLA (r_s = 0.60, df = 10, P < 0.05), which was best described by a quadratic function (RMSE =
277 6.96, df = 9; Figure 5a). The strength of this correlation, combined with the strength of correlation
278 between each of these traits and K_{flower}, strengthens the importance of these two water balance traits
279 in influencing floral hydraulic architecture. Within clades, the correlation between g_{min} and VLA was
280 significant only for the magnoliids (R^2 = 0.88, F = 30.82, df = 3, P = 0.012). No other correlations
281 between water supply and water loss traits were significant (Figure 5b-d).

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

286 than that of the magnoliids. Thus, most flowers, particularly of the monocots and eudicots, had
287 conductances in the low ends of the distributions for leaves and for stems. Only three species in
288 this combined dataset have had hydraulic conductance values measured for both leaves and flowers,
289 and for all of them K_{leaf} was higher than K_{flower} (*Calycanthus floridus* leaves and flowers averaged 13.44
290 (Nardini & Salleo 2000) and 10.38 mmol m^{-2} s^{-1} MPa^{-1}, respectively, *Magnolia soulangiana* leaves and
291 flowers averaged 7.87 (Nardini & Salleo 2000)and 3.51 mmol m^{-2} s^{-1} MPa^{-1}, respectively, and *Magnolia*
292 *grandiflora* leaves and flowers averaged 5.86 and 2.62 mmol m^{-2} s^{-1} MPa^{-1} (Feild et al. 2009),
293 respectively).
294
295
296 **Discussion**
297 Maintaining water balance in desiccating environments requires coordination between water
298 supply and water loss. As leaves or flowers transpire water, their water contents and water potentials
299 decline, which drives the movement of water along the soil-plant-atmosphere continuum. In order
300 to maintain turgor and physiological functioning, the capacity to transport liquid phase water should
301 be tightly coordinated with the rates at which water is lost to the atmosphere. In leaves, differences
302 between microenvironments, for example between sun and shade, induce coordinated changes in
303 traits associated with liquid phase and vapor phase conductances to water, lending strong support to
304 the idea that the need to maintain water balance is a critical determinant of leaf structure (Brodribb
305 & Jordan 2011). Although they are much more ephemeral than leaves, flowers must remain turgid
306 to attract pollinators and, as a result, may also need to maintain water balance throughout anthesis.
307 How they do this has been a point of controversy, with some suggesting that water is delivered
308 primarily by the phloem against the xylem water potential gradient (Trolinder et al. 1993; Chapotin
309 et al. 2003) and others showing that water is imported by the xylem (Feild et al. 2009; Feild et al.
310 2009).
311 In the present study, we took a different approach to examining variability in the hydraulic
312 capacity of recently opened flowers from a phylogenetically diverse set of species. Our results
313 showed that K_{flower} is linked to anatomical and physiological traits associated with water supply and
314 loss. K_{flower} increased significantly with increasing *VLA* (Figure 1), suggesting that the xylem is
315 involved in the delivery of liquid water to petals and tepals. K_{flower} also increased with increasing g_{min},
316 suggesting that the capacity for water transport through flowers is coordinated with rates of water
317 loss from them (Figure 2). The coordination between liquid phase water supply and vapor phase

318 water loss has been characterized for leaves (Brodribb & Jordan 2011), and the present study is, to
319 our knowledge, the first to demonstrate such coordination for flowers (Figure 5). Our results
320 further imply that the monocots and eudicots have minimized the hydraulic costs of flowers by
321 coordinated changes in water balance traits and K_{flower} (Figure 5). Despite being highly variable,
322 hydraulic conductances of flowers were generally lower than hydraulic conductances for leaves
323 (Figure 6). From the inflorescences of *Cornus florida* to the undifferentiated perianths of monocots,
324 there was remarkable convergence of K_{flower} across widely divergent morphologies and sizes among
325 the monocots and eudicots.
326
327 *Regulating water loss from flowers*
328 Rates of water loss from flowers can be affected by physiological processes, as well as
329 morphology and anatomy. More complex, three-dimensional morphologies with overlapping parts
330 would thicken boundary layers and reduce the gross transpiration flux. Our method for measuring
331 K_{flower} should be immune to boundary layer effects and allow us to elucidate the relationship between
332 structure and function. Although the abundance of stomata both within individual flowers and
333 among species is highly variable, floral stomata, unlike leaf stomata, may be limited in their ability to
334 curtail water loss from flowers (Hew et al. 1980; Teixido & Valladares 2014; Feild et al. 2009).
335 Although there were no significant correlations between stomatal traits and either K_{flower} or g_{min}, the
336 abundance and positioning of floral stomata, like foliar stomata (Sack et al. 2003), may nonetheless
337 influence water loss at least over microscopic spatial scales. Compared to leaves, lower stomatal
338 densities on flowers increase the relative importance of the cuticular pathway for water loss,
339 particularly among monocot and eudicot flowers, which had few, if any, stomata (Figure 4). As a
340 result, g_{min} was a strong predictor of K_{flower} among species (Figure 2). If it is a general pattern that
341 stomata on monocot and eudicot flowers do not close passively in response to environmental
342 conditions or actively in response to hormonal signaling (Hew et al. 1980; Teixido & Valladares
343 2014), then floral stomata, when they are present, may represent an open path for uncontrolled
344 water loss. While angiosperm leaves can actively close their stomata to prevent desiccation, floral
345 stomata, even when they do close, seem incapable of significantly curtailing water loss (Hew et al.
346 1980; Brodribb & McAdam 2011; McAdam & Brodribb 2012; Teixido & Valladares 2014). Stomata
347 provide an open pathway for water inside the flower to evaporate into the atmosphere, and the near
348 absence of stomata from many flowers may be an efficient way to prevent transpiration from tissues
349 incapable of assimilating CO_2. Thus, the role of stomata in regulating water loss from most flowers

350 may be not in their capability to close to prevent transpiration but rather in their near absence from
351 many flowers, which forces transpirational losses to be cuticular. In leaves, this cuticular pathway is
352 usually thought to be highly resistant to water loss.
353
354 *Supplying water to flowers*
355 For both flowers and fruits, there is controversy over the relative roles of the xylem and the
356 phloem in delivering water. The water content of reproductive organs results from a balance
357 between water supply by vascular tissues (xylem and phloem) and water losses to transpiration and
358 possible backflow to the stem. There has been substantially more work aimed at understanding
359 these dynamics in fruits. In various agricultural species, water flow to developing fruits early in
360 development is considered to come predominantly via the xylem, but this contribution diminishes
361 and is surpassed by the phloem contribution later in fruit development (Ho et al. 1987; Lang 1990;
362 Greenspan et al. 1994). However, more recent studies have called into question the idea that the
363 xylem becomes non-functional and that water import later in development is due solely to the
364 phloem (Choat et al. 2009; Windt et al. 2009; Clearwater et al. 2012; Clearwater et al. 2013). Instead,
365 water may be delivered by both xylem and phloem even if the xylem flow declines or fluctuates
366 throughout development.
367 With comparatively less work done on flowers, the controversy over xylem- and phloem-
368 delivery of water still remains unsolved. While the present study is incapable of determining
369 whether the xylem or the phloem is the predominant source of water to open flowers, our results do
370 suggest a role for the xylem, at least among some species. Among the magnoliids, increasing VLA
371 had a significant effect on K_{flower}, while it had little effect on K_{flower} of the moncots and eudicots.
372 (Figure 1). Increasing K_{flower} associated with increasing *VLA* strongly suggests that xylem is
373 functional, consistent with other studies measuring water potential gradients (Feild et al. 2009; Feild
374 et al. 2009). Interestingly, K_{flower} was insensitive to changes in *VLA* below a threshold of about 4 mm
375 mm^{-2} (Figure 1a). The species with *VLA* below this threshold were composed predominantly of the
376 monocots and eudicots. In a broader analysis of floral *VLA* variation, the vast majority of flowers
377 had *VLA* below 4 mm mm^{-2}, although species with floral *VLA* above 4 mm mm^{-2} appear in groups
378 across the angiosperm phylogeny (Roddy et al. 2013). Coincidentally, *Magnolia* species all had *VLA*
379 around this threshold 4 mm mm^{-2}, and inner whorl and outer whorl tepals of *M. grandiflora* vary both
380 in the direction of their water potential gradients and in *VLA;* inner whorl tepals had an average
381 *VLA* of 3.26 mm mm^{-2} and water potential gradients indicative of phloem-hydration and outer

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

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

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

414 and highlights, for the first time, that there may be variation in the hydraulic capacity of flowers.
415 Despite the coordination of K_{flower} with structural and physiological traits, precisely how flowers
416 remain turgid in desiccating microenvironments and the mechanisms of water import to flowers
417 remain unknown. Better characterization of the coordinated shifts in traits associated with
418 evolutionary transitions between the major angiosperm lineages could better elucidate how these
419 strategies may have evolved.
420
421 *Caveats about comparative physiology of non-homologous floral structures*
422 Measuring floral physiology at the broad phylogenetic scales used in the current study
423 requires comparing non-homologous structures. For example, the showy organs of flowers in our
424 dataset included undifferentiated tepals of the magnoliids and monocots, bracts of *Cornus florida*, and
425 petals of the eudicots (Table 1). Measuring and calculating K_{flower} and other traits required
426 normalizing flux measurements by an evaporative surface area. In the present study, we chose to
427 normalize by the showy display organs that would be attractive to pollinators responding to visual
428 cues. For these flowers, the showy organs also represent the majority of their evaporative surfaces
429 areas. Yet, other floral organs whose surface areas we did not measure could also contribute to
430 transpirational water losses. While comparing non-homologous structures is not ideal, these
431 structures nonetheless perform similar pollinator functions, which, we believe, warrants their
432 comparison. We are not ruling out the possibility that there may, in fact, be differences between
433 flowers associated with differences in perianth differentiation; indeed, our results suggest that among
434 clades differing in perianth differentiation there is significant variation in hydraulic architecture.
435 Future work could characterize more precisely how floral whorls may differ in hydraulic architecture
436 and function.
437
438 *Conclusions*
439 Flowers are one of the key innovations of the angiosperms and are incredibly diverse
440 morphologically, yet the physiological costs of flowers can limit the extent to which floral
441 morphology can be molded by pollinator selection. Reducing the physiological costs of flowers
442 would relax constraints on floral form. The diverse monocot and eudicot clades have traits
443 consistent with reduced hydraulic capacity compared to the magnoliids. Reductions in both water
444 loss and water supply among the monocot and eudicot clades may have relaxed physiological
445 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.

Acknowledgments

We thank P.V.A. Fine, D.D. Baldocchi, and D.D. Ackerly for useful comments on previous drafts of this manuscript, K.A. Simonin for insightful discussions, A. Schürkmann for technical assistance, and H. Forbes for facilitating access to plant material at the U.C. Botanic Garden. This work was funded by grants from the Jepson Herbarium and the Department of Integrative Biology, UC-Berkeley. A.B.R. was supported by a US NSF Graduate Research Fellowship.

463 **References**

464 Azad K., Sawa Y., Ishikawa T., Shibata H. (2007) Temperature-dependent stomatal movement in
465 tulip petals controls water transpiration during flower opening and closing. *Annals of Applied*
466 *Biology* **150,** 81-87.

467 Boyce C.K., Brodribb T.J., Feild T.S., Zwieniecki M.A. (2009) Angiosperm leaf vein evolution was
468 physiologically and environmentally transformative. *Proceedings of the Royal Society B* **276,** 1771-
469 1776.

470 Bradshaw H.D., Schemske D.W. (2003) Allele substitution at a flower colour locus produces a
471 pollinator shift in monkeyflowers. *Nature* **426,** 176-178.

472 Brodribb T.J., Feild T.S. (2010) Leaf hydraulic evolution led a surge in leaf photosynthetic capacity
473 during early angiosperm diversification. *Ecology Letters* **13,** 175-183.

474 Brodribb T.J., Feild T.S., Jordan G.J. (2007) Leaf maximum photosynthetic rate and venation are
475 linked by hydraulics. *Plant Physiology* **144,** 1890-1898.

476 Brodribb T.J., Jordan G.J. (2011) Water supply and demand remain balanced during leaf acclimation
477 of *Nothofagus cunninghamii* trees. *The New Phytologist* **192,** 437-448.

478 Brodribb T.J., Jordan G.J., Carpenter R.J. (2013) Unified changes in cell size permit coordinated leaf
479 evolution. *The New Phytologist* **199,** 559-570.

480 Brodribb T.J., McAdam S.A. (2011) Passive origins of stomatal control in vascular plants. *Science*
481 **331,** 582-585.

482 Chapotin S.M., Holbrook N.M., Morse S.R., Gutiérrez M.V. (2003) Water relations of tropical dry
483 forest flowers: Pathways for water entry and the role of extracellular polysaccharides. *Plant,*
484 *Cell & Environment* **26,** 623-630.

485 Choat B., Gambetta G.A., Shackel K.A., Matthews M.A. (2009) Vascular function in grape berries
486 across development and its relevance to apparent hydraulic isolation. *Plant Physiology* **151,**
487 1677-1687.

488 Clearwater M.J., Luo Z., Ong S.E., Blattmann P., Thorp T.G. (2012) Vascular functioning and the
489 water balance of ripening kiwifruit (*Actinidia chinensis*) berries. *Journal of Experimental Botany* **63,**
490 1835-1847.

491 Clearwater M.J., Ong S.E.C., Li K.T. (2013) Sap flow and vascular functioning during fruit
492 development. *Acta Horticulturae* **991,** 385-392.

493 Darwin C. (1888) *The various contrivances by which orchids are fertilised by insects.* John Murray, London.

494 Feild T.S., Brodribb T.J., Iglesias A., Chatelet D.S., Baresch A., Upchurch G.R., Gomez B., Mohr
495 B.A., Coiffard C., et al. (2011) Fossil evidence for cretaceous escalation in angiosperm leaf
496 vein evolution. *Proceedings of the National Academy of Sciences* **108,** 8363-8366.
497 Feild T.S., Chatelet D.S., Brodribb T.J. (2009) Ancestral xerophobia: A hypothesis on the whole
498 plant ecophysiology of early angiosperms. *Geobiology* **7,** 237-264.
499 Feild T.S., Chatelet D.S., Brodribb T.J. (2009) Giant flowers of southern magnolia are hydrated by
500 the xylem. *Plant Physiology* **150,** 1587-1597.
501 Feild T.S., Upchurch Jr G.R., Chatelet D.S., Brodribb T.J., Grubbs K.C., Samain M.-S., Wanke S.
502 (2011) Fossil evidence for low gas exchange capacities for early cretaceous angiosperm
503 leaves. *Paleobiology* **37,** 195-213.
504 Galen C. (2000) High and dry: Drought stress, sex-allocation trade-offs, and selection on flower size
505 in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *The American Naturalist* **156,** 72-
506 83.
507 Galen C., Sherry R.A., Carroll A.B. (1999) Are flowers physiological sinks or faucets? Costs and
508 correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* **118,** 461-470.
509 Greenspan M.D., Shackel K.A., Matthews M.A. (1994) Developmental changes in the diurnal water
510 budget of the grape berry exposed to water deficits. *Plant, Cell & Environment* **17,** 811-820.
511 Gullo M.A.L., Nardini A., Trifilò P., Salleo S. (2005) Diurnal and seasonal variations in leaf hydraulic
512 conductance in evergreen and deciduous trees. *Tree Physiology* **25,** 505-512.
513 Hew C.S., Lee G.L., Wong S.C. (1980) Occurrence of non-functional stomata in the flowers of
514 tropical orchids. *Annals of Botany* **46,** 195-201.
515 Higuchi H., Sakuratani T. (2005) The sap flow in the peduncle of the mango (*Mangifera indica* L.)
516 inflorescence as measured by the stem heat balance method. *Journal of the Japanese Society of*
517 *Horticultural Science* **74,** 109-114.
518 Higuchi H., Sakuratani T. (2006) Water dynamics in mango (*Mangifera indica* L.) Fruit during the
519 young and mature fruit seasons as measured by the stem heat balance method. *Journal of the*
520 *Japanese Society for Horticultural Science* **75,** 11-19.
521 Ho L.C., Grange R.I., Picken A.J. (1987) An analysis of the accumulation of water and dry matter in
522 tomato fruit. *Plant, Cell & Environment* **10,** 157-162.
523 Hopkins R., Rausher M.D. (2012) Pollinator-mediated selection on flower color allele drives
524 reinforcement. *Science* **335,** 1090-1092.

Kerstiens G. (1996) Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* **47,** 1813-1832.

Kolb K.J., Sperry J.S., Lamont B.B. (1996) A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *Journal of Experimental Botany* **47,** 1805-1810.

Lambrecht S.C. (2013) Floral water costs and size variation in the highly selfing *Leptosiphon bicolor* (*polemoniaceae*). *International Journal of Plant Sciences* **174,** 74-84.

Lambrecht S.C., Dawson T.E. (2007) Correlated variation of floral and leaf traits along a moisture availability gradient. *Oecologia* **151,** 574-583.

Lang A. (1990) Xylem, phloem and transpiration flows in developing apple fruits. *Journal of Experimental Botany* **41,** 645-651.

Lersten N.R., Wemple D.K. (1966) The discontinuity plate, a definitive floral characteristic of the Psoraleae (Leguminosae). *American Journal of Botany* 548-555.

McAdam S.A.M., Brodribb T.J. (2012) Stomatal innovation and the rise of seed plants. *Ecology letters* **15,** 1-8.

Münch E. (1930) *Die Stoffbewegungen in der Pflanze.*Gustav Fischer, Jena, Germany.

Nardini A., Gortan E., Salleo S. (2005) Hydraulic efficiency of the leaf venation system in sun- and shade-adapted species. *Functional Plant Biology* **32,** 953.

Nardini A., Salleo S. (2000) Limitation of stomatal conductance by hydraulic traits: Sensing or preventing xylem cavitation? *Trees* **15,** 14-24.

Nobel P.S. (1983) *Biophysical Plant Physiology and Ecology.* W.H. Freeman, San Francisco.

Noblin X., Mahadevan L., Coomaraswamy I.A., Weitz D.A., Holbrook N.M., Zwieniecki M.A. (2008) Optimal vein density in artificial and real leaves. *Proceedings of the National Academy of Sciences* **105,** 9140-9144.

Pittermann J. (2010) The evolution of water transport in plants: An integrated approach. *Geobiology* **8,** 112-139.

Rasband W.S. (2012) <http://imagej.nih.gov/ij/> Bethesda, MD, USA: National Institutes of Health.

R Core Team. (2012) <http://www.R-project.org/> Vienna, Austria: R Foundation for Statistical Computing.

Roddy A.B., Dawson T.E. (2012) Determining the water dynamics of flowering using miniature sap flow sensors. *Acta Horticulturae* **951,** 47-53.

556 Roddy A.B., Guilliams C.M., Lilittham T., Farmer J., Wormser V., Pham T., Fine P.V.A., Feild T.S.,
557 Dawson T.E. (2013) Uncorrelated evolution of leaf and petal venation patterns across the
558 angiosperm phylogeny. *Journal of Experimental Botany* **64,** 4081-4088.
559 Sack L., Cowan P.D., Jaikumar N., Holbrook N.M. (2003) The hydrology of leaves: Co-ordination
560 of structure and function in temperate woody species. *Plant, Cell & Environment* **26,** 1343-
561 1356.
562 Sack L., Frole K. (2006) Leaf structural diversity is related to hydraulic capacity in tropical rain forest
563 trees. *Ecology* **87,** 483-491.
564 Sack L., Melcher P.J., Zwieniecki M.A., Holbrook N.M. (2002) The hydraulic conductance of the
565 angiosperm leaf lamina: A comparison of three measurement methods. *Journal of Experimental*
566 *Botany* **53,** 2177-2184.
567 Scoffoni C., Pou A., Aasamaa K., Sack L. (2008) The rapid light response of leaf hydraulic
568 conductance: New evidence from two experimental methods. *Plant, Cell & Environment* **31,**
569 1803-1812.
570 Sprengel C.K. (1793) *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen.*
571 Friedrich Vieweg dem aeltern, Berlin.
572 Strauss S.Y., Whittall J.B. (2006) Non-pollinator agents of selection on floral traits. In: *Ecology and*
573 *Evolution of Flowers.* pp. 120-138. Oxford: Oxford University Press.
574 Teixido A.L., Valladares F. (2014) Disproportionate carbon and water maintenance costs of large
575 corollas in hot mediterranean ecosystems. *Perspectives in Plant Ecology, Evolution and Systematics*
576 **16,** 83-92.
577 Trolinder N.L., McMichael B.L., Upchurch D.R. (1993) Water relations of cotton flower petals and
578 fruit. *Plant, Cell & Environment* **16,** 755-760.
579 Whittall J.B., Hodges S.A. (2007) Pollinator shifts drive increasingly long nectar spurs in columbine
580 flowers. *Nature* **447,** 706-709.
581 Windt C.W., Gerkema E., Van As H. (2009) Most water in the tomato truss is imported through the
582 xylem, not the phloem: A nuclear magnetic resonance flow imaging study. *Plant Physiology*
583 **151,** 830-842.
584 Zwieniecki M.A., Boyce C.K. (2014) Evolution of a unique anatomical precision in angiosperm leaf
585 venation lifts constraints on vascular plant ecology. *Proceedings of the Royal Society B* **281,**
586 20132829.
587

Figure legends

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

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

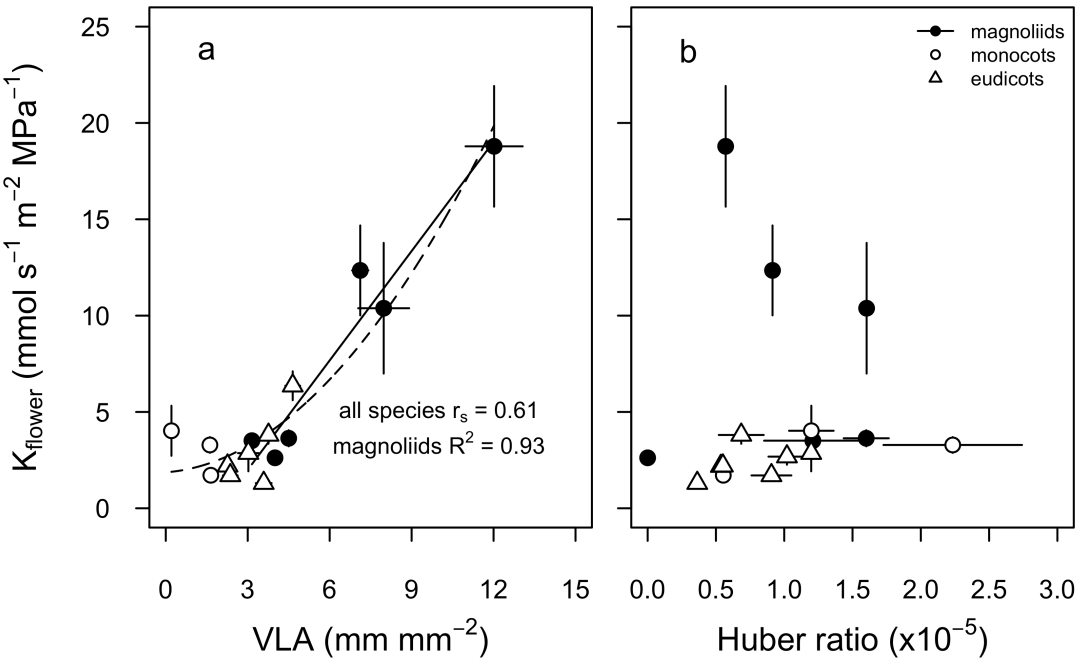
Figure 3. Lack of correlation among the water supply traits, *VLA* and Huber ratio.

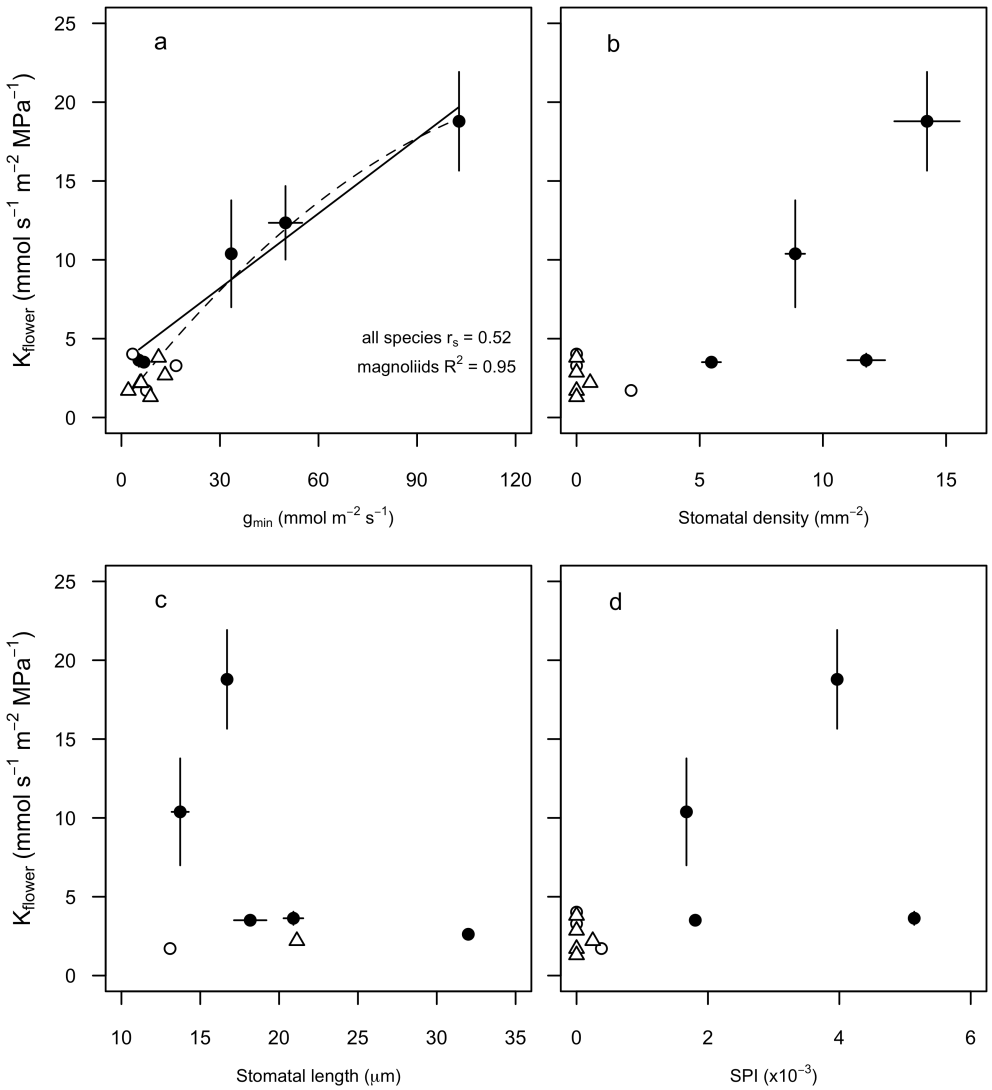
Figure 4. Correlations between g_{min} and (a) stomatal pore area index (SPI) and (b) stomatal density. Statistically significant curve fits are shown. Points symbols are the same as in Figure 1.

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

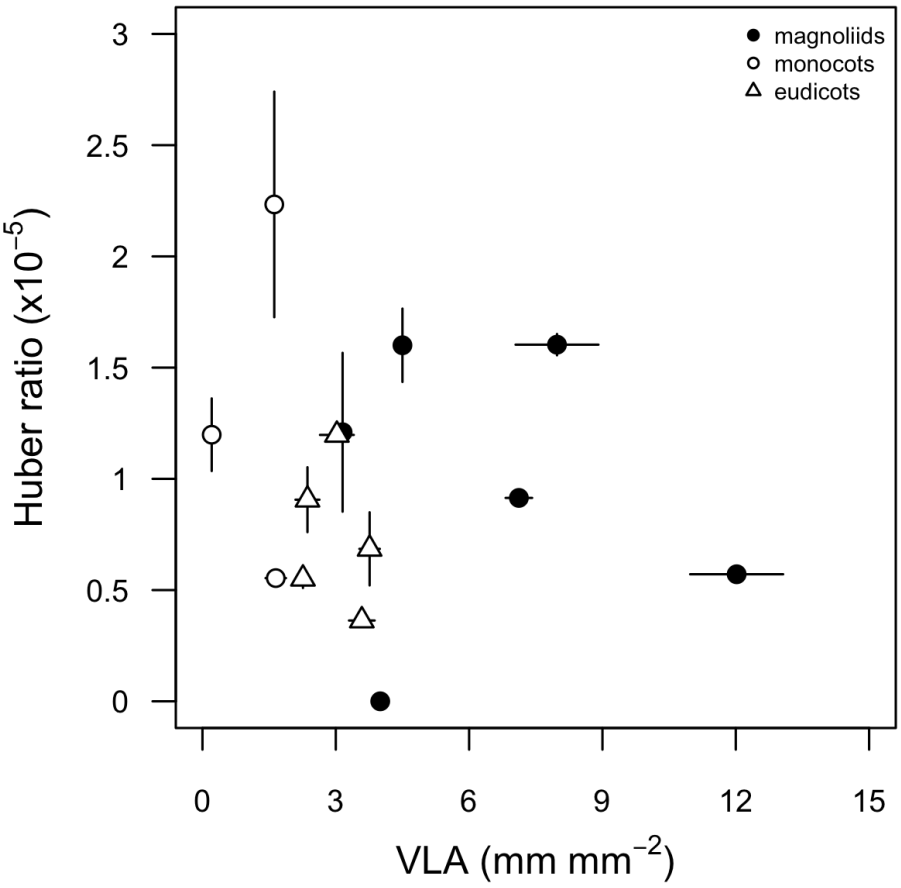
Figure 6. Variation among plant structures in hydraulic conductance normalized by the single-sided evaporative surface area (i.e. total leaf or flower area). Groupings were chosen to highlight the major differences within structures. Values below the bars indicate the number of species sampled in each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

623 Figure 1

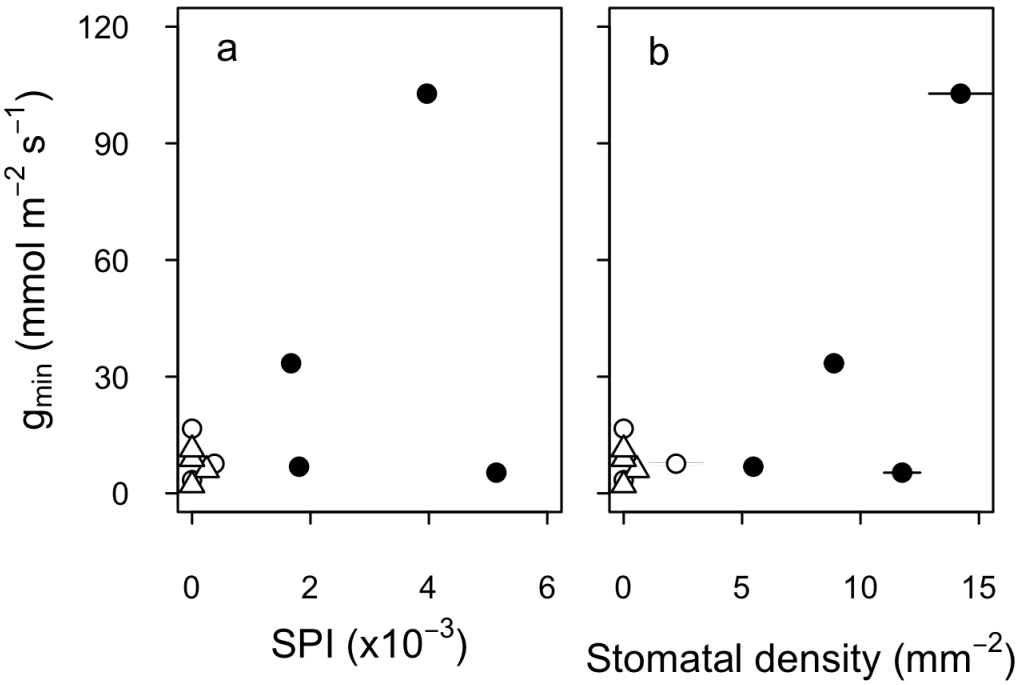

a
b
magnoliids
monocots
eudicots
all species r_s = 0.61
magnoliids R^2 = 0.93
K_{flower} (mmol s^{-1} m^{-2} MPa^{-1})
VLA (mm mm^{-2})
Huber ratio (x10^{-5})
0
5
10
15
20
25
0
3
6
9
12
15
0.0
0.5
1.0
1.5
2.0
2.5
3.0


a
b
c
d
all species r_s = 0.52
magnoliids R^2 = 0.95
K_{flower} (mmol s^{-1} m^{-2} MPa^{-1})
g_{min} (mmol m^{-2} s^{-1})
Stomatal density (mm^{-2})
Stomatal length (μm)
SPI ($x10^{-3}$)

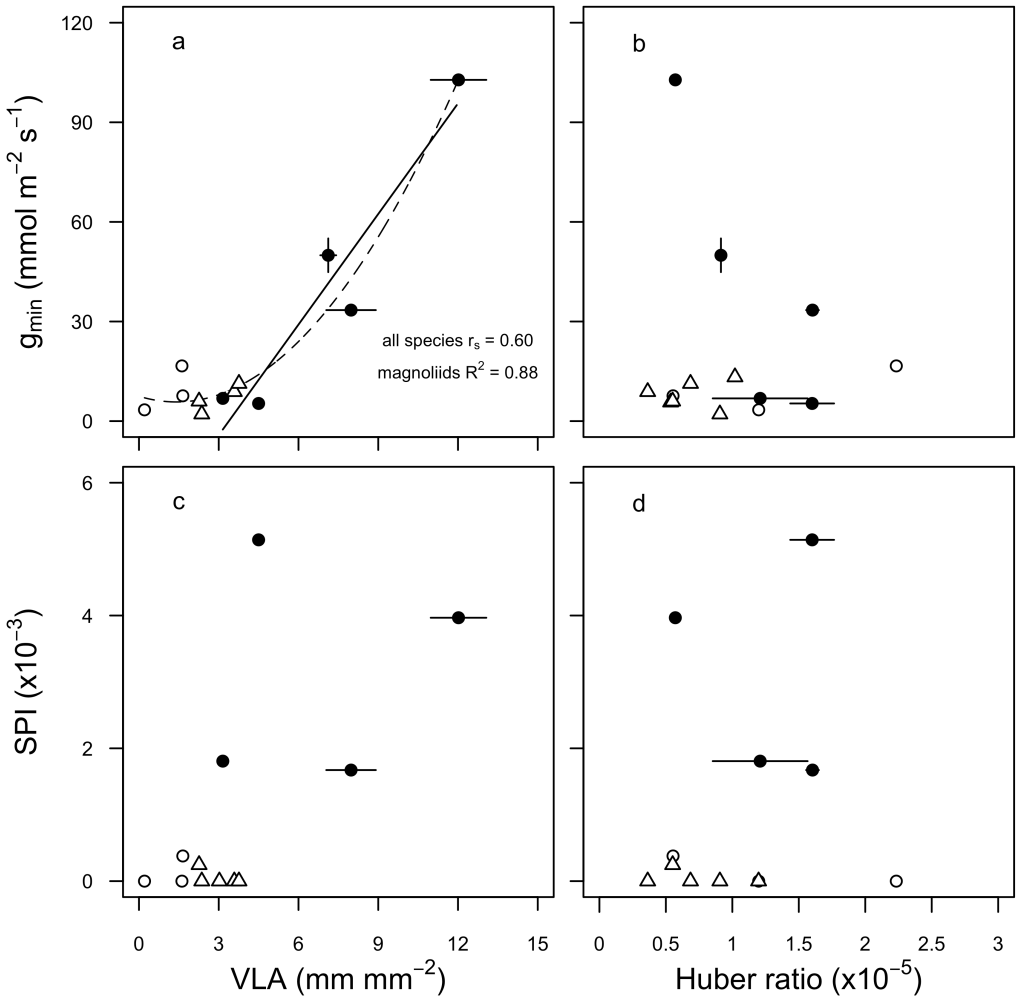
625 Figure 3


magnoliids
monocots
eudicots
Huber ratio (x10^{-5})
VLA (mm mm^{-2})
0
0.5
1
1.5
2
2.5
3
0
3
6
9
12
15

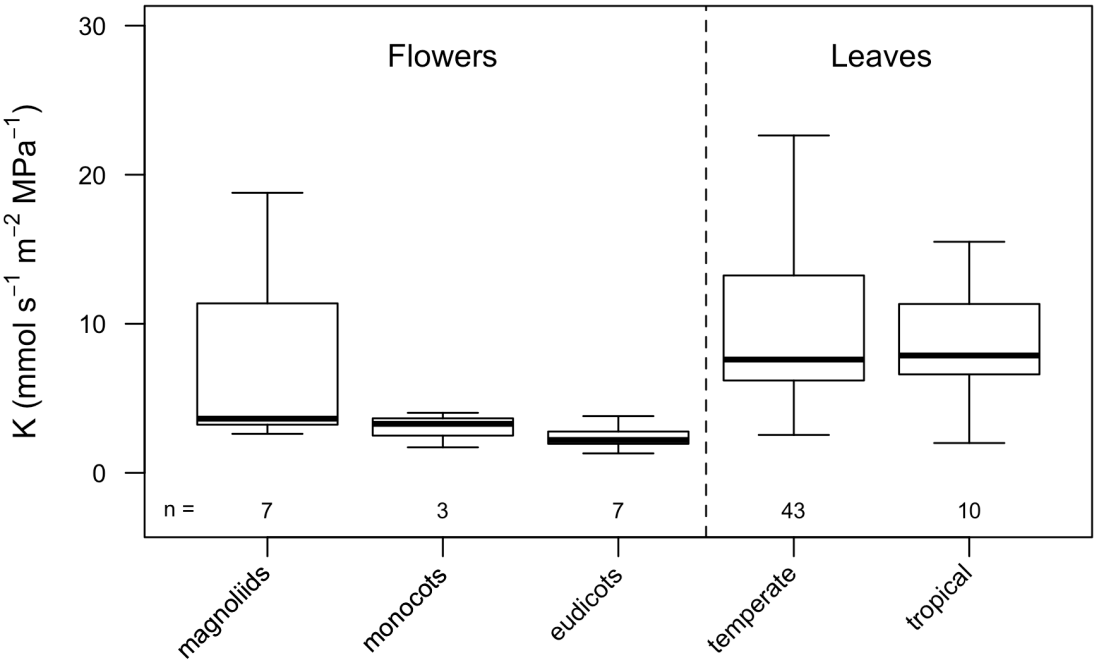
626 Figure 4
627
628



629 Figure 5


a
b
c
d
all species r_s = 0.60
magnoliids R^2 = 0.88
g_{min} (mmol m^{-2} s^{-1})
SPI (x10^{-3})
VLA (mm mm^{-2})
Huber ratio (x10^{-5})
0
30
60
90
120
2
4
6
3
9
12
15
0.5
1
1.5
2.5

630 Figure 6
631
632



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

635
636
637
638