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RESEARCH ARTICLE

Monocots and eudicots have more conservative flower water use strategies than basal angiosperms

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ABSTRACT

- Water balance is crucial for the growth and flowering of plants. However, the mechanisms by which flowers maintain water balance are poorly understood across different angiosperm branches.
- Here, we investigated 29 floral hydraulic and economic traits in 24 species from ANA grade, magnoliids, monocots, and eudicots. Our main objective was to compare differences in flower water use strategies between basal angiosperms (ANA grade and magnoliids) and derived group (monocots and eudicots).
- We found that basal angiosperms had richer petal stomatal density, higher pedicel hydraulic diameter, and flower mass per area, but lower pedicel vessel wall reinforcement and epidermal cell thickness compared to monocots and eudicots. We also observed significant trade-offs and coordination among different floral traits. Floral traits associated with reproduction, such as floral longevity and size, were strongly linked with physiological and anatomical traits.
- Our results systematically reveal the variation in flower economic and hydraulic traits from different angiosperm branches, deepening understanding of flower water use strategies among these plant taxa. We conclude that basal angiosperms maintain water balance with high water supply, whereas monocots and eudicots maintain a more conservative water balance.

INTRODUCTION

Flowers play an essential role in maintaining a species genetic stability, and their primary function is to facilitate pollination and reproduction (Primack 1985; Sargent & Ackerly 2008). Recent studies have shown that environmental conditions and resource availability significantly affect floral traits, although pollinators were traditionally considered to be the main drivers of floral evolution (Chapotin *et al.* 2003; Lambrecht & Dawson 2007; Roddy *et al.* 2021). For instance, water inputs during flowering are essential to maintain flower opening and produce nectar for pollinators (Ashman & Schoen 1994; Galen 2005; Lambrecht *et al.* 2011). Meta-analysis showed consistent decreases in flower size, flower number, and nectar volume in response to reduced water availability (Kuppler & Kotowska 2021). For many crop species, water stress during flowering can cause damage and yield loss (Hashem *et al.* 1998; Fang *et al.* 2010). Therefore, understanding how flowers regulate water-use strategies to adapt to their environments is crucial for better understanding floral evolution and plant–pollinator–environment interactions.

Floral water balance is critical to flower function. However, we still know very little about how water balance is maintained in flowers and whether it differs among taxa. Previous evidence found significant variation in regulation of flower water balance among major angiosperm clades (Chapotin *et al.* 2003; Feild *et al.* 2009a,b). Several studies have shown that extant basal angiosperm lineages require large amounts of water to maintain flower functioning (Feild *et al.* 2009a,b; Roddy *et al.* 2016, 2018). In contrast, in monocots and eudicots, flowers probably rely on stored water to maintain turgidity and delay desiccation (Feild *et al.* 2009b; Roddy *et al.* 2016, 2019, 2023; An *et al.* 2023). There is limited information on the diversity of floral hydraulic structure and function in basal angiosperms and their derived clades, specifically in relation to anatomy of the pedicel xylem.

Plants have long-term adaptations to different environmental conditions, forming a set of correlated traits (e.g., leaf morphology and stomatal structure) to regulate water use efficiency. Under drought conditions, plants reduce leaf size and stomatal opening to decrease the rate of water loss through transpiration and maintain water balance (Lawson & Blatt 2014; Sweet *et al.* 2017). Additionally, some plants reduce water loss by forming a wax layer on the leaf surface (Riederer & Schreiber 2001; Zeisler-Diehl *et al.* 2018). While there is abundant literature on leaf water use and protection strategies, our knowledge of water use strategies in flowers is limited. A previous study found that flowers tend to produce small and short corollas with a high water content to mitigate the water cost of flowering during dry seasons (Teixido *et al.* 2019). Flowers decrease water loss by reducing floral longevity under arid and hot conditions (Ashman & Schoen 1994; Teixido & Valladares 2015). However, it remains unclear how flowers adjust their water use strategies by altering their physiological and anatomical traits.

Floral longevity and size are significantly correlated with plant reproductive success and ecological adaptation (Primack 1985; Ashman & Schoen 1994; Kettle *et al.* 2011). Floral longevity and size are affected by environmental conditions (Lambrecht 2013; Song *et al.* 2022). For instance, high temperatures and drought can reduce floral longevity, while favourable environmental conditions can prolong it (Jorgensen & Arathi 2013; Teixido & Valladares 2015). Although long-lived flowers increase reproductive opportunities, they also require more carbon and water for maintenance (Ashman & Schoen 1994; Zhang *et al.* 2017; Li *et al.* 2022). Moreover, under reduced water supply, flower size consistently decreases (Kuppler *et al.* 2021; Kuppler & Kotowska 2021). This may be because, under drought conditions, water limits flower size as it becomes difficult to sustain the high water demand of large flowers (Lambrecht 2013). While pollinators typically prefer flowers with large corollas, the higher surface area of large corollas increases transpiration from leaves of the same plant, resulting in reduced leaf photosynthesis (Galen *et al.* 1999). Consequently, water consumption of flowers limits their size and hinders leaf functions (Galen *et al.* 1999). However, whether floral longevity and size coevolve with flower structural and physiological traits is still unclear.

Plant xylem anatomy affects water transport and hydraulic efficiency and safety (Tyree & Ewers 1991). Some studies have shown that leaf and stem vessels with larger diameters have increased hydraulic conductivity but are also more susceptible to cavitation (Knipfer *et al.* 2015; Brodribb *et al.* 2016). Similarly, the pedicel, an essential conduit for water transport into flowers, may be exposed to such risks. In fact, some studies have demonstrated that pedicels are more sensitive to cavitation than petioles and stems (Bourbia *et al.* 2020; Zhang *et al.* 2021; Harrison Day *et al.* 2022). Therefore, investigating the xylem anatomy of pedicels will help us better understand how flowers adapt to the local environmental conditions and their responses to climate change.

In this study, we measured 29 floral traits related to water transport, storage, and loss of 24 species from the ANA [Amborellales (A), Nymphaeales (N), Austroballeyales (A)] grade, magnoliids, monocots and eudicots. We address three questions and test corresponding hypotheses. (i) Do monocots and eudicots exhibit more conservative water use strategies compared to basal angiosperms? We hypothesize that basal angiosperms possess floral traits related to efficient water transport, such as higher pedicel theoretical conductivity, vessel diameter, vein density, and stomatal density, enabling them to maintain water balance through high water supply and water loss. Monocots and eudicots possess floral traits related to reducing water loss and increasing water storage, such as higher pedicel vessel density, epidermal cell thickness, and vessel wall reinforcement, allowing them to maintain water balance through more conservative strategies. (ii) Are there significant trade-offs or coordination between pedicel anatomical structure and function, and do these relationships differ among major evolutionary clades? Based on the leaf structure–function relationship, we argue that hydraulic efficiency of flowers may be closely related to xylem traits regulating water supply (e.g., pedicel vessel density and petal vein density) and petal and epidermal traits that regulate water loss (e.g., stomatal density and size, epidermal cell thickness, etc.). Therefore, we hypothesize that there may be a tradeoff between hydraulic efficiency and drought resistance in flowers. Additionally, because of environmental constraints, we hypothesize that the trade-offs between drought resistance and hydraulic efficiency are stronger in monocots and eudicots. (iii) Which floral physiological and anatomical traits are associated with reproduction-related floral traits? We hypothesize that flower longevity and size are significantly related to pedicel vessel size and flower carbon cost. Larger and longer-lived flowers increase the likelihood of reproductive success but require higher carbon and water maintenance costs.

MATERIAL AND METHODS

Study site and species

This study was conducted at Xishuangbanna Tropical Botanical Garden (XTBG), which is situated in Yunnan Province, China (21°55′ N, 01°15′ E, 570 m a.s.l.). XTBG is located at the northern boundary of the southeast subtropical zone and experiences a tropical monsoon climate. This region receives an average of 1859 h sunshine annually, with a mean annual temperature of 22.7 °C and rainfall of 1447 mm. The area has a distinct dry season from November to April and a rainy season from May to October.

We selected 24 monoecious species from the ANA grade, magnoliids, monocots, and eudicots for this study. According to the APG IV classification (Garnweidner-Holme *et al.* 2016), the major clades are the ANA grade, magnoliids, monocots, and eudicots. The early branching lineages are commonly called the ANA grade. The term “magnoliids” currently includes the Magnoliales, Laurales, Piperales, and Canellales. These species were chosen from 17 families to ensure a high phylogenetic diversity (Fig. 1; Table S1). All plants were cultivated outdoors under well-watered conditions and were randomly distributed at the XTBG. The selected species are widely distributed in southern Yunnan, and provided sufficient samples to measure the floral traits (Table 1).

Floral longevity and nutrient concentrations

We randomly marked 10–20 flower buds for each species and measured floral longevity (FL; days) from the moment flowers opened until the corolla fell off or became wilted and discoloured (Roddy *et al.* 2021).

To determine carbon and nitrogen content, healthy and fresh flowers (including receptacle, sepal, petal, stamen, and pistil) were first oven-dried at 70 °C for at least 48 h, ground into powder, and passed through a 60-mesh sieve. The flower carbon (C_{flower}; g·kg^{-1}) and nitrogen (N_{flower}; g·kg^{-1}) concentrations per mass were determined using a C-N elemental analyser (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany). The C/N ratio of flowers (C/N_{flower}) was then calculated.

Petal anatomy and morphology

We used the paraffin embedding method to prepare petal cross-sections, and captured images using a light microscope

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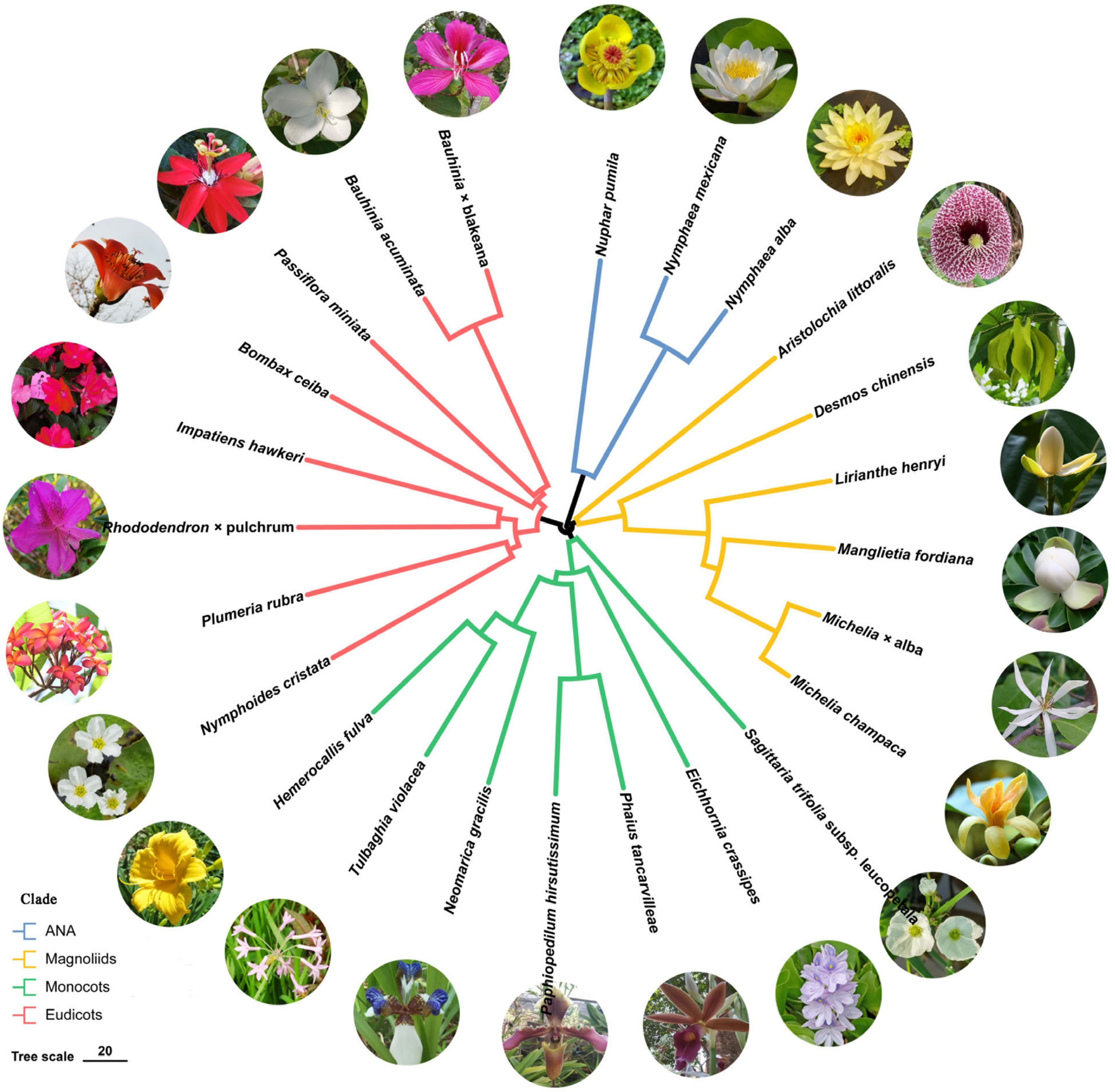

Fig. 1. Phylogenetic relationships of the 24 species studied. Branches were colour-coded to represent different clades: blue for ANA clade, yellow for magnoliids, green for monocots, and red for eudicots.

(Leica DM2500, Leica Microsystems, Wetzlar, Germany). For each species, we took 3–6 complete flowers with pedicels from different individuals and soaked them in FAA solution (formalin:acetic acid:70% alcohol: 5:5:90). We took the outermost whorl of petals to measure all petal anatomical traits. Petal sections measuring 1 × 1 cm, taken from the middle area near the midvein, were cut and placed in a 70% ethanol solution overnight. We found that petal tissues are more delicate than leaves, so the immersion time in high-concentration alcohol and hot paraffin needed to be reduced based on the leaf protocol. On the following day, the samples underwent dehydration in a series of alcohol solutions: 80% ethanol (1 h) — 85% ethanol (1 h) — 95% ethanol (1 h) — 100% ethanol (1 h) — 1:1 100% ethanol and limonene solution (overnight). On the third day, the samples were transferred to pure limonene solution (2 h) — pure limonene solution (2 h). After which the samples were embedded in a preheated embedding machine for approximately 3 h and then frozen. We used a rotary microtome (Leica DM2245, Leica Microsystems) for sectioning, followed by dewaxing, dehydration, staining, mounting, observation, and photography. Finally, we used the ImageJ software (National Institutes of Health, Bethesda, MD, USA) to measure petal thickness (PT; μm), adaxial epidermis thickness ($\mathrm{Ada}_{\mathrm{petal}}$; μm), abaxial epidermis thickness ($\mathrm{Aba}_{\mathrm{petal}}$; μm), and petal density (PD; $\mathrm{kg \cdot m^{-3}}$) = FMA × PT.

We collected three to six freshly opened flowers from different individuals of each species in the morning and measured fresh weight ($\mathrm{FW}_{\mathrm{flower}}$; g). The flowers were then immersed in water for a specific duration (4–12 h) to allow water absorption. We performed multiple weight measurements during this

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soaking period until the weight remained constant. This constant weight represented the saturated fresh weight of the flower (SW_{flower}; g). We utilized a flatbed scanner (Epson Perfection V850 Pro) to determine floral area (FA; cm^2) of all flower organs (receptacle, sepal, petal, stamen, and pistil). Subsequently, the flowers were dried at 70 °C for over 48 h to constant dry weight (DW_{flower}; g). Flower mass per area (FMA; $g \cdot m^{-2}$) was calculated as DW_{flower}/FA, flower dry matter content (FMDC; $g \cdot g^{-1}$) was calculated as DW_{flower}/SW_{flower}, and the relative water content of flowers (RWC_{flower}; %) was calculated as $(FW_{flower} - DW_{flower})/(SW_{flower} - DW_{flower})$.

We took the outermost whorl of petals to measure the vein density on the flatbed scanner at 3200 dpi. Petal vein density ($D_{vein,\ petal}$; $mm \cdot mm^{-2}$) was the total length per surface area. Then, we used a light microscope to count stomata in the upper and lower epidermis. We photographed 5–10 fields of view containing stomata to determine stomatal size. Petal stomatal size (SS_{petal}; μm^2) and epidermal cell size (ECS_{petal}; μm^2) were calculated using the ImageJ software. Stomatal size refers to the area enclosed by two guard cells, and epidermal cell size was calculated by dividing the area of the field of view by the number of epidermal cells within that area. Petal stomatal density (SD_{petal}; $no. \cdot mm^{-2}$) was calculated as the total number of stomata divided by the sample area. For cells that were only partially visible within the field of view, we only included those in the upper and left portions for analysis, while excluding cells in the lower and right portions.

Pedicel xylem anatomy and hydraulic efficiency

To examine xylem anatomy and hydraulic efficiency, we collected three to six freshly opened flowers with pedicels from different individuals of each species. We prepared cross-sections of the pedicels using the paraffin-embedding method. Pedicels were immersed in FAA solution then dehydrated and embedded. The embedded samples were sliced into 14–25 mm thick cross-sections using the rotary slicer. The sections were deparaffinized with limonene and stained with 0.5% Astral Blue and 1% Safranin-O in a 1:1 ratio. Subsequently, each part of the section was photographed with a light microscope. Finally, we utilized ImageJ to measure the cross-sectional area of the pedicel, the total number of vessels, and the long and short axes of vessel lumens. The diameter of each vessel in the pedicel ($D_{i,\ pedicel}$; μm) was calculated as follows:

$$D_{i,\ pedicel} = \left(\frac{32(ab)^3}{a^2 + b^2} \right)^{1/4}$$

where a and b are radii of the major and minor axes of each vessel lumen, respectively. The pedicel hydraulically weighted vessel diameter ($D_{h,pedicel}$; μm) was calculated according to Poorter *et al.* (2010):

$$D_{h,\ pedicel} = \left(\frac{\sum_{i=1}^{n} D_i^4}{n} \right)^{1/4}$$

where n is the total number of vessels in the cross-section. The theoretical specific hydraulic conductivity ($K_{t,\ pedicel}$; $kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$) of each pedicel was calculated according to the Hagen-Poiseuille equation (Tyree & Ewers 1991; Bourbia *et al.* 2020):

$$K_{t,\ pedicel} = \frac{\frac{\pi \rho}{128\eta} \sum_{i=1}^{n} \left(D_i^4 \right)}{A}$$

where π is the circular constant of 3.14, ρ is the fluid density (taken as $1000\,kg \cdot m^{-3}$), η is the viscosity of water ($1.002 \times 10^{-9}\,MPa \cdot s^{-1}$ at 20 °C), and A is the downstream projected flower area (m^2).

Mean diameters of ten largest and smallest vessels for each pedicel were defined as the maximum ($D_{max,\ pedicel}$; μm) and minimum ($D_{min,\ pedicel}$; μm) vessel diameter, respectively. Pedicel vessel density ($VD_{pedicel}$; $no. \cdot mm^{-2}$) was calculated as the number of vessels per unit pedicel cross-section area. Pedicel vessel lumen fraction ($VLF_{pedicel}$; %) was defined as the total vessel lumen area divided by the total cross-sectional area of the pedicel. Pedicel vessel wall reinforcement ($VWR_{pedicel}$) was calculated as the square of the ratio of pedicel vessel wall thickness ($VWT_{pedicel}$; μm) to vessel diameter ($D_{i,\ pedicel}$).

Measurement of water loss and drought tolerance in flowers

A relative water content of 70% is the threshold of physiological damage (Lawlor & Cornic 2002; Hao *et al.* 2010; Zhang *et al.* 2015). To examine the time required for drying of saturated flowers to 70% relative water content ($T_{70,\ flower}$; h), we cut the flower at the branch–pedicel junction and fully immersed the pedicel and perianth in water for 4–12 h. The saturated weight of the flower (SW_{flower}; g) was then recorded. In order to maintain constant conditions, the flowers were placed in a thermostatic incubator set at 25 °C and a relative humidity of 65%. We weighed the flowers each hour until no further weight change was observed. Subsequently, the flowers were dried at 70 °C for 48 h to obtain the dry weight (DW_{flower}; g). $T_{70,\ flower}$ was calculated by analysing the relationship between the relative water content and the time interval of each measurement.

We used a Model 5600 VAPOUR pressure osmometer (ELITechGroup, Logan, UT, USA) to measure the flower osmotic potential ($\psi_{osm,\ flower}$). Samples from the center of the midrib and margin of the outermost petal were collected using a perforator. The petal discs, wrapped in aluminium foil, were immersed in liquid nitrogen, and placed in the osmolality chamber. The osmolality was measured repeatedly until equilibrium was reached (with a difference of $<3\,mmol \cdot kg^{-1}$ between two measurements; Bartlett *et al.* 2012). We recorded the equilibrium osmolality in $mmol \cdot kg^{-1}$ and converted it to osmotic potential in MPa by multiplying by $-0.002437\,m^3\,MPa \cdot mol^{-1}$ using the Van't Hoff relationship (Laughlin *et al.* 2020). Flower water potential at the turgor loss point ($\psi_{tlp,\ flower}$; MPa) was calculated using the equation provided by Bartlett *et al.* (2012):

$$\psi_{tlp,flower} = 0.832\ \psi_{osm,flower} - 0.631$$

where $\psi_{osm,\ flower}$ is the flower osmotic potential.

Statistical analysis

We calculated the average values of floral traits in each species and performed a $\log_{10}$ transformation on the data to

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improve normality and homoscedasticity. Independent samples *t*-tests were used to analyse differences in floral traits between basal angiosperms (ANA grade and magnoliids) and derived groups (monocots and eudicots) using the *t*-test function in the 'stats' package. Considering that most ANA grade and monocots are herbaceous plants, while most magnoliids and eudicots are woody, we accounted for the influence of growth forms on floral trait divergence. Two-way ANOVA was utilized to examine differences in floral traits among major evolutionary clades, growth forms, and evolutionary clades with different growth forms. The relationship between paired floral traits was quantified using the *corr.test* function in the 'psych' package. To test the differences in these relationships among evolutionary clades, we conducted standardized major axis analysis (SMA) using the *sma* function in the 'smatr' package (Warton *et al.* 2012). Additionally, a principal components analysis (PCA) was performed using the *prcomp* function in the 'stats' package to determine the position of basal angiosperms, monocots, and eudicots in the multivariate floral trait space. Permutation multivariate analysis of variance (*PERMANOVA*) was used to assess whether plants from different evolutionary clades and growth forms occupy distinct positions in the multivariate trait space (number of permuted datasets = 999) with the *adonis* function in the 'vegan' package (Anderson 2001). We employed phylogenetic ANOVA and phylogenetically independent contrasts (PICs) to assess the influence of phylogeny on trait differences and associations (Felsenstein 1985). The phylogenetic tree for the selected species was constructed using the *phylo.maker* function in the 'V.PhyloMaker2' package (Jin & Qian 2022). Phylogenetic ANOVA was used to test the differences in 29 floral traits between basal angiosperms and derived groups using the *aov.phylo* function in the 'geiger' package (Pennell *et al.* 2014). Phylogenetically independent contrasts ($\mathrm{PIC_s}$) were performed using the *pic* function in the 'picante' package (Kembel *et al.* 2010). All analyses were performed in R version 4.2.3 (R Core Team 2023).

RESULTS

Comparison of floral traits among basal angiosperms and monocots and eudicots

For traits related to water transport, loss, and storage, basal angiosperms had higher $D_{\mathrm{h,pedicel}}$ ($P = 0.035$; Figures S1 and S2), $D_{\mathrm{max,\ pedicel}}$ ($P = 0.041$), $K_{\mathrm{t,\ pedicel}}$ ($P = 0.034$), $\mathrm{SD_{petal}}$ ($P = 0.001$; Figure S3), and $\mathrm{SPI_{petal}}$ ($P = 0.004$), but lower $\mathrm{Ada_{petal}}$ ($P = 0.001$; Figure S4), $\mathrm{Aba_{petal}}$ ($P = 0.021$) and $\mathrm{ECS_{petal}}$ ($P < 0.001$) than monocots and eudicots. Regarding resource acquisition and defence traits, basal angiosperms had higher FMA ($P = 0.011$), FDMC ($P = 0.002$), and PD ($P = 0.014$), but lower $\mathrm{VWR_{pedicel}}$ ($P = 0.007$) than monocots and eudicots (Table 2). The results of two-way ANOVA showed similar patterns to the independent-samples *t*-test results (Table S2). The differences in $\mathrm{VWR_{pedicel}}$ among basal angiosperms, monocots and eudicots became marginally significant;

Table 1. The ecological significance for each floral trait measured.

trait	abbreviation	ecological significance	unit
Floral longevity	FL	Resource capture	days
Floral area	FA	Resource capture	$\mathrm{cm^2}$
Flower mass per area	FMA	Resource capture and defence	$\mathrm{g{\cdot}m^{-2}}$
Flower dry matter content	FDMC	Resource capture and defence	$\mathrm{g{\cdot}g^{-1}}$
Carbon concentration per mass of flowers	$\mathrm{C_{flower}}$	Resource capture	$\mathrm{g{\cdot}kg^{-1}}$
Nitrogen concentration per mass of flowers	$\mathrm{N_{flower}}$	Resource capture	$\mathrm{g{\cdot}kg^{-1}}$
C/N ratio of flowers	$\mathrm{C/N_{flower}}$	Resource capture and defence	–
Petal thickness	PT	Water conservation	μm
Petal density	PD	Resource capture	$\mathrm{kg{\cdot}m^{-3}}$
Petal stomatal density	$\mathrm{SD_{petal}}$	Water loss	$\mathrm{no.{\cdot}mm^{-2}}$
Petal stomatal size	$\mathrm{SS_{petal}}$	Water loss	$\mathrm{\mu m^2}$
Petal guard cell length	$\mathrm{GCL_{petal}}$	Water loss	μm
Stomatal pore area index	$\mathrm{SPI_{petal}}$	Water loss	–
Petal epidermal cell size	$\mathrm{ECS_{petal}}$	Water conservation	$\mathrm{\mu m^2}$
Petal adaxial epidermis thickness	$\mathrm{Ada_{petal}}$	Water loss	μm
Petal abaxial epidermis thickness	$\mathrm{Aba_{petal}}$	Water loss	μm
Petal vein density	$D_{\mathrm{vein,\ petal}}$	Water transport	$\mathrm{mm{\cdot}mm^{-2}}$
Time required for drying of saturated flowers to 70% relative water content	$T_{\mathrm{70,\ flower}}$	Water loss	h
Flower water potential at turgor loss point	$\Psi_{\mathrm{tlp,\ flower}}$	Drought tolerance	MPa
Flower relative water content	$\mathrm{RWC_{flower}}$	Water conservation	%
Pedicel vessel density	$\mathrm{VD_{pedicel}}$	Water transport	$\mathrm{no.{\cdot}mm^{-2}}$
Pedicel vessel lumen fraction	$\mathrm{VLF_{pedicel}}$	Water transport	%
Pedicel hydraulic diameter	$D_{\mathrm{h,pedicel}}$	Water transport	μm
Pedicel mean vessel diameter	$\mathrm{MVD_{pedicel}}$	Water transport	μm
Pedicel maximum vessel diameter	$D_{\mathrm{max,\ pedicel}}$	Water transport	μm
Pedicel minimum vessel diameter	$D_{\mathrm{min,\ pedicel}}$	Water transport	μm
Pedicel theoretical hydraulic conductivity	$K_{\mathrm{t,\ pedicel}}$	Water transport	$\mathrm{10^{-4}\ kg{\cdot}m^{-1}{\cdot}s^{-1}{\cdot}MPa^{-1}}$
Pedicel vessel wall thickness	$\mathrm{VWT_{pedicel}}$	Drought tolerance	μm
Pedicel vessel wall reinforcement	$\mathrm{VWR_{pedicel}}$	Drought tolerance	–

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Table 2. Means ± SE of 29 floral traits for basal angiosperms, monocots and eudicots.

traits	basal angiosperms	monocots and eudicots	*t*	*P*	phylogenetic *P*
SD_{petal}	25.70 ± 7.87^{a}	2.18 ± 0.73^{b}	4.21	**0.001**	**0.01**
SS_{petal}	561.91 ± 72.46	620.41 ± 147.43	−0.97	0.322	0.509
GCL_{petal}	31.28 ± 2.17	25.71 ± 5.23	0.19	0.857	0.903
SPI_{petal}	$30425.99 \pm 13165.10^{a}$	3052.42 ± 1017.42^{b}	3.18	**0.004**	**0.050**
ECS_{petal}	1066.62 ± 145.58^{b}	3174.88 ± 531.29^{a}	−4.47	<**0.001**	**0.009**
$D_{vein,\ petal}$	1.65 ± 0.21	1.18 ± 0.11	2.07	0.055	0.199
FA	204.68 ± 77.17	66.58 ± 16.99	2.06	0.061	0.213
FMA	136.65 ± 51.51^{a}	44.75 ± 11.38^{b}	2.72	**0.011**	0.098
FDMC	0.13 ± 0.01^{a}	0.08 ± 0.01^{b}	3.67	**0.002**	**0.042**
C_{flower}	460.96 ± 6.10	449.97 ± 5.03	1.41	0.180	0.374
N_{flower}	22.74 ± 2.22	21.62 ± 2.04	0.52	0.626	0.76
C/N_{flower}	22.26 ± 2.61	23.98 ± 2.57	−0.33	0.756	0.835
$T_{70,\ flower}$	10.36 ± 2.43	8.94 ± 2.43	1.47	0.216	0.400
$\psi_{tlp,\ flower}$	-1.28 ± 0.03	-1.25 ± 0.05	−0.68	0.584	0.733
FL	4.14 ± 1.06	5.43 ± 2.28	21.72	0.468	0.646
Ada_{petal}	17.07 ± 1.67^{b}	28.38 ± 2.26^{a}	−3.94	**0.001**	**0.025**
Aba_{petal}	15.60 ± 1.21^{b}	21.53 ± 1.58^{a}	−2.67	**0.021**	0.131
PT	467.96 ± 126.47	395.90 ± 67.13	0.50	0.617	0.755
PD	0.28 ± 0.06^{a}	0.14 ± 0.04^{b}	2.91	**0.014**	0.107
RWC_{flower}	91.16 ± 1.98	87.77 ± 1.81	1.24	0.251	0.444
$VD_{pedicel}$	32.91 ± 11.17	46.91 ± 11.07	−0.93	0.367	0.552
$VLF_{pedicel}$	0.78 ± 0.19	0.60 ± 0.12	0.84	0.411	0.599
$D_{h,pedicel}$	20.93 ± 2.17^{a}	15.04 ± 1.98^{b}	2.54	**0.035**	0.163
$MVD_{pedicel}$	18.98 ± 2.10^{a}	14.20 ± 1.87^{b}	2.25	0.058	0.207
$D_{max,\ pedicel}$	27.80 ± 2.90	20.09 ± 2.86	2.50	**0.041**	0.17
$D_{min,\ pedicel}$	11.87 ± 1.29	10.10 ± 1.37	1.43	0.215	0.397
$K_{t,\ pedicel}$	1.10 ± 0.48^{a}	0.36 ± 0.08^{b}	2.37	**0.034**	0.161
$VWT_{pedicel}$	2.20 ± 0.19	2.24 ± 0.26	0.29	0.801	0.859
$VWR_{pedicel}$	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	−2.56	**0.007**	0.073

t- and *P*-values of independent samples *t*-test and phylogenetic *P*-values of phylogenetic ANOVA. Significant differences are indicated in bold. See Table 1 for trait abbreviations.

$MVD_{pedicel}$ and $K_{t,\ pedicel}$ were not significantly different after considering effects of the growth forms.

Phylogenetic ANOVA results were somewhat different to the results of traditional independent samples *t*-test (Table 2). Specifically, traits such as FMA, Aba_{petal}, PD, $D_{h,pedicel}$, $D_{max,\ pedicel}$, $K_{t,\ pedicel}$, and $VWR_{pedicel}$ had non-significant differences between basal angiosperms and monocots/eudicots. Additionally, results of Blomberg's *K* and *P* values produced significant phylogenetic signals in most structural traits of flowers (e.g., FMA, C_{flower}, SD_{petal}; Table S3).

Associations among floral traits

For associations among flower hydraulic structures, $K_{t,\ pedicel}$ was positively associated with $MVD_{pedicel}$ (Tables S4–S6, Fig. 2A), with a common slope and intercept but a different shift across basal angiosperms and monocots and eudicots (Table S7). $K_{t,\ pedicel}$ was negatively linked with $VD_{pedicel}$ in basal angiosperms (Fig. 2B) and $VWR_{pedicel}$ in monocots and eudicots (Fig. 2C), but was positively related to SD_{petal} in monocots and eudicots (Fig. 2D). The SD_{petal} was positively correlated with $VLF_{pedicel}$ in monocots and eudicots (Fig. 2E) but associated with FMA only in basal angiosperms (Fig. 2F).

For associations among traits related to flower drought tolerance, $\psi_{tlp,\ flower}$ was positively associated with $K_{t,\ pedicel}$ (Fig. 3A) and $VLF_{pedicel}$ (Fig. 3B) in monocots and eudicots, but not in basal angiosperms. The $\psi_{tlp,\ flower}$ was negatively connected with C_{flower} (Fig. 3C) in different evolutionary clade species, but basal angiosperms had a significantly higher slope than monocots and eudicots. The $T_{70,\ flower}$ was negatively correlated with $D_{vein,\ petal}$ (Fig. 3D), with a common slope, but basal angiosperms had a significantly higher intercept than monocots and eudicots and were positively related to FMA (Fig. 3E) and FL (Fig. 3F) in monocots and eudicots, but not in basal angiosperms.

For association among flower hydraulics and economic traits, FA was positively associated with $D_{max,\ pedicel}$ (Fig. 4A), with a common slope and intercept but a different shift across basal angiosperms and monocots and eudicots. FA was negatively associated with $VD_{pedicel}$ in monocots and eudicots (Fig. 4B) and was linked with $VWR_{pedicel}$ in basal angiosperms (Fig. 4C). FL was positively associated with RWC_{flower} in monocots and eudicots (Fig. 4D). Also, FL was positively correlated with ECS_{petal} in monocots and eudicots but was negatively related to ECS_{petal} in basal angiosperms (Fig. 4E). C_{flower} was negatively associated with $VLF_{pedicel}$ (Fig. 4F) in monocots and eudicots.

Multivariate analysis of floral traits

The PCA results showed that the first and second components accounted for 29.1% and 18.6% of the total variation in 29 floral traits across the basal angiosperms and monocots and

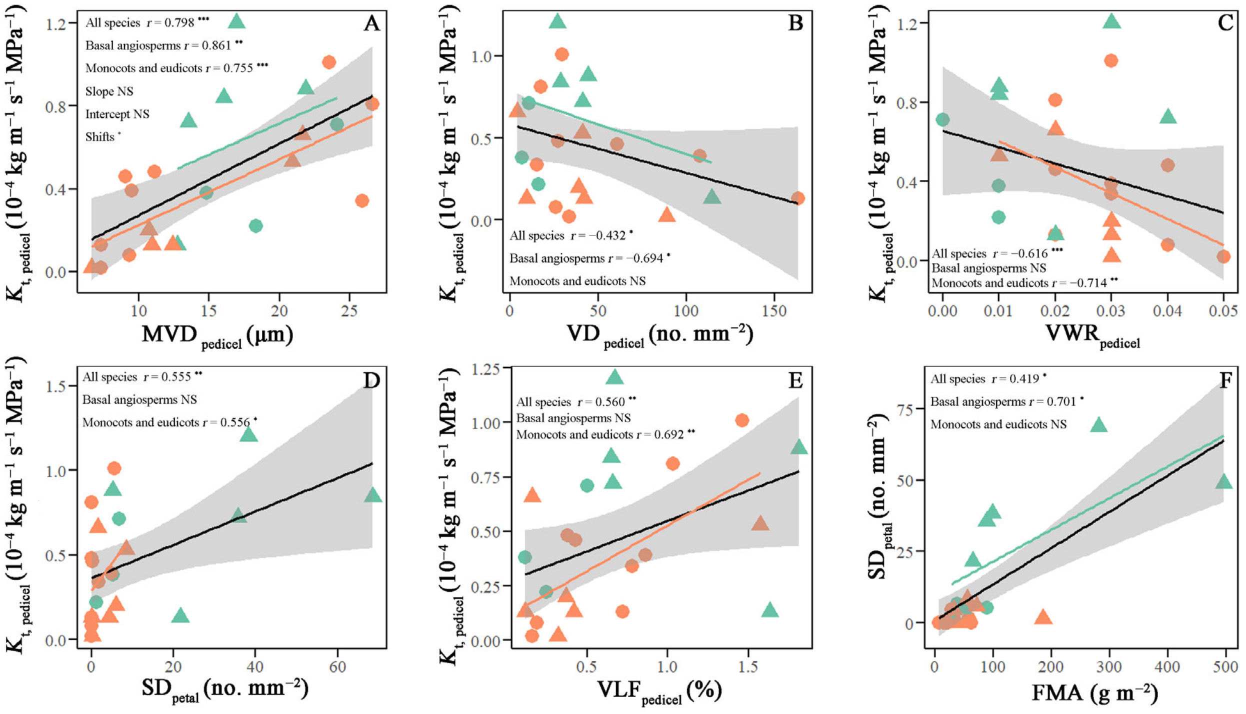

Fig. 2. Relationships of pedicel theoretical hydraulic conductivity ($K_{t,\ pedicel}$) with (A) pedicel mean vessel diameter ($MVD_{pedicel}$), (B) pedicel vessel density ($VD_{pedicel}$), (C) pedicel vessel wall reinforcement ($VWR_{pedicel}$), and (D) petal stomatal density (SD_{petal}), and the relationships of petal stomatal density (SD_{petal}) with (E) pedicel vessel lumen fraction ($VLF_{pedicel}$), and (F) flower mass per area (FMA) across basal angiosperms (green) and monocots and eudicots (orange). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

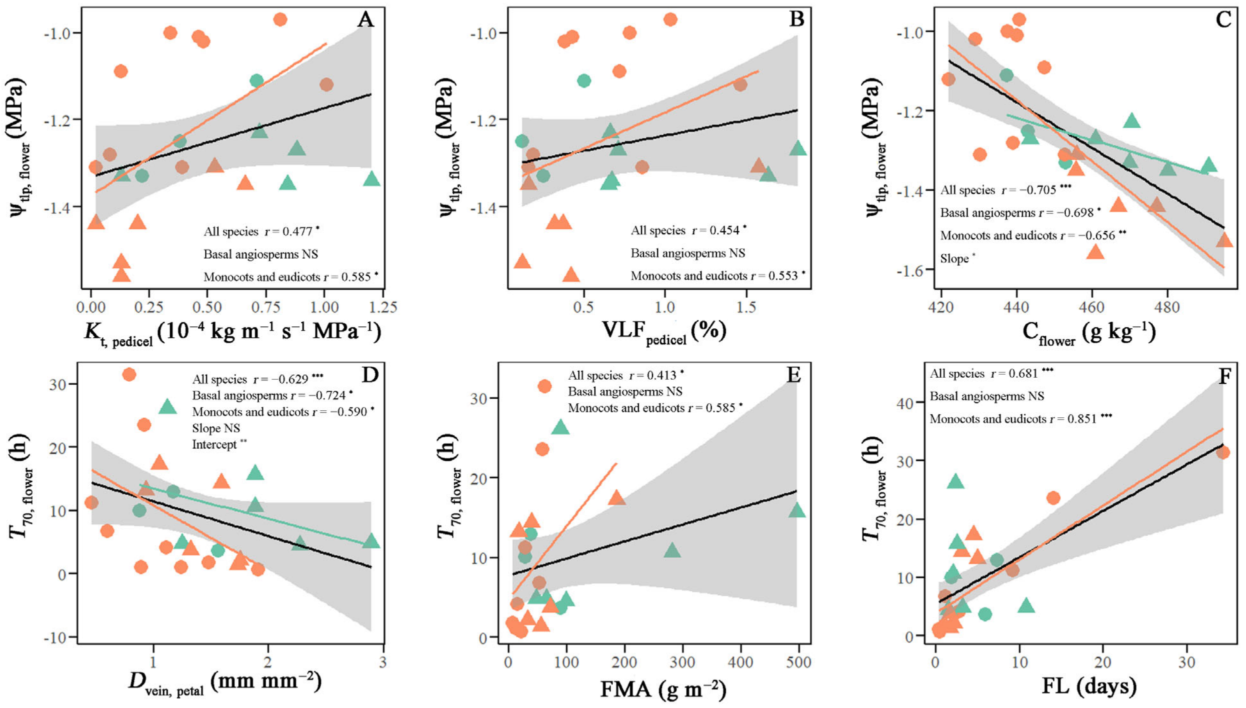

Fig. 3. Relationships of flower water potential at the turgor loss point ($\psi_{tlp,\ flower}$) with (A) pedicel theoretical hydraulic conductivity ($K_{t,\ pedicel}$), (B) pedicel vessel lumen fraction ($VLF_{pedicel}$), and (C) carbon concentration per mass of flowers (C_{flower}), and the relationships of $T_{70,\ flower}$ with (D) petal vein density ($D_{vein,\ petal}$), (E) flower mass per area (FMA), and (F) floral longevity (FL) across basal angiosperms (green) and monocots and eudicots (orange). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

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eudicots, respectively (Table S8; Fig. 5; Figure S5). The variation in the first axis was represented by a trade-off between floral hydraulic safety (e.g., $VWR_{pedicel}$ and $VD_{pedicel}$) and water transport efficiency (e.g., $K_{t,\ pedicel}$ and $MVD_{pedicel}$). The second axis was mainly represented by a trade-off between petal epidermal thickness and flower construction costs (Tables S9 and

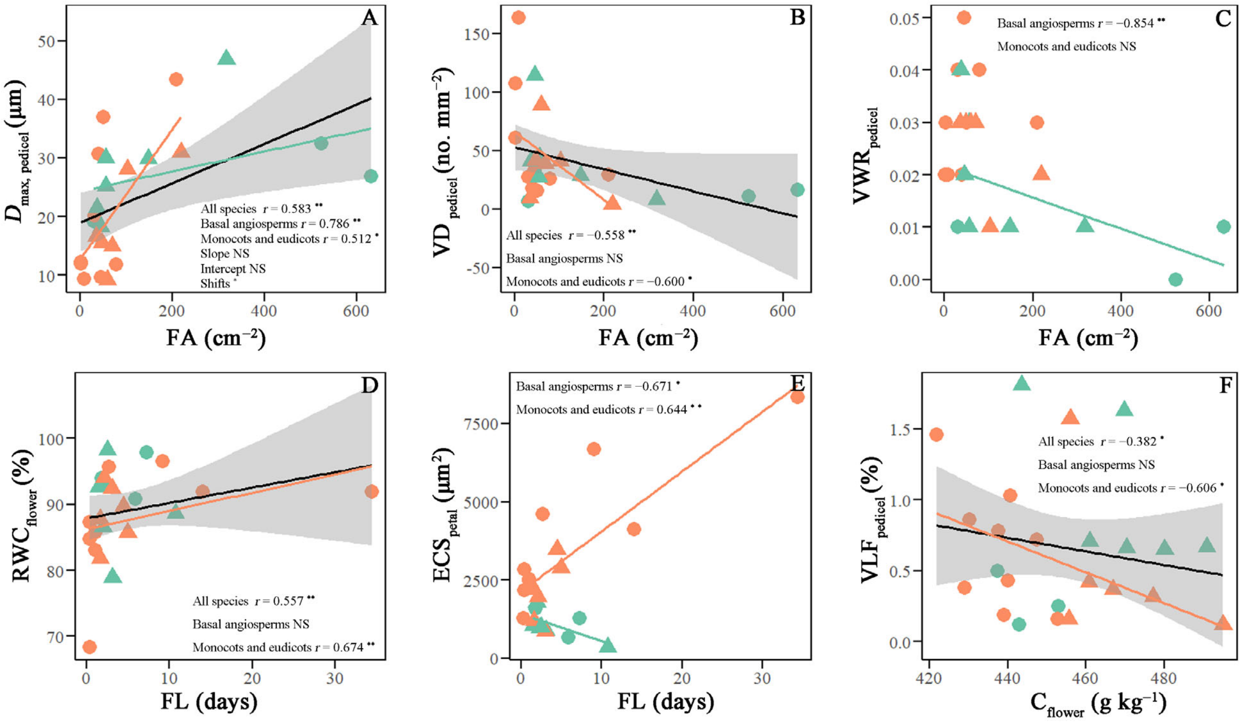

Fig. 4. Relationships of floral area (FA) with (A) pedicel maximum vessel diameter ($D_{max,\ pedicel}$), (B) pedicel vessel density ($VD_{pedicel}$), and (C) pedicel vessel wall reinforcement ($VWR_{pedicel}$), and relationships of floral longevity (FL) with (D) flower relative water content (RWC_{flower}) and (E) petal epidermal cell size (ECS_{petal}), and relationship of (F) carbon concentration per mass of flowers (C_{flower}) with pedicel vessel lumen fraction ($VLF_{pedicel}$) across basal angiosperms (green) and monocots and eudicots (orange). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

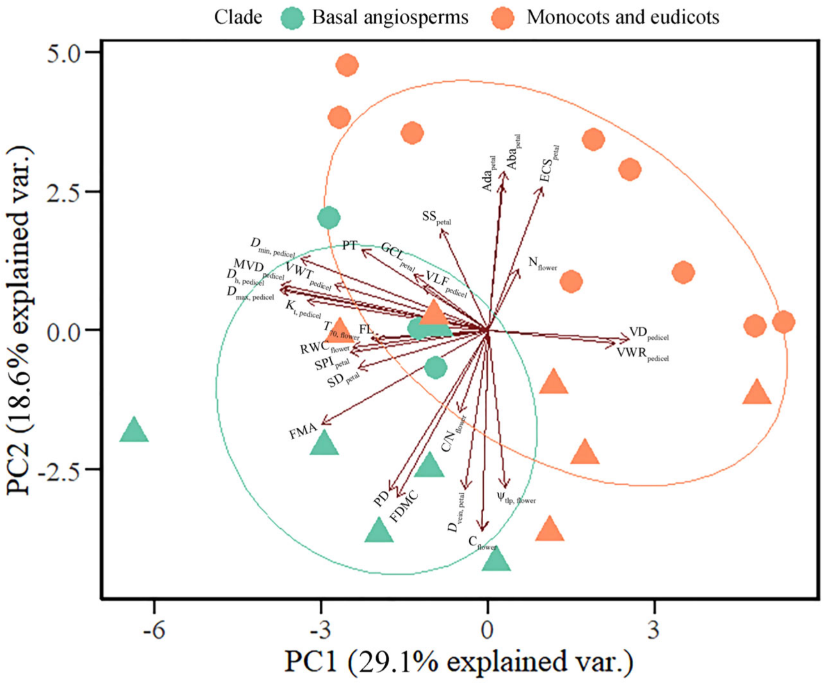

Fig. 5. Principal components analysis of 29 floral traits of 24 species. Green represents basal angiosperms, orange represents monocots and eudicots, circle represents herbaceous plants, and triangle represents woody plants.

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S10). Monocots and eudicots occupied a larger region in trait space than basal angiosperms. Flowers of basal angiosperms had higher $K_{t,\ pedicel}$, $D_{max,\ pedicel}$, $MVD_{pedicel}$, $D_{h,pedicel}$, and SD_{petal}, and lower $VD_{pedicel}$ and $VWR_{pedicel}$, whereas flowers of monocots and eudicots had the opposite trait values. The results of *PERMANOVA* showed that the multivariate trait space could be distinguished by the evolutionary clades rather than by growth forms (Table 3, Fig. 5).

DISCUSSION

Our study found that, despite sharing a common ancestry, there are notable differences in floral traits between basal angiosperms and derived taxa. Basal angiosperms maintained flower water balance through high water supply and water loss, as evidenced by higher values for traits such as petal stomatal density, stomatal pore area index, pedicel hydraulic diameter, maximum vessel diameter, and theoretical hydraulic conductivity. In contrast, monocots and eudicots exhibited relatively conservative flower water use strategies, with higher values for traits like petal epidermal cell size, petal adaxial epidermis thickness, and pedicel vessel wall reinforcement (Table 2; Table S2). These findings are consistent with previous research (Feild *et al*. 2009a,b; Roddy *et al*. 2016). However, the results of phylogenetic ANOVA showed that differences in some traits were reduced, and certain traits showed no significant variation (Table 2). This indicates that phylogeny may influence the results, further supporting our hypothesis that floral trait variation is pronounced among species from different evolutionary clades (with distant phylogenetic relationships). Similar observations have been reported by Feild *et al*. (2009a) and Roddy *et al*. (2016), who highlighted the shared physiological and ecological traits among many basal angiosperms. Moreover, there were significant differences in the coordination and trade-offs among floral traits in basal angiosperms, monocots and eudicots. These trait relationships further highlight the divergence of ecological adaptation strategies among different taxa.

Flowers of monocots and eudicots had relatively conservative water use strategies compared to those of basal angiosperms

Our findings indicate that flowers of monocots and eudicots tend to use water more conservatively compared to basal angiosperms, consistent with our first hypothesis. Basal angiosperms displayed floral trait values associated with higher water and carbon costs (Table 2). Conversely, monocots and eudicots had higher values for traits linked with lower carbon costs. The results of the PCA further supported these findings (Fig. 5). All of these findings support the "Xerophobia hypothesis", that early angiosperms are inherently drought-intolerant and require continuous high water supply to maintain their functionality (Feild *et al*. 2009a), presumably resulting in a high water and carbon costs. Previous studies have demonstrated that flowers of the Magnoliaceae and Calycanthaceae families have high transpiration rates and maintain a functional connection to the stem xylem for water supply (Feild *et al*. 2009a; Roddy *et al*. 2016, 2018). To support their high transpiration rates, flowers of basal angiosperms evolved an efficient water transport system, characterized by a high density of flower veins and stomata. In contrast, the flowers of monocots and eudicots consistently exhibited traits associated with low water flux and low carbon investment. Reduced stomatal density may be a key physiological innovation that triggers a cascade of effects on other floral traits. Since flowers are usual heterotrophs, they do not require high water evaporation rates to support photosynthesis, and relaxing this constraint allows the flower to reduce water loss by eliminating stomata (Roddy 2019). Most monocots and eudicots had few or no stomata in petals, which may serve as a compensatory mechanism to reduce water loss. Moreover, angiosperm leaves can actively close stomata to prevent desiccation (McAdam & Brodribb 2012), while the ability of petal stomata to do so may be limited (Hew *et al*. 1980). In monocots and eudicots, stomata may not play a significant role in actively regulating water loss from flowers (Zhang *et al*. 2018). Instead, water loss through the cuticle may have a stronger influence on the traits responsible for water supply and maintaining turgor pressure in these flowers (Zhang *et al*. 2017, 2018).

Table 3. PERMANOVA on the Euclidean distance of 29 floral traits for mean values of 24 species.

source	df	SS	R^2	*F*	*P*
Clades	1	15.325	0.204	6.077	**<0.001**
Growth forms	1	3.327	0.044	1.319	0.238
Clades × Growth forms	1	6.136	0.082	2.434	**0.047**
Residuals	20	50.435	0.670		
Total	23	75.210	1		

Significant differences are indicated in bold. See Table 1 for trait abbreviations.

df = degrees of freedom, SS = sum of squares, R^2 = variance explained, *F* = the *F* value of the model, *P* = the *P*-value of model.

It should be noted that growth forms can also influence the differences in floral traits among various evolutionary clades. In this study, most Nymphaeaceae and monocots were herbaceous, while most magnoliids and eudicots were woody. Woody and herbaceous plants have distinct hydraulic structures and functions (Joffre & Rambal 1993; Winkler *et al*. 2019). Woody plants generally have higher transpiration rates and, therefore, require more water compared to herbaceous plants (Joffre & Rambal 1993). Woody plants also exhibit increased drought tolerance than herbaceous plants (Winkler *et al*. 2019). However, the results of the *PERMANOVA* showed a significant separation of species from different evolutionary clades in the multidimensional floral trait space ($P < 0.001$), whereas species with different growth forms did not show a significant separation ($P = 0.238$; Table 3, Fig. 5). This suggests that the variations in floral traits were mainly caused by different evolutionary clades rather than different growth forms.

Differences in floral trait relationships among major evolutionary clades

In agreement with our second hypothesis, we observed variation in the relationships between pairwise floral traits across different evolutionary groups. This suggests potential differences in the adaptation strategy of flowers between basal angiosperms and more recently diverged monocots and eudicots. Specifically, we found that the correlation between pedicel theoretical hydraulic conductivity and pedicel vessel density was negative only in basal angiosperms, while the correlation

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between pedicel theoretical hydraulic conductivity and vessel wall reinforcement was negative only in monocots and eudicots (Fig. 2). This relationship indicates that, although there is a trade-off between hydraulic safety and hydraulic efficiency in flowers of different evolutionary clades, the factors restricting hydraulic efficiency are different among them. Additionally, we found a positive correlation between pedicel theoretical hydraulic conductivity and flower water potential at the turgor loss point, which was present only in monocots and eudicots (Fig. 3). This implies a stronger trade-off between drought tolerance and hydraulic efficiency in monocots and eudicots, while drought tolerance and hydraulic efficiency are decoupled in basal angiosperms. Previous studies have found that early angiosperms are inherently intolerant to drought and thrive in well-drained, disturbed microhabitats beneath moist forest canopies (Feild & Arens 2005, 2007; Feild *et al.* 2009a), which may explain why drought tolerance and hydraulic efficiency are decoupled in basal angiosperms.

We also observed a negative correlation between flower water potential at the turgor loss point and carbon concentration per mass, with monocots and eudicots having a steeper slope (Fig. 3). Since the higher the cost of carbon investment in flowers, the greater their tolerance to drought, this relationship indicates that drought tolerance in flowers is more sensitive to changes in carbon investment in monocots and eudicots. Interestingly, petal stomatal density was positively correlated with pedicel theoretical hydraulic conductivity and pedicel vessel lumen fraction only in monocots and eudicots (Fig. 2). Pedicel theoretical hydraulic conductivity and vessel lumen fraction are related to the pedicel hydraulic efficiency, and petal stomatal density is associated with the water loss rate from petals. These linkages suggests that petal stomatal density still plays a role in determining flower water use strategy, contrary to previous a report that there was no significant relationship between flower hydraulic conductivity and stomatal structure (Roddy *et al.* 2016). The time required for drying saturated flowers to 70% relative water content represents water retention time in flower tissues, and maintaining high flower mass per area and floral longevity requires abundant carbon and water resources. Here, time required for drying of saturated flowers to 70% relative water content was significantly and positively correlated with flower mass per area and floral longevity only in monocots and eudicots (Fig. 3). This indicates that in monocots and eudicots, the higher the construction cost of flowers, the longer the water remains in the tissues, whereas such a relationship does not exist in basal angiosperms. These findings indicate potential differences in the ecological adaptation strategies of flowers in response to changing environmental conditions across major evolutionary clades.

Reproduction-related traits are related to hydraulic traits in flowers

Our study revealed strong associations between hydraulic- and reproduction-related traits, consistent with our third hypothesis, suggesting that the water status of flowers can directly or indirectly impact plant reproduction. Floral longevity, which affects pollinator visits, pollen reception, and dispersal (Primack 1985; Ashman & Schoen 1994), was positively correlated with flower water loss rate (Fig. 3). Additionally, we observed that relative water content and petal epidermal cell size increased with flower longevity in monocots and eudicots (Fig. 4). A previous study found that flower structure–function relationships are related to flowering time and that the corolla buffers high evaporative demand during dry periods by increasing water content (Teixido *et al.* 2019). The prolonged existence of long-lived flowers might significantly deplete plant water and energy resources (Nobel 1977; Southwick 1984; Li *et al.* 2022). These findings indicate that flowers regulate the water demands of long-lived flowers by enhancing water retention within floral tissues or increasing water storage capacity. This evolutionary strategy may contribute to minimizing the transpiration budget of flowers, maintaining water balance in whole plants, and ensuring reproductive success under water-limited conditions (Galen 1999; Teixido & Valladares 2014).

Flower size plays a crucial role in reproductive ecology of angiosperms, as it influences pollinator attraction. A previous study on five species of Dipterocarpaceae demonstrated a positive correlation between pollen dispersal and flower size (Kettle *et al.* 2011). Our study observed an increase in pedicel maximum vessel diameter with increasing flower area (Fig. 4), suggesting that larger flowers require higher water availability, which aligns with previous research (Galen *et al.* 1999; Teixido & Valladares 2014; Ke *et al.* 2023). We also found a negative correlation between floral area and pedicel vessel density in monocots and eudicots, as well as between floral area and pedicel vessel wall reinforcement in basal angiosperms (Fig. 4). This finding suggests that larger flowers may be more susceptible to cavitation damage under water deficit conditions, consistent with the existing literature (Lambrecht 2013; Kuppler & Kotowska 2021). These findings further demonstrate that evaluating flower water use strategies is crucial in comprehending the impact of drought on floral traits and subsequent plant reproduction.

In summary, our study provides evidence that, compared to basal angiosperms, monocots and eudicots have a more conservative water use strategy in their flowers. There are strong relationships between flower hydraulic structure and function, but this relationship differs between basal angiosperms and monocots/eudicots. Moreover, there are strong linkages between reproduction-related and hydraulic traits in flowers, indicating that changes in water resource availability can significantly impact plant reproduction. In future studies, other important parameters, such as directly measured hydraulic conductivity, cavitation resistance, and pit membrane ultrastructure, should be considered to better understand the relationship between hydraulic structure and function in flowers, and how flowers respond to drought conditions.

AUTHOR CONTRIBUTIONS

YK and J-LZ conceived and designed the study. YK performed the experiments. YK, Y-BZ, F-PZ, and J-LZ analysed the data. YK wrote the draft manuscript. All authors read, commented on and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Basic information on the 24 species selected in this study.

Table S2. Two-way ANOVA results for each trait.

Table S3. Blomberg's *K* and *P*-values for phylogenetic signals of 29 floral traits.

Table S4. Coefficients of Pearson's correlation (lower left) and phylogenetic independent contrasts (upper right) for pairwise traits in 24 species.

Table S5. Coefficients of Pearson's correlation (lower left) and phylogenetic independent contrasts (upper right) for pairwise traits in basal angiosperms.

Table S6. Coefficients of Pearson's correlation (lower left) and phylogenetic independent contrasts (upper right) for pairwise traits in monocots and eudicots.

Table S7. SMA analysis of floral traits between basal angiosperms and monocots and eudicots.

Table S8. The eigenvalues for each set of principal components.

Table S9. Loadings of 29 floral traits on the first four principal components for 24 species.

Table S10. Bivariate relationships between 29 floral traits and the principal components with eigenvalues >1 for 24 species.

Figure S1. Pedicel anatomy of 12 woody plants from magnoliids to eudicots.

Figure S2. Pedicel anatomy of 12 herbaceous plants from Nymphaeaceae to eudicots.

Figure S3. Stomata of 24 species.

Figure S4. Petal anatomy of 24 species.

Figure S5. Principal components analysis (PCA) based on species trait values for 29 floral functional traits (A) and the loadings of basal angiosperms (green), monocots and eudicots (orange) along PCA axes (B).

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