

Functional types in the Bromeliaceae: relationships with drought-resistance traits and bioclimatic distributions

Jamie Males*  and Howard Griffiths

Department of Plant Sciences, University of Cambridge, Cambridge, UK

Summary

1. Neotropical Bromeliaceae occupy an exceptional diversity of habitats. The five principal functional types, which are defined by innovations such as Crassulacean acid metabolism (CAM), epiphytism, the tank growth form and neoteny, display distinct ecological water-use strategies.
2. The contribution of putative drought-resistance traits to the ecological differentiation of functional types has not previously been assessed, despite growing interest in the importance of these traits in other plant groups.
3. We formulated a set of hypotheses to be tested through a major survey of 376 bromeliad species (over 10% of the entire family) representing different functional types and ecologies. We quantified four drought-resistance traits: osmotic potential at full turgor (π_o), saturated water content (SWC), water mass per unit area (WMA) and dry leaf mass per unit area (LMA). For a subset of 308 species, relationships between drought-resistance traits and species bioclimatic envelopes were also analysed.
4. Saturated water content, WMA and LMA were closely inter-correlated, and there was weaker coordination with π_o , but the four traits differed significantly between functional types. Species of different functional types occupied distinct areas of bioclimatic space, and the relationships mapping drought-resistance trait values into bioclimatic space also varied between functional types.
5. We conclude that divergences in drought-resistance trait values form an integral part of the evolution of functional type distinctiveness and climatic niche differentiation in this megadiverse tropical plant family.
6. This study demonstrates how rapid, taxonomically extensive quantification of plant functional traits can provide important insights into the evolution of ecological diversity.

Key-words: adaptation, Bromeliaceae, diversity, drought resistance, functional types, water relations

Introduction

The Bromeliaceae is a large and extremely diverse family which includes several important examples of recent adaptive radiations, and is an emerging model system in plant evolutionary physiology and ecology (Givnish *et al.* 2014; Males 2016; Palma-Silva *et al.* 2016). The c. 3500 bromeliad species are distributed across the Neotropics and into adjacent temperate zones, and range from giant alpine succulents to epiphytes of tropical forests, some of which are neotenic and retain juvenile xeromorphic traits, while

others trap water and nutrients in impounding leaf bases (or 'tanks'). Bromeliads exhibit a variety of water uptake mechanisms, including root systems penetrating either terrestrial soil or canopy soil, and direct absorption of water trapped in tanks via specialised 'tank roots' or absorptive foliar trichomes (Benzing 2000). This range of water uptake mechanisms, coupled with other innovations such as epiphytism, succulence and Crassulacean acid metabolism (CAM), forms the basis of a spectrum of functional types and water-use strategies (Benzing 2000; Males 2016).

Several schemes have been proposed to describe bromeliad functional types (e.g. Pittendrigh 1948; Benzing 2000). Here, we use the scheme presented in Fig. 1, broadly

*Correspondence author. E-mail: jom23@cam.ac.uk

water content (SWC), which is defined as the quotient of area-specific water storage and area-specific dry mass (Ogburn & Edwards 2012). Another metric is the degree of succulence or water mass per unit area (WMA; Delf 1912), which is the product of LMA and SWC. Water mass per unit area therefore complements SWC by providing an area-specific measure of water storage.

We set out to test a set of core hypotheses relating to trait-mediated ecophysiological differentiation in the Bromeliaceae (Table 1), which address relationships between drought-resistance traits, between traits and bioclimatic factors and differences between functional types. To test our hypotheses, we undertook an extensive survey of the four drought-resistance traits (π_o , LMA, SWC, WMA). For a subset of 308 species, drought-resistance traits were compared with bioclimatic data associated with species' geographical ranges.

We found that mean values of all four drought-resistance traits showed ecologically relevant variation between functional types, with the tank epiphyte types being notably distinctive. Correlations were found among traits and between traits and bioclimatic factors, but were modulated

by functional type. Overall, the study highlights how complex interactions between anatomical and biochemical drought-resistance traits and morphological and physiological innovations have promoted ecological diversification in the bromeliads.

Materials and methods

PLANT MATERIAL

The 376 species included in this survey covered a broad taxonomic range, including representatives of seven out of the eight subfamilies in the Bromeliaceae. The only subfamily not represented was Lindmanioideae, which is rare in cultivation. All plant specimens used were in cultivation at one of four institutions: Cambridge University Botanic Garden (CUBG, UK), Royal Botanic Gardens Kew (RBGK, UK), Royal Botanic Gardens Edinburgh (RBGE, UK) and the Marie Selby Botanical Gardens (MSBG, FL, USA). Depending on origin, plants were grown either in glasshouses, outdoors under shade or outdoors without shade. All plants were well watered. Tropical glasshouse conditions were similar at each site, with daytime air temperature (T_{day}) maintained between 24 and 30 °C and night-time air temperature (T_{night}) between 16 and 22 °C. Daytime relative humidity (RH_{day}) was between 80 and

Table 1. Hypotheses tested in this investigation regarding trait–trait and trait–environment relationships

Hypothesis	Reasoning	References
SWC, WMA \propto π_o	Investment in water storage promotes drought avoidance, and alleviates selection for osmotically mediated drought tolerance	Bartlett <i>et al.</i> (2016), Males (2016)
SWC, WMA \propto LMA	Investment in storage succulence involves the production of thicker leaves with thick layers of hydrenchyma tissue	Vendramini <i>et al.</i> (2002), Males (2016)
$\pi_o \propto$ AI	More arid environments could select for investment in osmotically mediated drought tolerance	Bartlett, Scoffoni & Sack (2012b)
$\pi_o \propto$ 1/ P_{seas}	More seasonal rainfall could select for investment in osmotically mediated drought tolerance	Bartlett, Scoffoni & Sack (2012b)
SWC, WMA \propto 1/AI	More arid environments could select for hydraulic capacitance for drought avoidance	Arakaki <i>et al.</i> (2011)
SWC, WMA \propto P_{seas}	More seasonal rainfall could select for hydraulic capacitance for drought avoidance	Ellenberg (1981)
LMA \propto 1/AI	LMA mechanistically linked to SWC	Vendramini <i>et al.</i> (2002), Males (2016)
LMA \propto 1/ P_{seas}	LMA mechanistically linked to SWC	Vendramini <i>et al.</i> (2002), Males (2016)
SWC, WMA higher in CAM terrestrials than C ₃ terrestrials	Association of CAM bromeliads with more arid environments	Griffiths & Smith (1983)
SWC, WMA lower in tank epiphytes than terrestrials	Trade-off between internal and external hydraulic capacitance	Benzing (2000), Males (2016)
LMA lower in tank epiphytes than terrestrials	LMA mechanistically linked to SWC; selection for lower leaf construction costs in epiphytic niches	Benzing (2000), Vendramini <i>et al.</i> (2002), Males (2016)
π_o less negative in tank epiphytes than terrestrials	Resource limitation in epiphytic limitations reduces potential for osmolyte accumulation. External hydraulic capacitance also reduces selective pressure for drought tolerance	Martin <i>et al.</i> (2004), Males (2016)
SWC, WMA higher in CAM atmospheric epiphytes than in tank epiphytes	Selection for water retention in extreme, high-exposure microhabitats	Reyes-García, Mejía-Chang & Griffiths (2012)
LMA higher in CAM atmospheric epiphytes than in tank epiphytes	LMA mechanistically linked to SWC	Vendramini <i>et al.</i> (2002), Males (2016)

AI, aridity index; P_{seas} , precipitation seasonality; SWC, saturated water content; WMA, water mass per unit area; CAM, Crassulacean acid metabolism.

100%, while night-time RH (RH_{night}) was between 85 and 100%. For subtropical glasshouses, T_{day} was between 18 and 25 °C, and T_{night} between 10 and 18 °C. RH_{day} was between 50 and 80% and RH_{night} between 60 and 90%. Light microenvironments varied somewhat within glasshouses, depending on plant placement, but through a combination of natural and supplementary artificial illumination, daytime photosynthetic photon flux density (PPFD) was consistently in excess of levels known to be saturating for 50 of the 376 species (c. 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$; J. Males & H. Griffiths, unpubl. data). Outdoor environments at CUBG and MSBG (no outdoor-grown plants were sampled at RBGE or RBGK) were very different, although this did not lead to noticeable differences in trait values for congeneric species grown outdoors at either site (e.g. *Puya* spp.; historical meteorological data available online for CUBG at <http://www.botanic.cam.ac.uk/Botanic/Page.aspx?p=27&ix=2830> and for MSBG at <http://www.usclimatedata.com/cli/mate/sarasota/florida/united-states/usfl1072>).

A full list of species is included in Table S1, Supporting Information. For all measurements, fully expanded, non-senescent leaves were selected, generally corresponding to the central layer of leaves in rosette-forming species.

PSYCHROMETRIC DETERMINATION OF π_o

For each species, at least six replicate leaves from at least three individuals were allowed to reach full hydration in distilled water before leaf discs were cut from the centre of the lamina halfway along the leaf blade, avoiding the midrib in species where this was prominent. These were immediately frozen in liquid nitrogen or on dry ice and stored at -10 to -80 °C. During a measurement of π_o , leaf discs were punctured several times with a blade to promote maximal osmotic homogeneity within the sample, and then placed in a C-52 sample chamber attached to a Wescor Psypro system (Wescor, Inc., Logan, UT, USA). Samples were allowed to thaw and equilibrate with the air in the chamber before a psychrometric reading of π_o was taken. The time profile of the measurement process was optimised for each species.

SATURATED WATER CONTENT, LEAF MASS PER AREA AND WATER MASS PER AREA

Saturated water content was quantified for all species as a measure of succulence (Ogburn & Edwards 2012). Leaf discs of 5–20 mm radius were cut from the central portion of the lamina and allowed to reach full hydration in distilled water before turgid mass was recorded. They were then reweighed after complete desiccation in a drying oven at 80 °C and SWC was calculated as:

$$\text{SWC} = \frac{(\text{turgid mass} - \text{dry mass})}{\text{dry mass}}$$

Leaf mass per unit area was calculated using the same leaf discs. The dry mass of each leaf disc was divided by disc area to yield LMA. Twenty biological replicates were taken for each species, sampled from at least three individuals. Using SWC and

LMA data, WMA was calculated as the product of these two values.

FUNCTIONAL TYPE ASSIGNMENT AND COMPARISONS

Functional type assignment was based on the classification system of Pittendrigh (1948). Species were categorised as either C_3 terrestrials, CAM terrestrials, CAM tank epiphytes, C_3 tank epiphytes or CAM atmospheric epiphytes. Photosynthetic pathway determination was based primarily on the $\delta^{13}\text{C}$ values reported by Crayn *et al.* (2015). For 20 species in genera known to contain both C_3 and CAM species, there was no information available, and these were therefore assumed to use the same photosynthetic pathway as the majority of species in the same genus. Information on habit was obtained from the eMonocot portal (<http://e-monocot.org/>). Facultative epiphytes were recorded as epiphytes.

Decisions about which groups of functional types could be meaningfully compared were based on an assessment of the evolutionary hypotheses reported in phylogenetic analyses such as those of Givnish *et al.* (2011, 2014). Figure 1 shows an approximate representation of the phylogenetic relationships between bromeliad genera as supported by recently published analyses, with functional types assigned to each genus. Inspection of the phylogenetic patterning of functional types reveals a series of major transitions in functional type, displayed in Table 2.

Although transitional forms are present in some lineages, this scheme captures the essential trajectories that have characterised functional type evolution in the Bromeliaceae. Comparisons were therefore made between pairs of functional types linked by these evolutionary transitions. Differences in mean trait values between pairs were tested for by one-way ANOVA in R (R Development Core Team, 2008).

BIOCLIMATIC DATA

The Global Biodiversity Information Facility (GBIF) was interrogated for occurrence data for each species. All records were checked for quality, with records lacking adequate metadata, records that represented clear geographic outliers and duplicate records all being discarded. Sixty-eight species with insufficient reliable occurrence records were excluded from further analysis of climate relations. Using R, bioclimatic variables for each occurrence point for the remaining 308 species were extracted from a 2.5 arc min resolution raster dataset downloaded from the BIOCLIM website (Hijmans *et al.* 2005). We used precipitation seasonality (the coefficient of variation, %, in monthly precipitation) in our analysis because of the supposed relationship between seasonal rainfall and succulence (Ellenberg 1981). An aridity index was constructed as a proxy for evaporative demand, using the ratio of mean annual precipitation (MAP, mm) to mean annual temperature (MAT, °C). Lower aridity index values correspond to more water-limited conditions. For each species, the mean value of each climatic variable was calculated. Linear and nonlinear regressions were then performed in R to identify relationships

Table 2. Major transitions in functional type in the Bromeliaceae inferred from phylogenetic distribution of functional types (see Fig. 1)

Phylogenetic position of transition	Pre-transition functional type	Post-transition functional type
Base of Hechtioideae	C_3 terrestrial	CAM terrestrial
Within Puyoideae	C_3 terrestrial	CAM terrestrial
Base of/within early-diverging Bromelioideae	C_3 terrestrial	CAM terrestrial
Base of Tillandsioideae	C_3 terrestrial	C_3 tank epiphyte
Base of core Bromelioideae	CAM terrestrial	CAM tank epiphyte
Within <i>Tillandsia</i>	C_3 tank epiphyte	CAM atmospheric epiphyte

between these values and π_o , SWC, WMA and LMA. Principal components analysis (PCA) was also performed on all drought-resistance trait values and the first two principal components (PC1 and PC2) were plotted by functional type against species' mean bioclimatic scores.

Results

The variation in drought-resistance traits for all 376 species is displayed in density plots and bivariate plots in Fig. 2a,b.

Values of π_o indicated low solute concentrations overall, and ranged between -0.29 MPa in *Tillandsia lithophila* L. Hrom. (CAM atmospheric epiphyte) and -0.82 MPa in *Ochagavia litoralis* (Phil.) Zizka, Trumpler & Zöllner (C_3 terrestrial). SWC, WMA and LMA showed a relatively high degree of variation across all species. SWC values ran from 2.40 in *Vriesea gladioliflora* (H.Wendl.) Antoine (C_3 tank epiphyte) to 11.98 in *Puya humilis* Mez (C_3 terrestrial), while WMA values ranged between 117.72 g m $^{-2}$ in

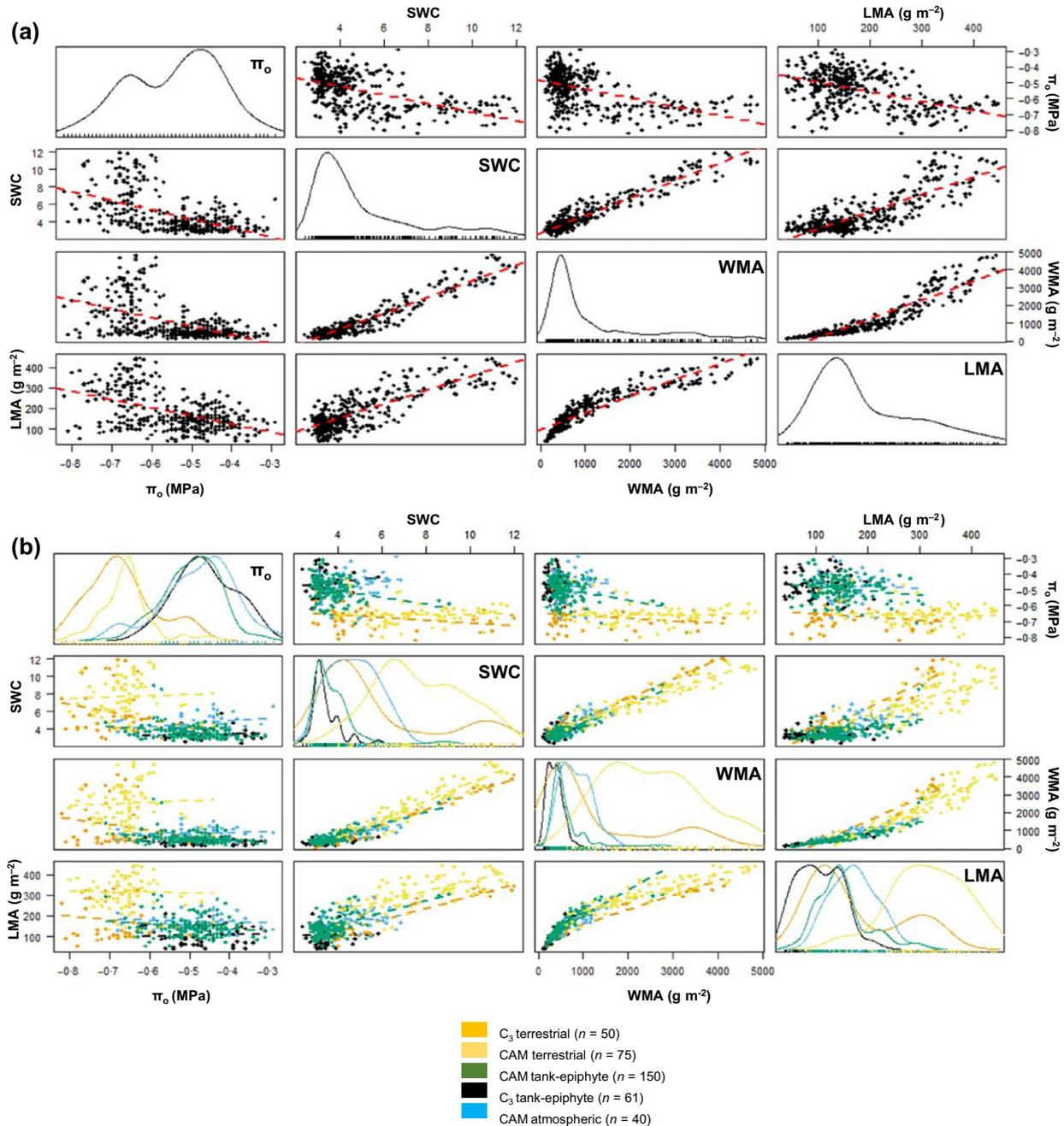


Fig. 2. Variation in drought-resistance traits (π_o , SWC, WMA, and LMA). (a) Results for all species ($n = 376$). Central diagonal panels show density plots for each trait; red dashed lines in bivariate panels indicate linear regression lines. (b) Results by functional type (see colour key in figure). Central diagonal panels show density plots for each trait by functional type; bivariate panels show all species plotted by functional type with dashed linear regression lines for each type. SWC, saturated water content; WMA, water mass per unit area; LMA, leaf mass per unit area.

Guzmania monostachia (L.) Rusby ex Mez (C₃-CAM tank epiphyte) and 4821.44 g m⁻² in *Hechtia glomerata* Zucc. (CAM terrestrial). LMA values between 38.28 g m⁻² in *G. monostachia* (C₃-CAM tank epiphyte) and 446.94 g m⁻² in *Neoglaziovia variegata* (Arruda) Mez (CAM terrestrial). The complete dataset is available in Table S1.

RELATIONSHIPS BETWEEN DROUGHT-RESISTANCE TRAITS

Pairwise relationships between the four drought-resistance traits are highlighted in Table 3 (see also Fig. 2a,b). Across the full species set, WMA was unsurprisingly strongly correlated with both SWC and LMA, since it is the product of these two variables. Log transformation improved the fit for the relationship between WMA and LMA, whereas linear regression provided the best fit for all other correlations. There were negative correlations between π_o and SWC and between π_o and WMA, suggesting that those species with a higher absolute water content at full turgor also tended towards higher solute concentrations. Similarly, there was a negative correlation between π_o and LMA. LMA and SWC showed a very strong positive correlation, consistent with the hypothesis that in the bromeliads the evolution of storage succulence (*sensu* Ihlenfeldt 1985) may have entailed an increase in leaf construction costs. These costs may be compensated for by the advantages of LMA in terms of both water storage and other factors such as leaf durability and life span in the harsh, often oligotrophic environments to which many of these species are native (Benzing 2000; Wright, Westoby & Reich 2002; Wright *et al.* 2004; Poorter *et al.* 2009). We note that the sign of the relationship between LMA and SWC (+ve) is the opposite of what might be expected to arise as an artefact of the methodologies used to derive LMA and SWC, which makes the observed correlation all the more compelling (Table 4).

Inspection of the bivariate plots with linear regression fitted by functional type (Fig. 2b) revealed that some of

the relationships between traits that were observed across the full species set are weak or undetectable within individual functional types. This was particularly true for correlations involving π_o , suggesting that interspecific variation in this trait within functional types may be decoupled from variation in the remaining three drought-resistance traits.

VARIATION IN DROUGHT-RESISTANCE TRAITS BETWEEN FUNCTIONAL TYPES

All four drought-resistance traits differed significantly between functional types (Fig. 3). Epiphytic functional types showed less negative values of π_o (Fig. 3a), while CAM terrestrials were most distinctive in terms of SWC (Fig. 3b), WMA (Fig. 3c) and LMA (Fig. 3d), with the highest median values. Variation within functional types was particularly high for π_o in the CAM atmospheric epiphytes and for LMA, SWC and WMA in the C₃ and CAM terrestrials.

Divergences in trait values between pairs of functional types between which bromeliad lineages have transitioned are summarised in Fig. 4. Compared with C₃ terrestrials, CAM terrestrials did not differ in π_o ($P = 0.306$, $F = 1.06$), but showed higher SWC ($P < 0.001$, $F = 23.20$), WMA ($P < 0.001$, $F = 40.50$) and LMA ($P < 0.001$, $F = 86.49$). This suggests that CAM terrestrials invest relatively more in drought avoidance through succulence, as we had hypothesised. CAM tank epiphytes meanwhile showed significantly less negative π_o than CAM terrestrials ($P < 0.001$, $F = 82.09$), as well as lower SWC ($P < 0.001$, $F = 146.70$), WMA ($P < 0.001$, $F = 412.20$) and LMA ($P < 0.001$, $F = 136.40$). C₃ tank epiphytes also displayed significantly less negative π_o than C₃ terrestrials ($P < 0.001$, $F = 45.64$), as well as lower SWC ($P < 0.001$, $F = 17.58$), WMA ($P < 0.001$, $F = 29.70$) and LMA ($P < 0.001$, $F = 16.16$). Tank epiphytism in both C₃ and CAM lineages therefore appeared to be associated with high π_o and low SWC, WMA and LMA, consistent with our hypothesis that tank-based external capacitance

Table 3. Correlations between drought-resistance traits across 376 bromeliad species based on linear regression except where stated

	SWC	WMA (g m ⁻²)	LMA (g m ⁻²)
π_o (MPa)	-, $P < 0.01$, $r^2 = 0.29$	-, $P < 0.01$, $r^2 = 0.27$	-, $P < 0.01$, $r^2 = 0.25$
LMA (g m ⁻²)	+, $P < 0.01$, $r^2 = 0.67$	+, $P < 0.01$, $r^2 = 0.90$ (log)	-
WMA (g m ⁻²)	+, $P < 0.01$, $r^2 = 0.91$	-	-

SWC, saturated water content; WMA, water mass per unit area; LMA, leaf mass per unit area.

Table 4. Correlation matrix for bioclimatic variables and drought tolerance traits for 308 bromeliads species

Bioclimatic variable	π_o (MPa)	SWC	LMA (g m ⁻²)
Aridity index	+, $P < 0.001$, $r^2 = 0.26$	-, $P < 0.01$, $r^2 = 0.20$	-, $P < 0.01$, $r^2 = 0.18$
Precipitation seasonality	-, $P < 0.001$, $r^2 = 0.13$	+, $P < 0.001$, $r^2 = 0.20$	+, $P < 0.001$, $r^2 = 0.12$

Relationships with $r^2 \geq 0.15$ are presented in boldface.

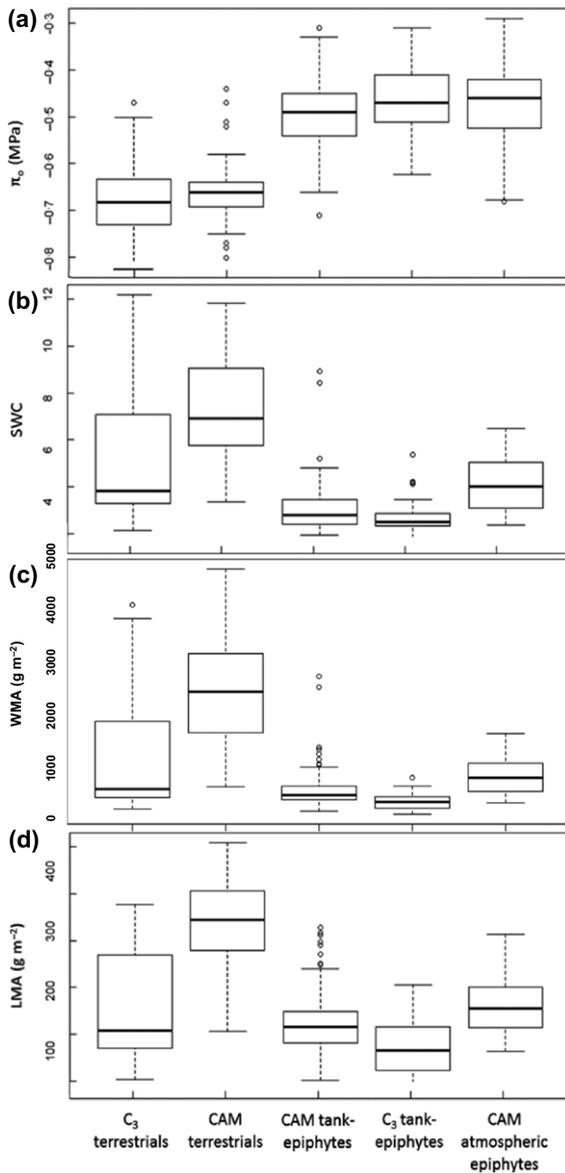


Fig. 3. Drought-resistance traits (a: π_0 ; b: SWC; c: WMA; d: LMA) by functional type. Sample sizes: C_3 terrestrials, $n = 50$; CAM terrestrials, $n = 75$; CAM tank epiphytes, $n = 150$; C_3 tank epiphytes, $n = 61$; CAM atmospheric epiphytes, $n = 40$. Boxes show median values and interquartile range (IQR), with whiskers of length $(1.5 \times \text{IQR})$ and outliers plotted as individual points. SWC, saturated water content; WMA, water mass per unit area; LMA, leaf mass per unit area; CAM, Crassulacean acid metabolism.

reduces the advantage of internal capacitance provided by succulence. Tillandsioid C_3 tank epiphytes and CAM atmospheric *Tillandsia* epiphytes did not differ in π_0 ($P = 0.759$, $F = 0.094$), but, as we had hypothesised, the atmosphericers displayed significantly higher SWC ($P < 0.001$, $F = 51.99$) and WMA ($P < 0.001$, $F = 91.78$), as well as LMA ($P < 0.001$, $F = 60.98$). This reflects the role of internal capacitance in allowing these plants to survive between infrequent pulses of moisture availability,

representing an associated transition to CAM and drought avoidance.

RELATIONSHIPS BETWEEN DROUGHT-RESISTANCE TRAITS AND BIOCLIMATIC FACTORS

The relationships between measured drought-resistance traits and bioclimatic occupancy (quantified with using species mean scores for the aridity index and precipitation seasonality) are presented in Figure 5. Relationships between variables with $r^2 \geq 0.15$ were considered relatively strong. Across all species for which bioclimatic data were available ($n = 308$), there was a positive relationship between π_0 and aridity index scores ($P < 0.001$, $r^2 = 0.26$), indicating that species native to more arid regions tend to display more negative π_0 . There was a weaker negative relationship between π_0 and precipitation seasonality scores ($P < 0.001$, $r^2 = 0.13$), offering moderate support to the hypothesis that more seasonal rainfall should select for greater cellular drought tolerance. Aridity index scores were negatively correlated with SWC (log, $P < 0.001$, $r^2 = 0.23$), WMA (log, $P < 0.001$, $r^2 = 0.22$) and LMA (linear, $P < 0.001$, $r^2 = 0.18$), implying that increased aridity selects for a higher degree of succulence and durability. Consistent with our expectations, there were stronger correlations between precipitation seasonality and SWC ($P < 0.001$, $r^2 = 0.20$) and WMA ($P < 0.001$, $r^2 = 0.17$) than with LMA ($P < 0.001$, $r^2 = 0.12$), suggesting that higher capacity for internal water storage becomes more advantageous when water availability is concentrated in limited temporal windows.

When PCA was performed on all log-transformed drought-resistance trait values, the first two principal components (PC1 and PC2) explained 77.24 and 16.49% of the total variance in the data respectively. Eigenvectors for SWC, WMA and LMA were closely aligned and approximately parallel to PC1, whereas the eigenvector for π_0 was almost orthogonal to the SWC–WMA–LMA grouping and not clearly aligned with either PC1 or PC2. Species scores for PC1 and PC2 were plotted against mean bioclimatic scores (for the aridity index and precipitation seasonality; Fig. 6a–d). Across all species, aridity index scores showed a strong negative correlation with PC1 scores ($P < 0.001$, $r^2 = 0.26$; Fig. 6a), but only a very weak positive correlation with PC2 scores ($P < 0.001$, $r^2 = 0.06$; Fig. 6b). Similarly, precipitation seasonality scores displayed a positive correlation with PC1 scores ($P < 0.001$, $r^2 = 0.18$; Fig. 6c), and a statistically significant but extremely weak correlation with PC2 scores ($P = 0.045$, $r^2 = 0.01$; Fig. 6d). Overall, species' positions along the PC1 axis therefore explained an important proportion of variation in bioclimatic affinities.

Discussion

Through a wide-ranging survey of four key drought-resistance traits, this investigation has generated extensive

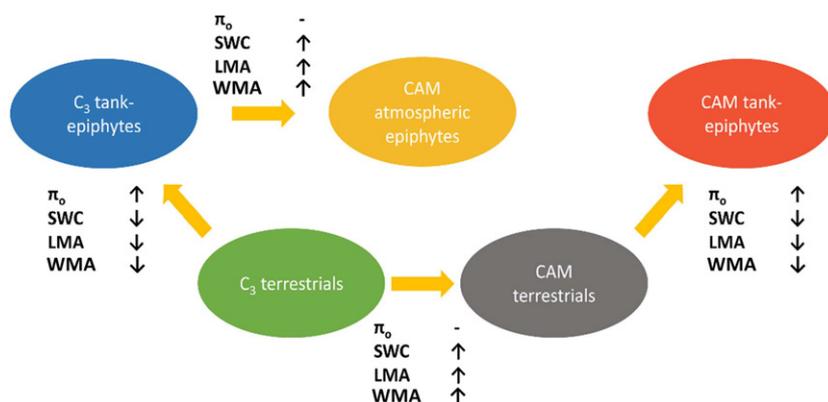


Fig. 4. Divergences in drought resistance traits between functional types in the Bromeliaceae.

evidence in support of trait-mediated ecophysiological differentiation in the Bromeliaceae, an ecologically important Neotropical family. More generally, it provides timely insights into the biochemical and structural basis of differentiation in water-use strategies in tropical herbaceous plants. The data also provided support for a series of specific evolutionary hypotheses (Table 1), which are further explored and contextualised here.

VARIATION IN DROUGHT-RESISTANCE TRAITS AMONG THE BROMELIADS

The extraordinary diversity of the Bromeliaceae is grounded in many forms of specialisation, from pollinator specificity (Pansarin & de Pedro 2016) to substrate specificity (Válka Alves, Kolbek & Becker 2008) and water-use strategies (Males 2016). Our broad survey of variation in a set of drought resistance predictors, π_o , SWC, WMA and LMA, has provided insights into the basis of diversity in water-use strategies.

Across the major functional types within the family, there is a relatively modest capacity for osmolyte accumulation (i.e. high values of π_o) and osmotic adjustment, consistent with some earlier studies (Smith *et al.* 1986; Nowak & Martin 1997; Martin *et al.* 2004; Ceusters *et al.* 2009). Bromeliads are therefore rather drought sensitive at the cellular level compared with other plant groups (e.g. Bartlett, Scoffoni & Sack 2012; Maréchaux *et al.* 2015). This is in accordance with their conservative water expenditure and relatively low productivity (J. Males & H. Griffiths, unpubl. data). Considering the drought resistance continuum running from drought tolerance to drought avoidance, all bromeliad species cluster towards the 'avoidance' end of the spectrum (Males 2016).

As hypothesised, the evolution of SWC and LMA appears to have been tightly coordinated across the bromeliad family, and the correlation between these traits suggests that succulence involves increased leaf construction costs. One noted effect of storage succulence is the decoupling of LMA from other leaf economic spectrum traits (Vendramini *et al.* 2002; Grubb *et al.* 2015; J. Males & H. Griffiths, unpubl. data). This arises because more storage-succulent leaves tend to be thicker, but that this elevated

thickness is dominated by specialised achlorophyllous hydrenchyma tissue. The relationship between LMA and leaf thickness is an important confounding factor in leaf trait studies (Witkowski & Lamont 1991; Niinemets 1999; Wilson, Thompson & Hodgson 1999), and will need to be studied further in the context of anatomical variation in the bromeliads. Because SWC is a measure of bulk water content and not exclusively of symplastic water (the pool referred to in definitions of succulence), it is possible that variation in apoplastic water storage, perhaps determined by differences in cell wall properties, also makes an important contribution to variation in SWC. This could provide an additional link between SWC and LMA, but will require further investigation.

Contrary to our hypothesis that drought avoidance and drought tolerance mechanisms would represent alternative strategies, the negative correlations between both SWC and WMA (the product of SWC and LMA) and π_o suggest that succulence cannot fully compensate for the potential for rapid loss of chlorenchyma water potential during periods of high evaporative demand. Acute, atmospherically driven water stress may select for drought tolerance, whereas drought avoidance mechanisms are likely to be more effective at coping with chronic water stress associated with gradual soil drying (or tank emptying).

Interestingly, some relationships between drought-resistance traits that were apparent across the full species set seemed to be driven by differences in trait combinations between functional types, and were not detectable within functional types. In particular, all correlations between π_o and other drought-resistance traits were clearly underpinned by differences in mean π_o between functional types, with little coordination between π_o and other traits within functional types. This observation leads to consideration of the extent and ecological relevance of variation in drought-resistance traits among functional types.

DROUGHT-RESISTANCE TRAITS IN THE ECOLOGICAL DIFFERENTIATION OF FUNCTIONAL TYPES

The water-use strategies of functional types were explained by differential investment in drought resistance mechanisms. Consistent with our hypothesis, those terrestrial

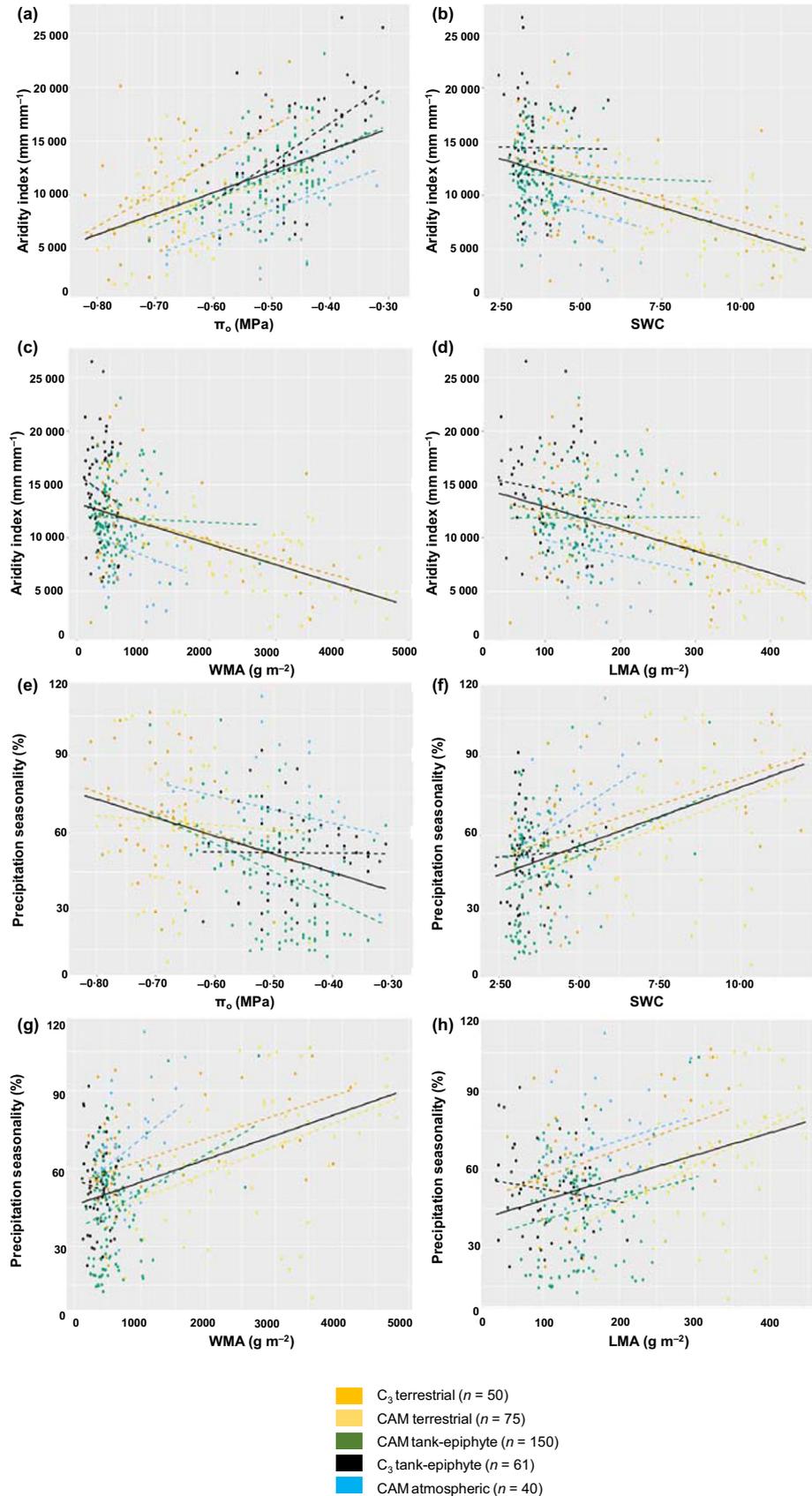


Fig. 5. (a–h) Relationships between species' mean values for drought-resistance traits (π_0 , SWC, WMA, LMA) and bioclimatic factors (aridity index and precipitation seasonality) across the full species set (*n* = 376). Dashed lines show linear regression by group; solid line shows linear regression across all species. SWC, saturated water content; WMA, water mass per unit area; LMA, leaf mass per unit area.

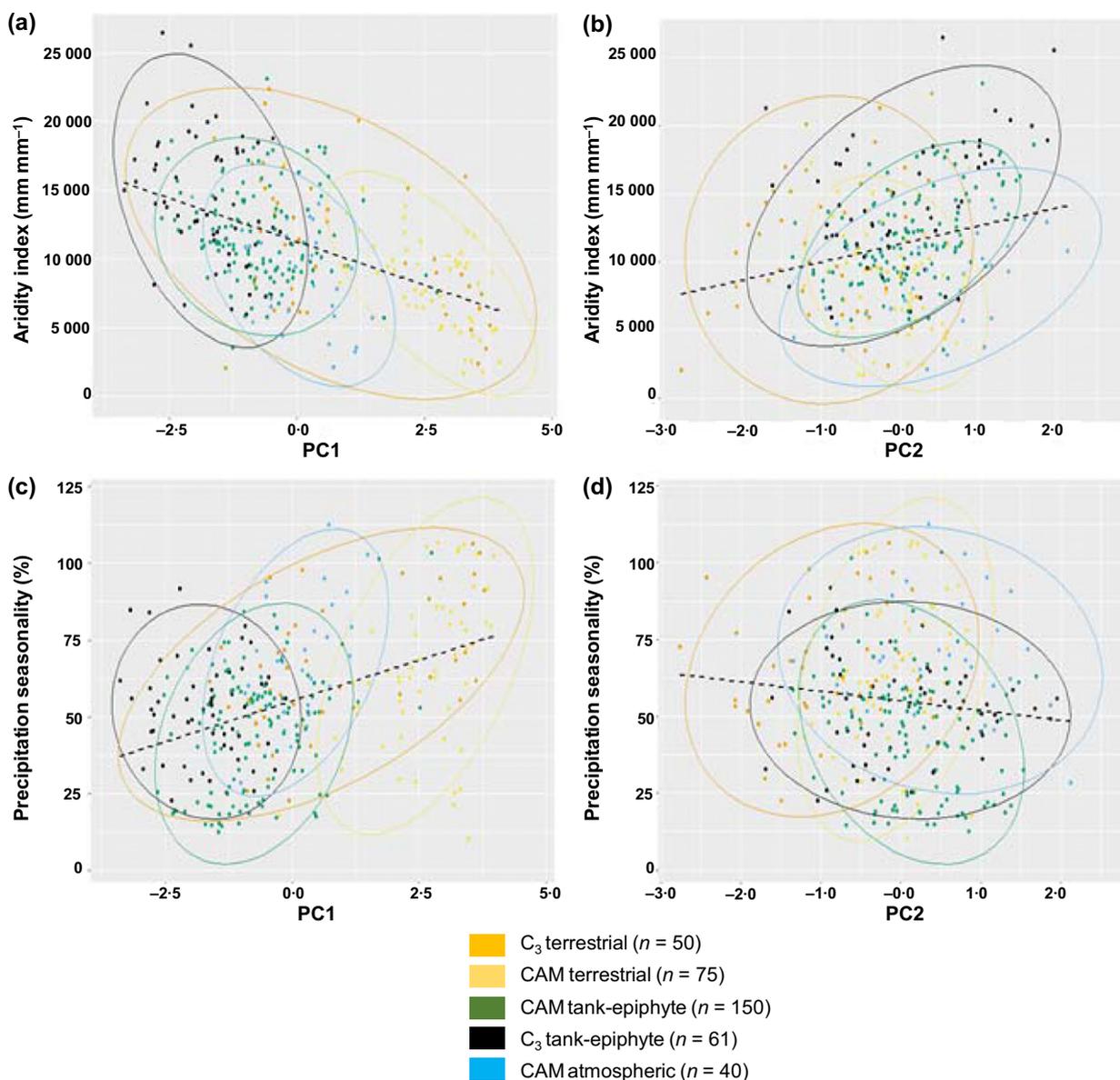


Fig. 6. (a–d) Relationships between species' principal component scores (PC1 and PC2) and bioclimatic scores (aridity index and precipitation seasonality). Species are plotted by functional type, with black dashed lines showing linear regression across all species ($n = 376$).

bromeliads with CAM tended to show a greater degree of succulence than C₃ species. Because these two syndromes (CAM and succulence) so often go hand-in-hand in plant evolution, it is difficult to say whether succulence preceded CAM or vice versa in the bromeliads. Detailed investigation of the genetic history of specific lineages may help in the negotiation of this perennial impasse (Heyduk *et al.* 2016).

The convergent evolutionary origins of the tank epiphyte functional type in C₃ and CAM bromeliad lineages are associated with similar changes in drought-resistance traits. According to our hypothesis, the tank growth form reduces reliance on internal hydraulic capacitance. Our data show that this, perhaps coupled with resource limitation in epiphytic niches selecting for reduced construction costs

(linked with lower LMA), has favoured reduced leaf succulence in tank epiphytes relative to terrestrial species. The tank itself functions as an alternative drought avoidance adaptation. How this distinctive drought resistance syndrome of tank epiphytes relates to their heteroblastic development from tank-less juvenile forms should be investigated further, particularly given the importance of drought-related juvenile mortality in bromeliad demography (Hietz, Ausserer & Schindler 2002; Winkler, Hülber & Hietz 2005). Other traits in epiphytic bromeliads that are likely to enhance the conservatism of water use and facilitate drought avoidance include low stomatal conductance and leaf hydraulic conductance (North *et al.* 2013, 2015; Males & Griffiths, 2017). We hypothesised that this complex of drought avoidance traits could reduce the need for

drought tolerance mechanisms in tank epiphytes, which was supported by the less negative values of π_0 in tank epiphytes than in terrestrials. This reduced reliance on osmotically mediated drought tolerance could be important for nutrient-limited epiphytic niches (Wanek & Zotz 2011) by minimising the necessary investment in osmolyte storage relative to allocation for growth or reproduction.

It was hypothesised that CAM atmospheric epiphytes would display particularly high values of SWC and WMA, since they depend on water storage between brief, often infrequent pulses of water availability (Benzing 2000; Reyes-García, Mejía-Chang & Griffiths 2012). This contention was strongly supported by the data, which also showed that many atmospheric epiphytes also display high values of LMA, perhaps partly as by-products of dense cell packing conferred by the neotenic retention of juvenile leaf anatomy (Till 1992; Benzing 2000). The genetic basis and detailed physiological implications of neoteny in *Tillandsia* merit further research. The absence of a significant difference in π_0 values between C_3 tank epiphytes and CAM atmospheric epiphytes can be explained by the continued pressures of resource limitation and the intensified investment along the drought avoidance trajectory in atmospheric epiphytes. Atmospheric *Tillandsia* species generally have reduced leaf surface areas, and are densely covered in large absorptive trichomes, which, by shielding the stomata, are generally supposed to act secondarily as barriers to water loss (but see Benz & Martin 2006).

INTERACTIONS BETWEEN DROUGHT-RESISTANCE TRAITS AND BIOCLIMATIC FACTORS

The correlations between species' mean values of π_0 , SWC, WMA and LMA with scores for the aridity index and precipitation seasonality support the notion that physiological traits underpin adaptations for particular bioclimatic envelopes. We had specifically hypothesised that π_0 would be positively correlated with aridity index scores and negatively correlated with precipitation seasonality scores due to selection for drought tolerance in more arid and seasonal environments. The data not only supported this hypothesis, but also supported the competing hypothesis that the same environments would select for succulence (i.e. drought avoidance). This again suggests that at the level of the whole family, while drought avoidance mechanisms may be the dominant components of species' water-use strategies, there is an important undercurrent of complementary investment in drought tolerance mechanisms. Given the strong coordination of SWC with LMA, it seems likely that the negative correlation between LMA and aridity index scores was associated with investment in thicker hydrenchyma tissue in species native to more arid environments. However, the possibility that some component of LMA (e.g. leaf thickness, leaf density) could be an important determinant of bromeliad drought responses in its own right could be explored in greater detail in the future through more detailed structural characterisation.

Some of the large amount of variance (particularly within functional types) that could not be accounted for in relationships between drought-resistance traits and bioclimatic factors can be attributed to interactions with other drought-resistance traits, and the fact that these analyses were performed on plants grown under garden conditions. We recognised that an important limitation of the sampling design utilised here is that it is unclear how different trait values might be under natural conditions, or the extent to which differences between functional types would be exacerbated or diminished. One possibility is that, by contrast with our well-watered garden specimens, plants growing under natural conditions might be exposed to more frequent water limitation, which could promote stronger expression of drought-resistance traits. Although the limited data available suggest that bromeliads have little capacity for osmotic adjustment (e.g. Smith *et al.* 1986), morphological and anatomical plasticity in response to environmental variation has been observed in a number of bromeliads (e.g. Scarano *et al.* 2002; Cavallero, López & Barberis 2009). A comprehensive assessment of the extent and ecophysiological importance of phenotypic plasticity in bromeliads is much needed, along with detailed mechanistic case studies of how anatomical and physiological traits contribute to the determination of bromeliad species distributions.

The relationships between drought-resistance traits and bioclimatic scores were clearly modulated by functional type identity. Thus, innovations such as CAM, epiphytism and the impounding tank have interacted with differential investment in drought-resistance traits to facilitate the occupancy of distinct water-limited climate space for each functional type (Fig. 5). While previous treatments of bromeliad trait evolution have emphasised the undeniable importance of origins of innovations in facilitating evolutionary diversification through the exploitation of ecological opportunities (Givnish *et al.* 2011, 2014; Donoghue & Sanderson 2015), quantitative traits have likely also played a role in the generation of species-level diversity through ecological differentiation. Future research could explore this possibility in a more explicit phylogenetic framework by incorporating a comprehensive estimation of bromeliad phylogenetic relationships with ancestral state reconstruction and analysis of trait covariation and diversification dynamics.

In summary, our unique and extensive dataset demonstrates the involvement of four drought-resistance traits in ecological differentiation in the Bromeliaceae. We have shown that these traits are correlated across a diverse species set, differ between functional types and relate to bioclimatic limitations on species distributions. Furthermore, functional type identity modulates the relationships between drought-resistance traits and bioclimatic properties. Future work should seek to determine the molecular and genetic basis for variation in drought-resistance traits, as this would not only provide a more complete picture of the evolution of the extensive ecological diversity displayed

by the bromeliads, but could also inform efforts to improve drought resistance of commercially important species such as pineapple.

Authors' contributions

J.M. conceived the study and collected and analysed the data; J.M. and H.G. prepared the manuscript. Both authors contributed to the drafts and gave final approval for publication.

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Data accessibility

All data used in this manuscript are present in the manuscript and its supporting information.

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Full listing of traits for all species examined in this investigation.