

**Structure-function relationships in the water-use strategies and ecological diversity of the
Bromeliaceae**

Jamie Oliver Males

Clare College, University of Cambridge

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Summary

The Bromeliaceae is one of the largest and most ecologically diverse angiosperm families in the Neotropics. In recent years, this family has begun to emerge as a model system for the study of plant evolutionary ecology and physiology, and major advances have been made in understanding the factors involved in episodes of rapid diversification and adaptive radiation in specific bromeliad lineages. However, despite a long tradition of ecophysiological research on the Bromeliaceae, an integrative, evolutionarily-contextualised synthesis of the links between anatomical, physiological, and ecological aspects of bromeliad biology has hitherto been lacking. The overarching aim of this research project was therefore to use new quantitative data representing a wide range of bromeliad taxonomic and functional groups to elucidate how variation in leaf traits connected by structure-function relationships influences ecological differentiation among bromeliad taxa. Special emphasis was placed on hydraulic and water relations traits because of fast-paced contemporary developments in these fields. The methodologies employed included an assessment of the diversity of bromeliad hydrological habitat occupancy, quantification of key anatomical and physiological traits and their correlations, investigation of the links between vascular and extra-xylary anatomy and hydraulic efficiency and vulnerability, quantification of stomatal sensitivity to leaf-air vapour pressure deficit and stomatal kinetics, and a case study of trait-mediated niche segregation among congeneric epiphytic bromeliad species on the Caribbean island of Trinidad. The results highlight how divergences in a range of continuous and categorical anatomical traits underpin differences in physiological capacities and sensitivities, which in turn determine environmental relations and ecological distinctiveness. This research project therefore provides critical insights into the mechanistic basis of evolutionary diversification in a highly ecologically important family. It also represents the most comprehensive analysis of the significance of trait variation for ecological differentiation across any major radiation of herbaceous angiosperms.

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Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit for the relevant Degree Committee.

Jamie Males

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Summary

The Bromeliaceae is one of the largest and most ecologically diverse angiosperm families in the Neotropics. In recent years, this family has begun to emerge as a model system for the study of plant evolutionary ecology and physiology, and major advances have been made in understanding the factors involved in episodes of rapid diversification and adaptive radiation in specific bromeliad lineages. However, despite a long tradition of ecophysiological research on the Bromeliaceae, an integrative, evolutionarily-contextualised synthesis of the links between anatomical, physiological, and ecological aspects of bromeliad biology has hitherto been lacking. The overarching aim of this research project was therefore to use new quantitative data representing a wide range of bromeliad taxonomic and functional groups to elucidate how variation in leaf traits connected by structure-function relationships influences ecological differentiation among bromeliad taxa. Special emphasis was placed on hydraulic and water relations traits because of fast-paced contemporary developments in these fields. The methodologies employed included an assessment of the diversity of bromeliad hydrological habitat occupancy, quantification of key anatomical and physiological traits and their correlations, investigation of the links between vascular and extra-xylary anatomy and hydraulic efficiency and vulnerability, quantification of stomatal sensitivity to leaf-air vapour pressure deficit and stomatal kinetics, and a case study of trait-mediated niche segregation among congeneric epiphytic bromeliad species on the Caribbean island of Trinidad. The results highlight how divergences in a range of continuous and categorical anatomical traits underpin differences in physiological capacities and sensitivities, which in turn determine environmental relations and ecological distinctiveness. This research project therefore provides critical insights into the mechanistic basis of evolutionary diversification in a highly ecologically important family. It also represents the most comprehensive analysis of the significance of trait variation for ecological differentiation across any major radiation of herbaceous angiosperms.

1. General Introduction

Content from this chapter is published in Males (2016)

1.1 Introduction to the Bromeliaceae as a study system

1.1.1 Phylogenetic context

The Bromeliaceae Juss. is a large and diverse monocotyledonous family with a centre of diversity in the northern Andes (Benzing, 2000; Givnish et al., 2011). It includes one commercially important species, the pineapple (*Ananas comosus* (L.) Merr.), but a number of bromeliad species are also of ethnobotanical significance, including the fibre-yielding *Neoglaziovia variegata* (Arruda da Camara) Mez. Some species and hybrid bromeliads (notably of *Guzmania*, *Vriesea*, and *Tillandsia* parentage) are also popular houseplants and are grown commercially under glasshouse conditions in regions including northern Europe, the USA and Australia. From a phylogenetic perspective, the bromeliad family occupies an interesting position as an early-diverging taxon in the Poales (Bouchenak-Khelladi et al., 2014), the monocot order that includes the most agriculturally important plant family, the Poaceae. Divergence time estimates suggest that the stem lineage of the Bromeliaceae diverged from the Typhaceae approximately 100 Mya, while the crown radiation of the family began approximately 19.5 Mya (Bouchenak-Khelladi et al., 2014; Givnish et al., 2011). Relative to all other families in the Poales, the crown groups of the Bromeliaceae are therefore very recent.

Eight subfamilies are currently recognised within the Bromeliaceae: Brocchionideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Navioideae, Pitcairnioideae, Puyoideae and Bromeliadeae (Fig. 1.1.1; Givnish et al., 2011). Phylogenetic relationships among the Bromeliaceae are increasingly well-resolved at the generic and infra-generic level, but many groups remain problematic due to limited sequence diversity associated with the family's very recent evolutionary radiation. The Core Bromelioideae (*Aechmea* Ruiz & Pav. – *Wittrockia* Lindm. in Fig. 1.1.1) is a notable example, and taxonomic concepts in this clade will require reworking as new evidence continues to come to light.

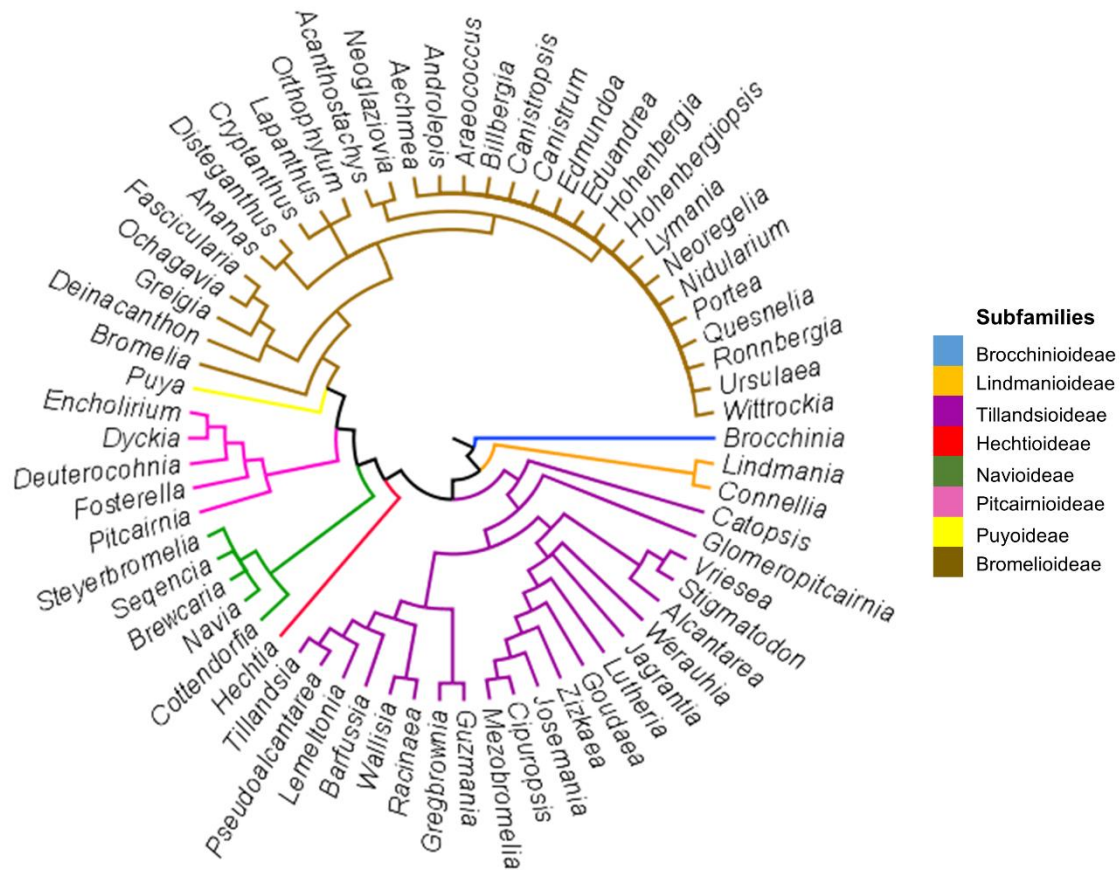


Figure 1.1.1. Genus-level phylogeny of the Bromeliaceae based on topological consensus of recent molecular phylogenetic analyses (Schulte et al., 2009; Givnish et al., 2014; Silvestro et al., 2014; Barfuss et al., 2016).

1.1.2 Key innovations, key landscapes, and adaptive radiation

The discourse surrounding the evolutionary history of the Bromeliaceae has been dominated by discussion of the roles of (1) orogenic and physiographic change in the Neotropics, and (2) morphological and physiological ‘key innovations’ driving invasion of unsaturated niche space with subsequent diversification and adaptive radiation (Gentry, 1982; Givnish et al., 2014; Silvestro et al., 2014). A range of putative key innovations have been identified in the Bromeliaceae. They include ornithophilous pollination, entangling seeds, absorptive foliar trichomes, CAM, epiphytism, and the tank growth form, in which a rosette of leaves with overlapping bases forms a tank that can trap both water and leaf-litter. Givnish et al. (2014) used a variety of methodologies to analyse the contribution of these innovations to the evolutionary dynamics of the bromeliads, concluding that epiphytism, the tank growth form, ornithophilous pollination, and montane habitats (‘key landscapes’) were associated with significantly increased net diversification rates, and suggesting mechanisms for these correlations.

Donoghue and Sanderson (2015) introduced the term ‘confluence’ to denote the coming together of organismal and environmental factors at a critical point in the history of a biological lineage to promote increased net diversification. Rapid diversification must be distinguished from the phenomenon of radiation, as the crown species richness of a lineage may accrue either quickly or slowly (Givnish, 2015). Furthermore, not all radiations automatically qualify as *adaptive* radiations, as this phrase should be reserved for cases in which there is real evidence of niche differentiation between crown taxa (Givnish, 2015). In the Bromeliaceae, there is, however, evidence of both explosive diversification and true adaptive radiation. A notable example of the latter is provided by species-level niche differentiation in nutritional strategy in *Brocchinia*. (Givnish et al., 1997, 2014; Givnish, 2015). New quantitative data and critical assessment of water-use strategies in other radiations will help to determine the degree of ecophysiological niche differentiation and the validity of considering these radiations to be adaptive. It may also assist in establishing whether anatomical constraints on physiology can explain why some lineages, referred to by Donoghue and Sanderson (2015) as ‘depauperons’, have not radiated and remain species-poor.

1.1.3 Morphological and ecophysiological diversity of functional types

Bromeliad growth forms range from the massive arborescent Queen-of-the-Andes (*Puya raimondii* Harms) to the strongly morphologically reduced Spanish moss (*Tillandsia usneoides* (L.) L.). The environmental space inhabited by bromeliads is correspondingly diverse (Benzing, 2000). Some *Puya* species and Bromelioideae have invaded high Andean tundra, bogs and *páramos*, while others occur in Mediterranean scrub or exposed temperate coastal sites (e.g. *Ochagavia* spp.; Zizka et al., 2009; Jabaily and Sytsma, 2013). Neotropical dry forests and thickets are rich in both terrestrial and epiphytic bromeliads (e.g. *Neoglaziovia*, *Tillandsia* spp.; Versieux and Wendt, 2007). Nebulophytic *Tillandsia* spp. are often the dominant vegetation on the dunes of South American and Mexican coastal deserts (Rundel and Dillon, 1998; Pinto et al., 2006), and species such as *Bromelia humilis* Jacq. occur on the sodium-rich substrates of the *salinas* that are inimical to most flora (Lee et al., 1989). Epiphytic bromeliads are often a major component of the biomass of Neotropical wet forests, and different bromeliad floras are found in lowland, montane and elfin formations of contrasting physiognomy and climate (e.g. Sugden and Robins, 1979; Steege and Cornelissen, 1989). Many early-diverging bromeliads are restricted to poorly-developed, oligotrophic substrates on *tepuis* (Givnish et al., 1997; Huber, 2006), and the saxicolous (lithophytic) habit has evolved repeatedly within the family (Benzing, 2000). There are some rheophytic species experiencing periodic submersion in the genera *Dyckia*, *Guzmania* and *Pitcairnia* (Luther and Norton, 2008; Voltolini et al., 2009; Saraiva et al., 2015).

A scheme to classify the ecophysiological syndromes or functional types in the bromeliads was developed by Pittendrigh (1948). These functional types are closely connected with water-use strategies (Males, 2016). Working on the continental South American island of Trinidad (see **Chapter 6**), Pittendrigh identified the following series of functional types: Type I *Soil-Root*; Type II *Tank-Root*; Type III *Tank-Absorbing Trichome*; and Type IV *Atmosphere-Absorbing Trichome*. Benzing (2000) modified Pittendrigh's classification to separate *Tank-Absorbing Trichome* Bromelioideae (which are mostly CAM) and Tillandsioideae (mostly C₃) into Types III and IV, respectively, leaving the atmospheric species as Type V. In this research project, the following functional types were distinguished: C₃ mesic terrestrials; C₃ succulent terrestrials; CAM terrestrials; C₃ tank-epiphytes; CAM tank-epiphytes; and CAM atmospheric epiphytes. The rationale behind dividing C₃ terrestrials into mesic and succulent groups was that succulent species were expected to display distinctive leaf anatomy and physiology (Males, 2016). All CAM terrestrials (including soil-root and tank-root species) were grouped together because it was expected that the primary physiological differences between these groups would lie in root tissues, whereas this research project focussed on leaf traits. Table 1.1.1 provides a conspectus of the distribution of the functional types recognised in this project across bromeliad genera.

| Subfamily | Genus | Functional types |
|-----------------|---|---|
| Brocchinioideae | <i>Brocchinia</i> | C ₃ mesic terrestrial, C ₃ tank-epiphyte* |
| Lindmanioideae | All | C ₃ mesic terrestrial |
| Tillandsioideae | All except <i>Tillandsia</i> <i>Tillandsia</i> | C ₃ tank-epiphyte [†] C ₃ tank-epiphyte; CAM atmospheric epiphyte |
| Hechtioideae | <i>Hechtia</i> | CAM terrestrial |
| Navioideae | All | C ₃ mesic terrestrial |
| Pitcairnioideae | All | C ₃ mesic terrestrial; CAM terrestrial |
| Puyoideae | <i>Puya</i> | C ₃ succulent terrestrial [†] ; CAM terrestrial |
| Bromelioideae | <i>Ananas, Bromelia, Cryptanthus,</i> <i>Disteganthus, Deinacanthon,</i> <i>Fascicularia, Greigia, Lapanthus,</i> <i>Neoglaziovia, Ochagavia</i> Core Bromelioideae | CAM terrestrial; C ₃ succulent terrestrial CAM tank-epiphyte; C ₃ tank- epiphyte |

Table 1.1.1. Distribution of ecophysiological functional types across genera of the Bromeliaceae. **Brocchinia* C₃ tank-epiphytes are not true epiphytes but are saxicolous and hydraulically independent of the growth substrate. [†]Includes C₃-CAM intermediate species.

1.2 Current understanding of structural and functional aspects of bromeliad water relations and hydraulics

Many traits that influence either directly or indirectly the water economy of bromeliads have been identified in the literature, but little attempt has been made to quantify these across large, phylogenetically-diverse samples of species. As many bromeliads have evolved unusual traits that may cause them to deviate from standard soil–plant–atmosphere hydraulics models, developing a holistic understanding of their water relations is a peculiar challenge (Males, 2016). However, addressing this issue will provide critical insights into the unique success of bromeliads in so many stressful regions of plant niche space. Improved understanding of the controls on water fluxes through bromeliads could also be of broader significance, since bromeliads frequently represent a large fraction of the vascular epiphytic biomass in Neotropical forests and are therefore likely to represent an important component of hydrological and nutrient cycles (Nadkarni, 1984; Veneklaas et al., 1990; Ingram and Nadkarni, 1993; Freiberg and Freiberg, 2000; Hölscher et al., 2004; Köhler et al., 2007; Díaz et al., 2010; Gehrig-Downie et al., 2011). A summary of previous studies of bromeliad water relations and hydraulics will highlight the key knowledge gaps and provide a baseline for the investigations presented in this thesis.

1.2.1 Roots and stems

Relatively little attention has been paid to the structure and function of bromeliad roots. Root to shoot biomass ratio (R: S) varies widely; atmospheric or nebulophytic *Tillandsia* spp. sometimes lack roots entirely in the mature state, whereas C₃ mesic terrestrial *Pitcairnia* spp. produce extensive adventitious rooting systems (Lüttge et al., 1986; Benzing, 2000). As reflected in the functional type series, bromeliad evolution is characterized by a progressive loss of dependence on roots for water absorption in parallel with increased dependence on absorptive trichomes (Pittendrigh, 1948; Benzing, 2000). Terrestrial species are rooted in the soil and are presumed to obtain the majority of their water from this source, via root vasculature that is fairly typical of monocots, with vessel elements equipped with scalariform end-plates (or simple end-plates in *Pitcairnia*; Tomlinson, 1969). The availability of soil water varies enormously across the different habitats of terrestrial bromeliads, from the frequently inundated flood plain soils inhabited by hydrophytic *Dyckia distachya* Hassler and *Pitcairnia flammeea* Lindl. to the dry, well-drained soils associated with many Chilean *Puya* spp. (Benzing, 2000; Rocha-Pessôa and Rocha, 2008; Voltolini et al., 2009). There is a dearth of quantitative data regarding variation in the absorptive capacity of roots within and between terrestrial species, whereas it has generally been assumed that the roots of most epiphytic species make a negligible contribution to plant water balance in comparison with foliar trichomes

(Benzing, 2000). Meanwhile in atmospheric *Tillandsia* spp., the cable-like, rot-resistant roots are so heavily sclerified as to preclude any significant absorptive role and they function simply as holdfasts to maintain connection with the phorophyte (Benzing, 1978, 2000; Reinert and Meirelles, 1993).

The role of specialised roots in tank-root CAM terrestrial species warrants particular note. These species, all belonging to the early-diverging Bromelioideae, display a rudimentary tank-forming habit with multiple low-volume axillary tanks in individual leaf bases, rather than the large central tank of the CAM tank-epiphyte Core Bromelioideae (Pittendrigh, 1948; Benzing, 2000). The tanks are explored by foraging adventitious roots, rendering the plant less dependent on soil roots for water acquisition and nutrition (Pittendrigh, 1948; Lee et al., 1989).

Bromeliad stems are generally reduced in size relative to the foliage, but have the typical monocot structure (Tomlinson, 1969). The picture is complicated only by the often highly extensive leaf traces grouped into collateral bundles and the intracauline roots that may penetrate the stem for some distance before exiting (Tomlinson, 1969; Benzing, 2000). Increased proportional investment in stems is observed in the Andean *Puya* spp., in which stem-succulence is presumed to fulfil a number of functions including water storage to buffer against temperature-limited soil water availability (Benzing, 2000). In stark contrast to the stem-succulent condition in *Puya*, stems of neotenic atmospheric epiphytes such as *Tillandsia usneoides* are sometimes reduced to sclerotic connections between ramets (Benzing, 2000).

1.2.2 Shoot architecture

The ancestral bromeliad shoot architecture is presumed to be a typical liliaceous rhizomatous rosette (Benzing, 2000). From this, a range of morphologies in different lineages has arisen, each with distinct adaptations relating to water-use strategies (Smith, 1989; Benzing, 2000). Among these, the evolution of the phytotelm or tank form was a major advance that allowed epiphytes access to a more reliably continuous water supply (Schulte et al., 2009; Givnish et al., 2014; Silvestro et al., 2014). Several properties of the tank determine its contribution to whole-plant water relations. The orientation of the rosette strongly affects the efficiency of precipitation interception, and most mature tank bromeliads display negative geotropism (Adams and Martin, 1986c). The efficiency of water retention is determined by the tightness of the overlap between leaf bases. Proto-tanks in groups such as the early-diverging Bromelioideae are relatively inefficient at retaining water because of limited overlap and hence these plants typically continue to depend partly on soil water and are slow-growing stress-tolerators with less ecological flexibility than Core Bromelioideae equipped with a well-developed central tank (Benzing, 2000; Schulte et al., 2009;

Silvestro et al., 2014). The volume of the tank relative to trichome-mediated absorptive capacity and leaf area is of obvious importance in maintaining hydration (Zotz and Thomas, 1999; Schmidt and Zotz, 2001; Zotz and Hietz, 2001). Tank surface area is also important, as evaporative loss of water from tanks may be significant during periods of intense or extended vapour pressure deficit (Zotz and Thomas, 1999; Mejia-Chang, 2007). Some xeromorphic tank-epiphytes display tubular tanks with a large volume but low exposed surface area, limiting evaporative loss (e.g. *Aechmea nudicaulis* (L.) Griseb.; Benzing, 2000).

Beyond its relation to tank volume and consequent influence over nutritional status and photosynthetic capacity (Zotz, 1997; Zotz et al., 2011), shoot size is of manifold importance for both tank-forming and tank-less species. Many size-related effects operate via the surface area to volume ratio (SA: V), an important determinant of potential transpirational or peristomatal water loss and light-quenching efficiency. Reduced size and SA: V have evolved through neoteny in Tillandsioideae, with profound ecophysiological consequences (Benzing and Ott, 1981; Adams and Martin, 1986a; Benzing, 2000). Neotenic *Tillandsia* spp. are in the mature state phenotypically comparable with the seedlings of non-neotenic Tillandsioideae, whereas the latter undergo a dramatic developmental transition to a tank-forming morphology in the mature state (Benzing, 2000). This was historically regarded as a classic example of heteroblasty, but evidence from large, multi-species datasets suggests that the transition is a slower process better described as ontogenetic drift, with plant size being an important confounding effect (Zotz et al., 2004; Meisner et al., 2013). However, it remains true that the water-acquisition strategies of juvenile individuals of these species are generally unlike those of mature individuals. Dew-fed juveniles do not possess an impounding rosette and their narrow leaves (which minimize SA: V) are more uniformly covered in absorptive trichomes than rain-fed adults, resembling the mature state of neotenic atmospheric species (Adams and Martin, 1986b; Reinert and Meirelles, 1993; Schmidt and Zotz, 2001). Maturation involves the development of the impounding tank, the establishment of opposing gradients of stomatal and trichome density along leaf axes and the broadening of leaf blades.

1.2.3 Leaf morphology and structure

The leaves of bromeliads vary considerably in size, shape and density (Benzing, 2000). As in other plant lineages, mesic, low-light environments such as forest floor habitats have selected for thin, low-density leaves with a large surface area for water and light interception (Gilmartin, 1983; Valladares et al., 2002). At the opposite end of the scale, highly exposed, upper canopy microhabitats have selected for small, dense leaves with a low SA: V ratio (Benzing, 2000). Rosettes

of many narrow rolled or terete leaves with dense trichome layers allow nebulophytic *Tillandsia* spp. to maximise uptake of transiently available water (Gilmartin, 1983; Martorell and Ezcurra, 2007).

Bromeliad leaves display a number of tissue-level adaptations relevant to water relations. The mesophyll is often traversed by conspicuous axial air lacunae, usually occurring in alternation with vascular bundles (Tomlinson, 1969). The dimensions of these lacunae vary widely, but they are most well-developed in the C₃ tank-epiphyte Tillandsioideae. The role traditionally ascribed to these lacunae is the aeration of tissue submerged below the water-line in tank-forming species (Tomlinson, 1969; Varadarajan, 1986), although this situation does not explain their presence in many lineages lacking central tanks. Indeed, given their frequent occurrence in families across the Poales, it seems likely that the propensity for aerenchyma formation is plesiomorphic in Poales and that lacunae have been occasionally co-opted for more specialized functions in specific lineages (Tomlinson, 1969; Jung, Lee and Choi, 2008). Beyond the possible importance of lacunae in the aeration of submerged tissues in bromeliad leaves, they may serve as reservoirs for respiratory CO₂ accumulation prior to fixation by phospho-*enol*-pyruvate carboxylase in CAM-cycling (Griffiths, 1988; Sage and Khoshravesh, 2016), and as important conduits for vapour-phase transport of H₂O (Sheriff, 1977, 1984; Rockwell et al., 2014; Buckley, 2015).

Bromeliad leaves typically express some degree of succulence. This is often associated with an adaxial layer of differentiated water storage parenchyma (hydrenchyma), sometimes in conjunction with an additional, thinner abaxial layer (Tomlinson, 1969; Benzing, 2000). The hydrenchyma provides a reservoir of water to buffer against reduced availability in epiphytic, lithophytic, xeric and seasonal environments. This form of succulence based on tissue differentiation ('storage succulence' *sensu* Ihlenfeldt, 1985) is associated with drought avoidance rather than drought tolerance because of the ability of the hydrenchyma to release water to the chlorenchyma when the water potential of that tissue decreases during drought (Nowak and Martin, 1997; Benzing, 2000; Martin et al., 2004). Both the ratio of chlorenchyma to hydrenchyma and the leaf volume must also be considered in determining the volume and availability of the reservoir of internally stored water for buffering against fluctuations in transpirational demand and avoiding drought (Ogburn and Edwards, 2012; Griffiths, 2013). A different form of succulence ('all-cell succulence' *sensu* Ihlenfeldt, 1985) exists in some CAM bromeliads, notably among the Bromelioideae. Here, in addition to the hydraulic capacitance afforded by the hydrenchyma, voluminous chlorenchyma cells may store large quantities of water with the organic acids associated with CAM. Chlorenchymatous succulence often occurs alongside reduced investment in hydrenchyma, but there exists a spectrum involving varying degrees of investment in hydrenchymatous and chlorenchymatous succulent tissues. In an evolutionary context, succulence, alongside other xeromorphic traits, has been credited with

facilitating the successful invasion of the South American 'dry diagonal' by terrestrial Pitcairnioideae (Santos-Silva et al., 2013), and is associated with CAM, xerophytism, and epiphytism in other bromeliad lineages (Benzing, 2000).

1.2.4 Leaf vasculature and hydraulic conductance

Bromeliads xylem traces are dominated by tracheids rather than vessel elements (Tomlinson, 1969; Carlquist, 1975, 2012). Vessel elements are in fact absent from the stems and leaves of most species (Tomlinson, 1969; Carlquist, 1975). The xylem elements are also highly reduced in diameter, limiting their conducting capacity (Tomlinson, 1969). The vasculature of the leaves of neotenic *Tillandsia* spp. comprises highly reduced phloem and just a few narrow tracheids, an arrangement that probably arose via heterochronic effects (Carlquist, 2009), and which Benzing (2000) argued is compatible with reduced dependence on axial water transport due to the efficiency of trichome-mediated water uptake. Additionally, the xylem is surrounded by an 'endodermoid' layer or parenchymatous sheath of low permeability, restricting exchange of water to and from the xylem (Tomlinson, 1969). With these considerations in mind, we should expect bromeliads to demonstrate relatively low rates of xylem hydraulic conductance and vein leakiness, contributing to low total leaf hydraulic conductance (K_{leaf}). This expectation has been corroborated by reports that axial K_{leaf} in *Guzmania lingulata* (L.) Mez is even lower than in many gymnosperms and ferns and much lower than in monocots with less conservative water use, such as many grasses (Martre et al., 2000; Maherali et al., 2008; North et al., 2013).

Measured leaf xylem tensions in bromeliads are consistently low compared with other plant groups, but comparable with observations for other vascular epiphytes (Smith and Lüttge, 1985; Smith et al., 1986; Martin et al., 2004). Smith et al. (1986) reported that two terrestrial species of Bromelioideae in Trinidad displayed sufficiently low xylem tensions during the dry season that they must have been functionally independent of the substrate, using instead water stored in tanks and hydrenchyma to maintain low xylem tension. As these plants had developed soil-penetrating roots, they presumably were able to express a seasonally induced capacity to modify their dependence on soil water uptake. The precise mechanisms for such an effect in the bromeliads have not been established, but could involve the rectifier-like conductivity reported in phylogenetically diverse desert succulents, facilitated by suberisation and reduction in root-specific aquaporin activity (Nobel and Sanderson, 1984; López and Nobel, 1991; North and Nobel, 1991, 1992; Nobel and Cui, 1992; Martre et al., 2001; North et al., 2004).

Vulnerability to xylem cavitation is an important limitation on leaf physiological capacity (Tyree and Sperry, 1989) and is closely related to symplast drought tolerance (Vilagrosa et al., 2010). Because of the low xylem tensions and transpiration rates in bromeliads, the threat of cavitation may be low under most conditions. This is compounded by the narrow lumens of the conducting elements of bromeliad xylem, as there is a well-established correlation between xylem conduit diameter and hydraulic vulnerability (Scoffoni et al., 2016). Bromeliad species capable of hydraulically isolating themselves from their environment during periods of drought might be expected to show particularly limited exposure to routine embolism (Smith et al., 1986). Species with smaller leaves and higher major vein density may show especially low vulnerability (Scoffoni et al., 2011; Sack et al., 2012), although as discussed above, neotenic species with strong capacity for foliar uptake may be insensitive to xylem embolism anyway.

1.2.5 *Stomata and trichomes*

Bromeliad stomata are typically arranged on the abaxial surface in irregular intercostal series (Tomlinson, 1969). Stomatal density varies greatly between species and groups, displaying a weak inverse correlation with trichome density across the ecophysiological functional types. These stomatal to trichome density ratios (S: T) reflect evolutionary shifts in water-use strategies, with lower S: T values representing more conservative water use (Benzing, 2000).

Stomata of bromeliads are complex and highly diverse structures, but their functional biology remains understudied. Fundamentally, the behaviour of bromeliad stomata must answer to the same challenge confronting all land plants: CO₂ for photosynthetic assimilation can only be gained from the atmosphere at the expense of lost water vapour (Manzoni et al., 2011). Guard cells of angiosperm stomata lie at the nexus of a host of signalling pathways and integrate this information to adjust pore aperture in such a way as to optimize carbon gain against water loss (Schroeder et al., 2001). Bromeliad guard cells are frequently subtended by a pair of enlarged epidermal or hypodermal subsidiary cells that may almost occlude the substomatal cavity (Tomlinson, 1969). This situation has led some authors to speculate that these cells have taken over part of the function of the guard cells, although as yet there is little evidence (Tomlinson, 1969; Martin and Peters, 1984; Benzing, 2000). Transitions in stomatal complex morphology appear to coincide with key divergences in the bromeliad phylogenetic tree, such as at the bases of Core Tillandsioideae and Core Bromelioideae, and display convergence in lineages that have invaded similar environmental space (Tomlinson, 1969).

An early report on gas exchange by the atmospheric epiphyte *Tillandsia recurvata* (L.) L. was suggestive of the operation of a feed-forward response in stomatal conductance to ambient humidity (Lange and Medina, 1979) and similar observations have been made in *Aechmea nudicaulis* (Griffiths et al., 1986). Various mechanisms for this response have been proposed following reports of its occurrence in many plant groups (Peak and Mott, 2011; Bauer et al., 2013) and it is a phenomenon of potentially profound ecological significance. Direct measurements of the strength of this effect in bromeliads of different functional types have not been reported, but it might be hypothesized that stomata of atmospheric species, which inhabit the most water-limited, pulse-supplied microhabitats, would be most sensitive to declining humidity in order to conserve captured water (Reyes-García et al., 2012).

The absorptive trichomes found in most bromeliad lineages and their valve-like absorptive function have long been a source of fascination for plant scientists (Mez, 1904; Tietze, 1906). The peltate multicellular foliar trichome is a synapomorphy for the family and it has been suggested that the origin of the absorptive function may relate to the association of early-diverging bromeliads (e.g. Brocchinioideae) with oligotrophic habitats on the inselbergs of the Guayana Shield (Medina, 1974; Givnish et al., 1997). Moving from the terrestrial through to the epiphytic and atmospheric bromeliad functional types, trichomes make an increasingly important contribution to total water (and mineral) uptake. A number of secondary characters must be accounted for when considering the contribution of trichomes to plant water balance. These include trichome density and the kinetics of trichome-mediated absorption, which may be associated with anatomical detail specific to individual lineages (Benzing, 2000).

Trichome density is highly variable across the family, ranging from zero in some *Pitcairnia* spp. to ca. 100% cover of the leaf surface in many atmospheric *Tillandsia* spp. (Tomlinson, 1969). Dense trichome cover may help to reduce transpiration and photoinhibition through increased reflectance (Benzing et al., 1978; Pierce et al., 2001; Pierce, 2007). The maximization of water absorption through the presence of a dense array of trichomes is critical to the success of atmospheric bromeliads in pulse-supplied microhabitats (Benzing, 2000). Trichome-mediated water exchange between atmosphere and plants is complex. Martin and Schmitt (1989) identified two separate pools of water in *T. usneoides*: an epidermal pool associated with hydration of the trichome layer at night followed by dehydration after dawn and an internal mesophyll pool being supplied by trichomes but depleted by both stomatal- and trichome-mediated water loss. This effect has subsequently been noted in investigations of gas exchange and oxygen isotope signature in *T. usneoides* (Haslam et al., 2003; Helliker and Griffiths, 2007; Helliker, 2011, 2014) and similar observations have been made in *T. recurvata* (Schmitt et al., 1989).

The absorptive capacity of bromeliad trichomes, in terms of water and mineral nutrients, varies considerably between and within species (Benzing, 1970; Benzing et al., 1976; Sakai and Sanford, 1980; Brighigna et al., 1988; Owen et al., 1988; Papini et al., 2010). In Tillandsioideae, trichomes are highly absorptive across the entire leaf surface, whereas in Bromelioideae there is evidence to suggest that trichomes on the leaf sheath, frequently inundated by tank water, are more absorptive than those that occur at lower density on the exposed leaf blade (Benzing, 1970; Benzing et al., 1976).

1.2.6 Cell and tissue water relations

Tissue-level succulence and capacitance is defined at a cellular level by a set of key parameters: cell size, cell shape and cell wall elasticity. Cell size is a critical parameter because it controls the volume of water the cell can hold and the number of membranes water molecules that has to cross when traversing a given distance of symplast and therefore affects the resistance of this pathway. Cell size is additionally linked to nuclear DNA content, which may be subject to selective pressures during the terrestrial-epiphytic transition because of reduced phosphorus availability (Zotz, 2004; Chase et al., 2005; Zotz and Richter, 2006; Leitch et al., 2009; Winkler and Zotz, 2009; Zotz and Asshoff, 2010; Wanek and Zotz, 2011). The hydrenchyma cells of bromeliads are often hexagonal or rectangular in cross-section, this regular shape allowing them to be densely packed, thereby improving tissue-level capacitance and water conservation (Nelson et al., 2005). The elasticity of the walls of a cell, quantified as Young's elastic modulus (ϵ), defines the capacity of the cell to undergo mechanical deformation. Non-photosynthetic hydrenchyma cells typically have highly elastic cell walls (low ϵ) that enable them to undergo reversible shrinkage or deformation, preferentially releasing water to buffer metabolism in photosynthetic chlorenchyma cells during drought (Ekern, 1965; Stiles and Martin, 1996; Nowak and Martin, 1997). There is only one published value of bulk ϵ for a bromeliad: 3.3 MPa in *Tillandsia utriculata* L. (Stiles and Martin, 1996). This low value is in accordance with a drought tolerance strategy involving reductions in relative water content, but maintenance of Ψ_{leaf} . In contrast, it has long been established that some bromeliads are able to avoid internal drought during periods of desiccating conditions by minimizing water loss to extremely low levels (Sideris and Krauss, 1928, 1955; Benzing and Burt, 1970; Martin and Adams, 1987).

All published measurements of the osmotic potential of bromeliad cell sap suggest that solute concentrations are low, especially in CAM species and epiphytes (Harris, 1918; Biebl, 1964; Griffiths et al., 1986; Lüttge et al., 1986; Smith et al., 1986; Martin, 1994; Nowak and Martin, 1997;

Zotz and Andrade, 1998; Martin et al., 2004). The capacity for osmotic adjustment to drought appears limited (Martin et al., 2004). However, as bulk osmotic potential represents a major determinant of Ψ_{TLP} (Bartlett et al., 2012b), it should be expected that Ψ_{TLP} would be modest in most bromeliads.

1.2.7 *Photosynthetic pathways*

Approximately 50% of bromeliads use CAM photosynthesis, following multiple independent origins in the family (Crayn et al., 2004, 2015). In certain bromeliad lineages, CAM appears to be associated with increased diversification rates, although it does not seem to represent a driver of diversification across the family as a whole (Givnish et al., 2014; Silvestro et al., 2014). CAM has generally been interpreted as an adaptation to environments characterized by high evaporative demand, and is therefore intimately associated with plant water relations (Kluge and Ting, 1978; Osmond, 1978; Lüttge, 1987, 2010; Winter and Smith, 1996; Cushman, 2001; Cushman and Borland, 2002; Herrera, 2009). In CAM species, nocturnal opening of stomata allows gas exchange to occur when the leaf-atmosphere vapour pressure deficit is reduced because of lower ambient temperatures, thereby solving the dilemma of ‘desiccation or starvation’ for a C_3 plant under water-limited conditions (Lüttge, 1997; Males and Griffiths, 2017b). Obligate CAM bromeliads typically occur in more arid habitats and microhabitats than their C_3 relatives, including water-limited epiphytic niches (Medina, 1974; Medina et al., 1977; Griffiths and Smith, 1983; Crayn et al., 2004, 2015). Because CAM species require large, highly vacuolate cells for nocturnal acid accumulation, they typically display succulence and densely packed mesophyll (Maxwell et al., 1997; Nelson et al., 2005; Nelson and Sage, 2008).

CAM is often highly plastic within species and individuals, with the strength of the expression of each characteristic CAM phase being controlled by recent environmental conditions (Cushman, 2001; Dodd et al., 2002; Haslam et al., 2003; Owen and Griffiths, 2013). Many bromeliads demonstrate two alternative forms of CAM, known as CAM-cycling and -idling (Sipes and Ting, 1985; Griffiths, 1988; Loeschen et al., 1993; Martin, 1994). CAM-cycling, thought to be the least evolutionarily advanced state along the CAM continuum (Silvera et al., 2010), involves nocturnal fixation of respiratory CO_2 behind closed stomata accompanying typical diurnal C_3 fixation. The capacity for CAM-cycling may exist in bromeliad taxa currently considered as C_3 plants because of the difficulty of identifying such capacity in rapid screens of carbon isotope values. CAM-idling, conversely, is an extreme form of CAM in which the stomata remain continuously closed and the only fixation is of respired CO_2 at night. CAM-idling therefore cannot make a net contribution to the carbon balance of a plant. In

many cases, CAM-cycling and -idling appear to be reversibly inducible, and drought-inducible CAM has been observed in some species such as the terrestrial succulent *Puya floccosa* (Linden) E.Morren ex Mez (Herrera et al., 2010). In another instance of intraspecific variability in CAM expression, CAM activity has been shown to vary with elevation in *Puya chilensis* Molina (Quezada et al., 2014). There are many examples of CAM bromeliads in perhumid cloud forests, where the classical benefit of CAM in improving WUE is negated by the constantly low VPD. Pierce et al. (2002) suggested that the benefit of CAM in such environments might lie in the plasticity it affords in photosynthetic responses to variability in leaf wetness, including the capacity to engage in recycling of respiratory CO₂.

1.2.8 *Life history traits*

Dependence on water availability is a critical component of the bromeliad regeneration niche (Mondragón et al., 2004; Winkler et al., 2005; Bader et al., 2009; Goode and Allen, 2009; Mantovani and Iglesias, 2010; Montes-Recinas et al., 2012; Wagner et al., 2013). In some epiphytic species, germination and seedling growth is enhanced by the presence of specialized water-uptake structures (Wester and Zotz, 2011), and a degree of drought tolerance is a key component of the adaptive strategy of epiphytic seedlings (Adams and Martin, 1986b; Bader et al., 2009). Less is known regarding the ecophysiology of seedling establishment in terrestrial species, but many principles from the epiphytic context may apply equally to those terrestrial species associated with water-limited habitats.

1.2.9 *Exposure and habitat*

The degree to which bromeliads are exposed to intense sunlight, temperature-related evaporative demand, and desiccating winds varies widely between habitats and microhabitats. For terrestrial species, the most important influences are probably latitude, altitude, aspect and the density of any higher forest or scrub canopy. In the case of lithophytic species the impact of aspect may be accentuated by the fact that these tenacious plants may grow on vertical rock faces and it has been suggested that the thermal capacity of the substrate may be an important modulator of transpirational water loss by lithophytes (Szarzynsky, 2000). With epiphytes, the vertical stratification and horizontal physiognomic complexity of canopies are critical considerations. Trunk and low-canopy epiphytes are generally exposed only to still, humid air, but moving up through tree-crowns to the uppermost canopy epiphytes will be increasingly exposed to high light and wind (Pittendrigh, 1948). This vertical exposure gradient is steepest in forest occurring on windward slopes and ridge tops and is associated with a characteristic turnover of species that are

progressively more tolerant of water limitation (Pittendrigh, 1948; Graham and Andrade, 2004; Cascante-Marín et al., 2006; Reyes-García et al., 2008). Similar turnover between different functional groups occurs along elevational and topographical transects, interacting with local wind and precipitation patterns (Sugden, 1981). An additional determinant of exposure-related structuring of epiphytic bromeliad distributions is the degree of deciduousness in phorophytes and neighbouring trees. Seasonally dry tropical forest tree communities often contain many deciduous species with canopies that may remain bare following leaf abscission for up to several months (Bullock and Solis-Magallanes, 1990). Epiphytes residing in the crowns of these trees must be capable of acclimating to the dramatically changed conditions during the dry season. *Guzmania monostachia* achieves this in seasonally deciduous forests in Trinidad by inducing CAM and both anatomical and biochemical photoprotective mechanisms (Maxwell et al., 1992, 1994; 1999).

1.3 Project objectives

The Bromeliaceae represent an increasingly popular model system in plant evolutionary ecology and physiology (Palma-Silva et al, 2016), but the foregoing discussion makes it clear that there are major knowledge gaps regarding the anatomical basis of bromeliad water relations and hydraulic traits, and how these traits relate to the contrasting ecological characteristics of the functional types. The aims of this research project were therefore to characterise the structure-function relationships underpinning the water relations and hydraulic physiology of the bromeliads, and to frame the findings in an ecological and evolutionary context. To meet these overarching aims, the following specific questions were considered:

- 1) What is the quantitative diversity of the hydrological habitats inhabited by bromeliads?
- 2) How does diversity in leaf structural and functional traits relate to divergences in water-use strategies between bromeliads of contrasting functional types?
- 3) How do specific structure-function traits influence plant-environment interactions?
- 4) To what extent can divergences in ecophysiological functional traits explain the spatial distributions of bromeliads at the landscape and regional levels?

1.4 Thesis structure

Following this **General Introduction**, five data chapters are presented. A **General Discussion** of the results is then provided, which aims to present a comprehensive synthesis of structural-functional evolution in the Bromeliaceae. The workflow of this research project is summarised in Fig. 1.2.1.

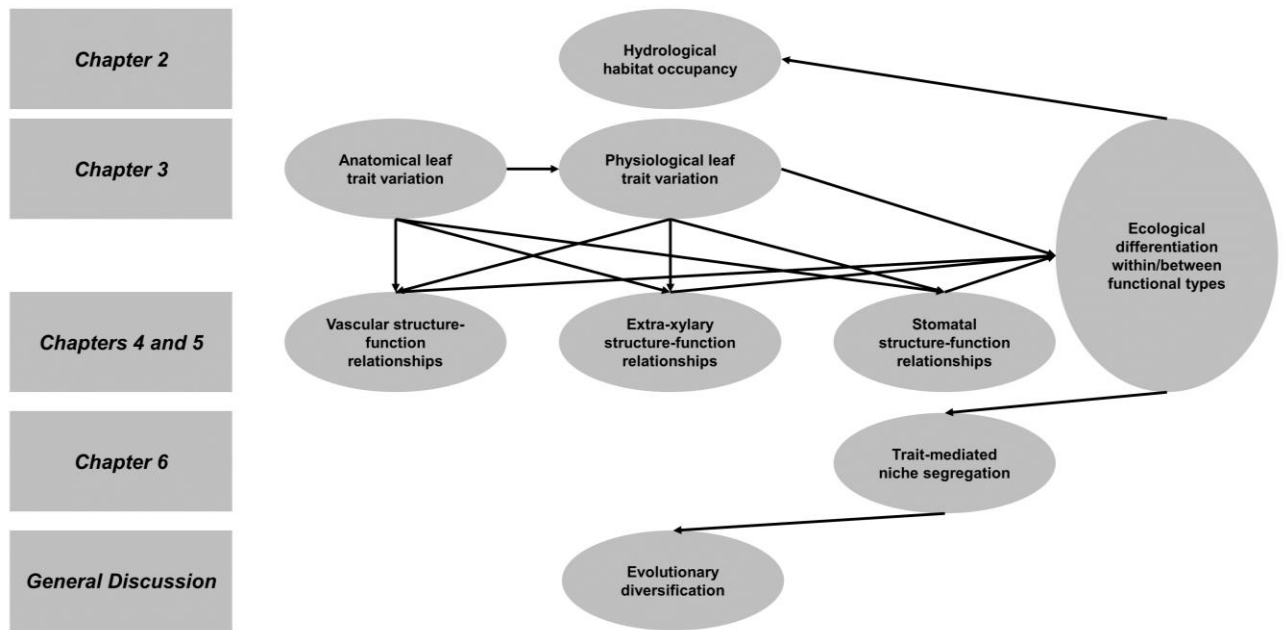


Figure 1.2.1. Research project workflow.

In **Chapter 2**, an assessment is made of the pattern and diversity of hydrological habitat space occupancy in terrestrial Bromeliaceae. This is achieved through analysis of distributional and bioclimatic data, and provides insights into the nature of interspecific variation in hydrological habitat mean and range. Variation in these properties is discussed with reference to existing knowledge of bromeliad functional traits, and the potential for improved understanding of bromeliad-environment interactions through structure-function characterisation of environmental niches is highlighted.

Chapter 3 presents the results of a survey of leaf anatomical, economic and hydraulic traits across an ecologically-diverse range of 50 bromeliad species. It also shows the findings of a survey of a more limited set of key traits associated with drought resistance across a much wider range of species. Finally, it delivers an analysis of the integration of leaf anatomical traits and physiological functions in the characteristic functional types of the bromeliads.

In **Chapter 4**, the results of an investigation into the structural basis of variation in the hydraulic physiology of a set of representative bromeliad species of different ecophysiological types are presented.

In **Chapter 5**, the results of an enquiry into interspecific variation in stomatal conductance and their sensitivity to environmental perturbations are presented. The anatomical basis of this variation is

discussed alongside its significance for physiological function in dynamic microenvironments and ecophysiological differentiation.

Chapter 6 explores the ecological relevance of the structure-function relationships identified in previous chapters by establishing the role of hydraulic traits in driving niche differentiation and altitudinal segregation in *Aechmea* species (CAM tank-epiphytes) occurring in Trinidad. The results of distribution mapping and species distribution modelling are presented. Anatomical and physiological characterisation of the species is then discussed, demonstrating the importance of divergence in a set of key traits in promoting niche differentiation.

In the **General Discussion**, the findings of the individual data chapters are drawn together and combined with additional qualitative and quantitative observations to generate a synthetic account of how the evolution of structural and functional traits has been coupled to the evolutionary history of the Bromeliaceae.

2. Hydrological habitat position and range in terrestrial bromeliads

2.1 Introduction

The purpose of this chapter is to provide insights into the following question posed in the **General Introduction**: *What is the quantitative diversity of the hydrological habitats inhabited by bromeliad species?*

The concept of the ecological niche has been articulated in many ways (see discussion in Whittaker et al., 1973; Leibold, 1995; Soberón, 2007), and frequent cases of terminological ambiguity or vagueness have prompted statements such as “I can’t define the niche but I know it when I see it” (Godsoe, 2010), and even occasional calls to abandon the term altogether (McInerny and Etienne, 2012). However, one longstanding definition which has remained popular is that proposed by Hutchinson (1957), who described the niche as an n -dimensional hypervolume corresponding to the available resource space as limited by environmental constraints. When applied alongside appropriate consideration of additional contextual factors, this resource-based view of the niche has proved a powerful tool for probing the controls of species distributions (Pulliam, 2000), and the evolution of ecological and species diversity (Holt, 2009). The environmental constraints recognised by the Hutchinsonian niche concept are often subdivided between biotic and abiotic factors. While the former category includes factors such as pollination and dispersal syndromes, the latter is principally associated with edaphic and climatic factors. This investigation focusses on a subset of climatic factors describing environmental water availability, which, as outlined in the **General Introduction**, is of special importance in bromeliad evolutionary ecology (Males, 2016). For the purposes of this chapter, the term ‘hydrological niche’ is used to describe the component of the overall ecological niche that is defined by water availability factors. A species’ hydrological niche is determined by physiological requirements in terms of both the absolute quantity of available moisture and its annual temporal distribution (Silvertown et al., 1999; Ogle and Reynolds, 2004; Araya et al., 2011). Interspecific variation in hydrological niches can be expressed in terms of the position of the niche (i.e. the location in multidimensional niche space associated with maximal fitness) and the niche width (i.e. the region of multidimensional niche space across which fitness is significantly greater than zero).

It is important to distinguish between the concepts of ‘niche’ and ‘habitat’ (Whittaker et al., 1973; Kearney, 2006). The fundamental niche can be considered as an integrative emergent property of trait-mediated plant-environment interactions. Complete understanding of the niche *per se* therefore requires labour-intensive quantification of plant traits and process-based relationships

with environmental tolerance. By contrast, quantification of the climatic properties of the habitat space occupied by species, which is partly determined by the fundamental niche but also by extraneous environmental factors, is often comparatively straightforward and can provide correlative insights into environmental tolerance. Under this approach, the concepts of habitat position and habitat range represent the equivalents of niche position and niche width.

Measurements of habitat space and niche space are thus interrelated, and can shed light on each other. The rapidity with which the habitat-based approach can be achieved makes it suitable for taxonomically broad-ranging comparative analyses, provided that its limitations are acknowledged.

Although it is recognised that the bromeliads occupy a broad range of hydrological habitat space (Benzing, 2000; Males, 2016), this diversity has not been quantified across a wide range of species and examined in terms of trait-driven differences between taxonomic and functional groups. More complete, quantitative understanding of bioclimatic patterns in bromeliad species distributions is fundamental to understanding the relevance of divergences in ecophysiological traits for niche evolution and differentiation, and the degree of environmental specialisation at different taxonomic levels (Silvertown et al. 2006). Addressing this critical knowledge gap offers opportunities not only for providing a firmer grounding for future assessments of the ecological relevance of innovations in structural-functional traits in the bromeliads, but also for exploring how this important family can shed light on broader questions in plant biogeography.

While the scope of a hydrological habitat quantification exercise could encompass the entire bromeliad family, this investigation focusses on the terrestrial and saxicolous bromeliad lineages. In terrestrial species, distributions are expected to be less strongly affected by micro-environmental factors than for epiphytic bromeliads (Pittendrigh 1948; Benzing 2000), and are therefore more amenable to analysis of environmental habitat occupancy (*sensu* Whittaker et al. 1973; Kearney 2006) based on distributional data. With the focal taxa thus defined, the overall aims were as follows:

- To quantify the hydrological habitat position and range of terrestrial bromeliad species of contrasting taxonomic and functional groups with respect to a series of water-related environmental variables;
- To quantify the extent of hydrological habitat overlap within taxonomic groups;
- To suggest character changes associated with variation in hydrological habitat position and range within and between taxonomic and functional groups;
- To examine the evolution of hydrological habitat position in selected genera;
- To determine the relationship between hydrological habitat position and range.

These aims were addressed by using distributional data to quantify relevant bioclimatic variables for reliable presence points for a total of 564 species. Mean values and ranges of bioclimatic variables were subjected to PCA to explore variation in multivariate hydrological habitat occupancy between taxonomic and functional groups, and differences in habitat properties within and between species groups were related to divergences in life-history, morphology and other factors. Finally, a range of approaches were taken to determine the degree of coordination in the evolution of habitat properties and their integration in the evolutionary diversification of the terrestrial bromeliads.

2.2 Methods

2.2.1 Taxon sampling

All terrestrial (and saxicolous) bromeliads in the following taxonomic groups were considered in this investigation: Brocchinioideae (*Brocchinia*); Lindmanioideae (*Connellia*, *Lindmania*); Hechtioideae (*Hechtia*); Navioideae (*Brewcaria*, *Cottendorfia*, *Navia*, *Sequencia*, *Steyerbromelia*); Pitcairnioideae (*Deuterocohnia*, *Dyckia*, *Encholirium*, *Fosterella*, *Pitcairnia*); Puyoideae (*Puya*); and early-diverging Bromelioideae (*Ananas*, *Bromelia*, *Cryptanthus*, *Deinacanthon*, *Disteganthus*, *Fascicularia*, *Greigia*, *Neoglaziovia*, *Ochagavia*, *Orthophytum*). A complete list of currently recognised (May 2016) species names for each of these genera was generated using The Plant List (2016).

2.2.2 Collection and processing of distributional data

Species names were used to query the Global Biodiversity Information Facility (GBIF) for distributional data. For species for which three or more georeferenced presence points were available, data were downloaded and subjected to manual quality control. Any outlying points obviously outside of the native range of the species (e.g. on another continent) were removed, as were those corresponding to the geographical locations of herbaria or living collections. Duplicate records and those with insufficient supporting metadata were also removed. The total number of species used in subsequent analyses was 564, as listed in **Appendix 2.1**.

2.2.3 Bioclimatic data

Bioclimatic layers (mean annual precipitation, MAP; precipitation in driest month, P_{dry} ; and precipitation seasonality, P_{seas}) were downloaded from the Bioclim database (Hijmans et al., 2007) at 30 arc-second resolution. Aridity index, actual evapotranspiration (AET) and potential evapotranspiration (PET) layers were obtained from the CGIAR-CSI portal (Zomer et al., 2007, 2008).

The bioclimatic variables selected for hydrological habitat position and range estimation are shown, with the rationale for their inclusion, in Table 2.2.1.

| Variable | Defintion | Rationale | Source |
|-------------------|---|---|--------------------------------------|
| MAP | Mean annual precipitation, mm | Proxy for the absolute quantity of water available during each year. Species may differ in the absolute quantity of water required to maintain turgor and transpiration. | Bioclim (Hijmans et al., 2007) |
| AI | Aridity index, mm mm ⁻¹ | Proxy for the degree of dryness. Species may respond differently to chronic water deficit depending on morphological, anatomical and physiological specialisation. | CGIAR-CSI (Zomer et al., 2007, 2008) |
| AET/PET | Actual evapotranspiration/potential evapotranspiration, mm mm ⁻¹ | Proxy for plant water supply relative to demand. Species may differ in their requirements depending on water-use and hydraulic characteristics. | CGIAR-CSI (Zomer et al., 2007, 2008) |
| P _{dry} | Precipitation in driest month, mm | Proxy for the absolute degree of water limitation during the dry season. Species may differ in the minimum quantity of dry-season precipitation required to maintain physiological functions. | Bioclim (Hijmans et al., 2007) |
| P _{seas} | Precipitation seasonality, % | Proxy for the intensity of the dry season relative to the remainder of the year. Species may differ in their requirement for environmental equability throughout the year. | Bioclim (Hijmans et al., 2007) |

Table 2.2.1. Bioclimatic variables used in hydrological habitat position and range analysis.

A script was compiled in R (R Development Core Team, 2008) using the ‘raster’ package (Hijmans and van Etten, 2012) to enable automated retrieval of the value of each bioclimatic variable at each presence point. For each species, the mean and range was then calculated across all values of each bioclimatic variable.

2.2.4 Assessment of hydrological habitat position and range

The mean and range of individual bioclimatic variables were utilised as univariate indicators of species’ hydrological habitat position and range. To estimate multivariate hydrological habitat position scores, the mean values of all environmental variables were log-transformed and subjected to principal components analysis (PCA) in R. Species scores in the climate space defined by the first two principal components (PC1, PC2) were then used as comparative estimates of hydrological habitat position scores. The same PCA-based procedure was used to calculate estimates of multivariate hydrological habitat ranges.

An alternative approach would have been to undertake species distribution modelling (SDM), which uses presence (and optionally absence) data to estimate species' bioclimatic envelopes and predict the distribution of probability of occurrence over a specified geographical range. One advantage of SDM is that it can provide insights into the relative importance of different environmental factors in constraining species distributions. However, SDM depends on many assumptions and must be performed in a careful and time-consuming manner, such that it was not feasible to perform SDM for such a large set of species as was used in this investigation. Further discussion of SDM is provided in **Chapter 6**, where it is used to estimate the bioclimatic envelopes of a small set of species in the context of landscape-level habitat partitioning.

2.2.5 Assessment of overlap between univariate habitat indicator ranges

In order to determine the extent of hydrological habitat overlap within taxonomic groups, a custom-designed function based on the 'proxy' and 'stats' packages was implemented in R. The script generated pairwise distance matrices for all species within a taxonomic group, with the distance function set to calculate the absolute extent of overlap between minimum and maximum indicator values for either species. The mean and standard deviation were calculated across the entire resultant matrix to provide measures of the extent and variation in pairwise species-level overlap. For each group, the mean extent of overlap was then normalised by dividing by the mean species-level range in that indicator variable in that taxonomic group. Because of the collinearity between many of the indicator variables (see **Appendix 2.2**), only AI and P_{seas} were used for habitat overlap analysis.

2.2.6 Relationships between species richness and diversity in hydrological habitat position and range across genera

The 'convhulln' function from the R package 'geometry' was used to calculate the area of the smallest convex hull covering all species scores for each genus represented by three or more species in the PC1-PC2 space performed on the hydrological habitat position and range datasets for all species. This provided measures of the diversity of hydrological habitat position and range in each genus. Species richness values for each genus were obtained from the Bromeliad Taxon List (Butcher and Gouda, 2017).

2.3 Results

Taxonomic and geographic coverage of distributional data are reported in **Appendix 2.2**.

2.3.1 Variation in hydrological habitat position and range between taxonomic groups

Bioclimatic scores averaged across species within each genus showed a large amount of variation in median scores and ranges. Figures displaying this variation are available in **Appendix 2.3**. When PCA using bioclimatic data for all species was performed, PC1 and PC2 explained 77.6% and 12.2% of the total variance in the data respectively. The alignment of bioclimatic variable loadings reflected two major axes of variation corresponding to overall environmental moisture (MAP, AI, AET/PET) and precipitation seasonality (P_{seas} , P_{dry}). Taxonomic groups showed some overlap, but tended to cluster towards different areas of the climate space (Fig. 2.3.1a). The early-diverging Brocchinioideae and Lindmanioideae were restricted to the area of climate space associated with relatively high overall moisture and low/moderate precipitation seasonality. Species of the genus *Hechtia* (Hechtioideae) were located exclusively in moderately/highly seasonal, low/moderate moisture environments. Navioideae were principally clustered in the same area of climate space as Brocchinioideae and Lindmandioideae, with one important exception being the species *Cottendorfia florida* Schult & Schult.f. Species in the two early-diverging genera of the Pitcairnioideae (*Pitcairnia* and *Fosterella*) occupied a wide area of climate space, but did not occur in areas characterised by very low overall moisture. These areas were however occupied by Xeric Clade Pitcairnioideae, which covered a similarly broad region of climate space to that occupied by *Pitcairnia* and *Fosterella*. Although there was some overlap with the latter, the region occupied by the Xeric Clade was shifted towards the drier end of the overall moisture axis. *Puya* spp. (Puyoideae) covered a roughly comparable area of climate space to that occupied by Xeric Clade Pitcairnioideae, although they did not occur in very strongly seasonal environments. Among the terrestrial Bromelioideae, the basal C₃ genera were confined to relatively high-moisture environments with moderate levels of seasonality, while CAM genera occupied a wider range of climate space that included considerably more seasonal and arid environments. Further PCAs and more detailed description of variation within taxonomic groups are available in **Appendix 2.4**.

Comparisons between functional rather than taxonomic groups showed extensive within-group variation, but some differences between groups could also be discerned. C₃ mesic terrestrials generally occurred in environments with higher overall moisture and lower seasonality. C₃ succulent and C₃-CAM terrestrials, while showing extensive overlap with C₃ mesic terrestrials, also occurred in drier and more seasonal environments. Meanwhile CAM terrestrials showed the broadest ranging

habitat occupancy in the climate space, occurring in all environments except those with the very highest overall moisture and lowest seasonality.

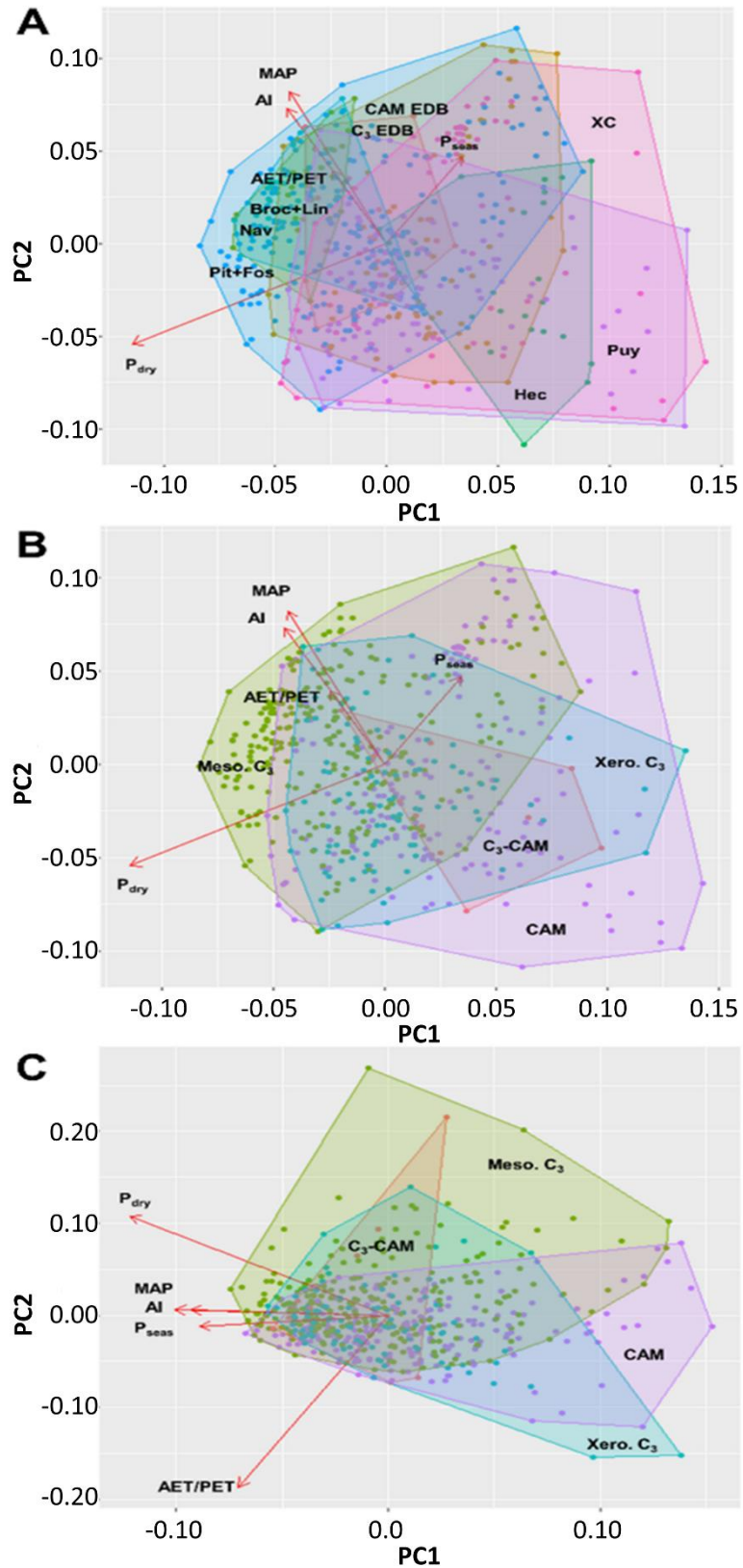


Figure 2.3.1. (overleaf) PC1-PC2 biplots for hydrological habitat occupancy properties of 564 terrestrial bromeliad species by taxonomic group or functional type. Arrows show bioclimatic variable loadings. a) PC1-PC2 biplot based on PCA of mean values of bioclimatic variables (mean annual precipitation, MAP; aridity index, AI; ratio of actual to potential evapotranspiration, AET/PET; precipitation of driest month, P_{dry} ; precipitation seasonality, P_{seas}). Species scores are plotted and grouped by taxonomic group, with separate convex hulls covering all species belonging to the following groups: Brocchiniaceae and Lindmaniaceae (Broc + Lin); Hechtioideae (Hec); Navioideae (Nav); *Pitcairnia* and *Fosterella* (Pit + Fos); Xeric Clade Pitcairnioideae (XC); Puyoideae (Puy); C₃ early-diverging Bromelioideae genera (C₃ EDB); and CAM early-diverging Bromelioideae genera (CAM EDB). b) PC1-PC2 biplot based on PCA of mean values of bioclimatic variables (MAP, AI, AET/PET, P_{dry} , P_{seas}). Species scores are plotted and grouped by functional group, with separate convex hulls covering all species belonging to the following functional groups: C₃ mesic terrestrial (Meso. C₃); C₃ succulent terrestrial (Xero. C₃); C₃-CAM succulent terrestrial; and CAM succulent terrestrial. c) PC1-PC2 biplot based on PCA of ranges of bioclimatic variables (MAP, AI, AET/PET, P_{dry} , P_{seas}). Species scores are plotted and grouped by functional group, with separate convex hulls covering all species belonging to the following functional groups as in (b).

PCA on hydrological habitat range data for all species identified three independent axes of variation in the bioclimatic variables: 1) AET/PET; 2) P_{dry} ; and 3) MAP, AI and P_{seas} (Fig. 2.3.1b). The loadings for AET/PET and P_{dry} were orthogonal, with the loadings for the third, multifactorial axis located approximately midway between. Functional groups showed a high degree of overlap at the centre of PC1-PC2 space, suggesting that relatively broad hydrological habitat ranges occur in all taxonomic groups and are associated with tolerance of variation in a range of bioclimatic factors. However, the loadings for species in different functional groups radiated differentially into the areas of the PC1-PC2 space associated with narrow habitat ranges, in a manner suggestive of contrasting environmental drivers of hydrological habitat range among different groups. Overall, more succulent species (C₃ succulent terrestrials and CAM terrestrials) appeared to be more prone to limitation in habitat range by P_{dry} .

PCA on habitat range data identified two major axes of variation: overall moisture (MAP, AI, AET/PET) and seasonality (P_{dry} , P_{seas} ; Fig. 2.3.1c). The broadest habitat ranges occurred in *Ananas*, *Neoglaziovia* and *Bromelia* spp., while narrow ranges occurred in *Cryptanthus* spp. and *Orthophytum* spp. Within *Bromelia*, the geographically widespread species *B. pinguin* L. showed one of the broadest habitat ranges. *B. grandiflora* Mez displayed a particularly narrow habitat range with respect to seasonality but not overall moisture, whereas the converse was true for *B. arenaria* Ule. In *Cryptanthus*, species including *C. pseudopetiolaris* Philcox showed relatively broad habitat ranges. The miniaturised species *C. micrus* Louzada, Wand. & Versieux appeared to be limited by seasonality,

while the broad-leaved *C. capitellatus* Leme & L.Kollmann was strongly restricted to a narrow range of overall moisture. In *Orthophytum*, *O. saxicola* (Ule) L.B.Sm. showed one of the broadest habitat ranges, comparable to that of *C. pseudopetiolaris*, while the strongly caulescent species *O. zanonii* Leme was limited by seasonality and *O. rubiginosum* Leme by overall moisture.

2.3.2 Relationships between hydrological habitat position and range

Regression analyses were performed between the species-specific mean scores and ranges for each bioclimatic variable across the whole dataset and within taxonomic and functional groups. Across the whole dataset ($n = 564$), the only strong correlation between mean scores and ranges for a bioclimatic variable was for P_{dry} (+^{ve}, $r^2 = 40.31$, $p < 0.001$). This relationship suggests that species adapted to low levels of precipitation during the driest part of the year are strongly specialised and restricted to such environments, whereas species adapted to higher levels of precipitation during the driest part of the year are more tolerant of a wider range of levels. Consistent with this hypothesis, there were significant but much weaker correlations between mean and range for MAP (+^{ve}, $r^2 = 6.27$, $p < 0.001$) and for AI (+^{ve}, $r^2 = 11.10$, $p < 0.001$). In the case of AET/PET, there was a very weak negative correlation ($r^2 = 3.01$, $p < 0.001$), with a steep decline in range occurring at the very highest mean values. The weakness of these correlations suggests that very different degrees of specialisation can coexist under any given environment.

2.3.3 Hydrological habitat overlap analysis

The results of hydrological habitat overlap analysis, using the univariate indicators AI and P_{seas} , are displayed in Table 2.3.1. In terms of AI, the lowest levels of univariate habitat overlap occurred in the C₃ early-diverging Bromelioideae and the Navioideae, while the highest levels occurred in the Xeric Clade Pitcairnioideae and the *Pitcairnia-Fosterella* grade. The ranking of taxonomic groups by univariate habitat overlap in terms of P_{seas} was slightly different, with the most noticeable contrast being the shift in the position of Navioideae to very low levels of overlap. Navioideae species therefore appear to be unusual in overlapping considerably in their AI ranges but not in P_{seas} ranges.

| Taxonomic group | Overlap | AI (mm mm ⁻¹) | | Overlap | P _{seas} (%) | |
|--|---------|---------------------------|--------------|---------|-----------------------|--------------|
| | | Mean range | Overlap/Mean | | Mean range | Overlap/Mean |
| Brocchinioideae - Lindmanioideae | 403.49 | 5911.66 | 0.068 | 1.960 | 21.86 | 0.090 |
| Hechtioideae | 106.34 | 5026.96 | 0.021 | 1.800 | 18.40 | 0.098 |
| Navioideae | 826.78 | 4710.64 | 0.176 | 1.260 | 16.65 | 0.076 |
| <i>Pitcairnia</i> – <i>Fosterella</i> | 509.97 | 10187.66 | 0.050 | 4.210 | 32.94 | 0.128 |
| Xeric Clade | 554.83 | 3955.18 | 0.140 | 6.321 | 23.33 | 0.271 |
| Puyoideae | 499.21 | 7549.20 | 0.066 | 3.594 | 31.10 | 0.116 |
| C ₃ early-diverging Bromelioideae | 87.88 | 12449.85 | 0.007 | 1.083 | 36.54 | 0.030 |
| CAM early-diverging Bromelioideae | 252.93 | 5551.77 | 0.046 | 2.320 | 29.13 | 0.080 |

Table 2.3.1. Mean species-level range overlap for aridity index (AI) and precipitation seasonality (P_{seas}) within taxonomic groups, showing absolute values and values normalised by mean species-level variable range.

2.3.4 Relationship between hydrological habitat range and geographical range

Across all species ($n = 564$), linear regression revealed strong positive correlations between log-transformed geographical range size and ranges for each bioclimatic indicator variable: AI ($r^2 = 0.48$, $p < 0.001$), AET/PET ($r^2 = 0.31$, $p < 0.001$), MAP ($r^2 = 0.52$, $p < 0.001$), P_{dry} ($r^2 = 0.41$, $p < 0.001$), and P_{seas} ($r^2 = 0.62$, $p < 0.001$). These relationships are illustrated in Fig. 2.3.2. There was no significant difference in mean geographical range size between functional types (AOV: $F = 0.62$, $p = 0.600$).

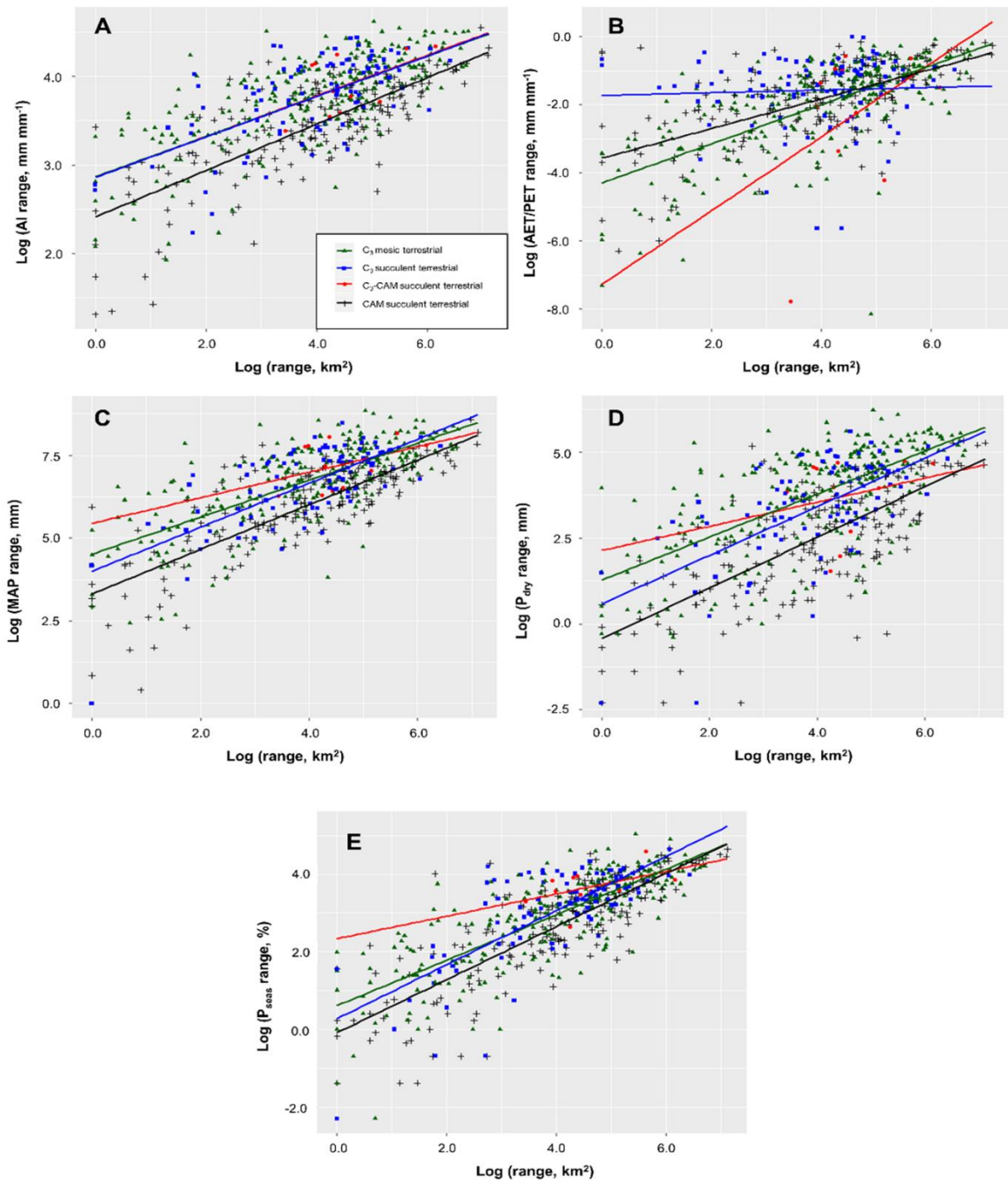


Figure 2.3.2. Relationships between log-transformed geographical range sizes (km²) and ranges of bioclimatic indicator variables: a) aridity index, AI; b) ratio of actual to potential evapotranspiration, AET/PET; c) mean annual precipitation, MAP; d) precipitation of driest month, P_{dry}; e) precipitation seasonality, P_{seas}. Lines show linear regression for each functional type (see legend in (a)).

2.3.5 Relationships between species richness and diversity in hydrological habitat position and range across genera

Across all 19 genera represented by three or more species in the bioclimatic datasets, there was a strong positive correlation between diversity in hydrological habitat position and range ($r^2 = 0.62$, $p < 0.001$). Genera which showed greater diversity in hydrological habitat position therefore tended also to show greater variety in hydrological habitat range. Following log-transformation, there were additionally strong positive correlations between species richness and range in hydrological habitat position ($r^2 = 0.53$, $p < 0.001$) and between species richness and range in hydrological habitat range ($r^2 = 0.79$, $p < 0.001$). Thus larger genera tended to show significantly greater diversity with respect to both hydrological habitat position and range.

2.4 Discussion

The analyses of biogeographical and bioclimatological patterns in the terrestrial bromeliads presented here provide timely clarification of critical questions in the evolution of a major plant radiation. While some fundamental biogeographical and ecological hypotheses were clearly supported by the data gathered here, others were surprisingly contradicted. These fresh insights not only help to improve our understanding of the evolutionary ecology of the important bromeliad family, but also represent significant contributions to the discourse surrounding core concepts in biogeographical patterns and processes in tropical herbaceous angiosperms.

2.4.1 Diversity and drivers of hydrological habitat position scores

Consistent with their broad geographic range and noted ecological diversity, the terrestrial bromeliads show a wide variety of hydrological habitat position scores. Despite extensive hydrological habitat overlap, there was evidence of differentiation among taxonomic and functional groups, which can be related to existing knowledge of differences in life-history, morphological and ecophysiological traits. Understanding how functional traits underpin plant-environment interactions and thus define the species' environmental niche is crucial for efforts to predict the responses of species and communities to climate change (Violle and Jiang, 2009).

Guiana Shield lineages (Brocchinioideae, Lindmanioideae, Navioideae) were almost universally constrained to a common area of high-moisture, low-seasonality climate space. These groups, which include some of the earliest-diverging bromeliads, lack innovations such as succulence, CAM, or root vessels that might have enhanced their capacity to invade other regions of climate space (Givnish et al., 2011, 2014). While *Connellia* spp. are able to survive in somewhat drier habitats than most

Guiana Shield species, perhaps due to their reduced, stiff foliage, the only species to have truly escaped this narrow area of climate (and geographical) space is *Cottendorfia florida*, a rhizomatous, drought-deciduous pyrophyte native to the Brazilian Cerrado. Heavy investment in subterranean storage structures and adaptive responses to seasonal stresses and fires enable this species to thrive under very different climatic regimes from those to which its closest relatives are adapted (Benzing, 2000). It is also notable that among *Brocchinia* species, tank-forming species such as *B. hechtioides* Mez and *B. reducta* Baker were associated with relatively low levels of moisture availability, where the ability to capture water in tanks could be advantageous (Givnish et al. 1997; Benzing 2000).

Perhaps the most comparable group to the Guiana Shield bromeliads is the *Pitcairnia-Fosterella* grade (Pitcairnioideae), which also comprises C₃ mesic terrestrials, but is substantially more diverse in terms of hydrological habitat occupancy. This diversity could relate to the origin of root vessels in *Pitcairnia*, which presumably facilitates more efficient root-mediated water uptake (Tomlinson, 1969; Males, 2016), and the greater evolutionary lability in leaf form seen in *Pitcairnia* and *Fosterella*. It is perhaps significant that among *Pitcairnia* spp., many species occurring in high-moisture environments display broad, (pseudo-)petiolate leaf-blades, whereas those from the driest and most seasonal environments frequently display highly reduced, sometimes spinose, linear leaf-blades (Males, 2017a). Similarly, convergent cases of petiolate leaf morphology in the genera *Cryptanthus* and *Disteganthus* appeared to be associated with high levels of moisture, and the same is probably true for the strongly petiolate *Bromelia scarlatina* (Henriq. ex Linden) E.Morren ex C.H.Morren, for which sufficient distributional data were not available for analysis but which is closely related to the *B. tubulosa* L.B.Sm., which was associated with higher moisture levels than any of its congeners. The same results would probably be found for rare petiolate epiphytes such as the endangered *Aechmea tayoensis* Gilmartin (IUCN, 2016; cf. placement in *Ananas* in Sass and Specht, 2010), which is restricted to high-rainfall regions of Ecuador, and *Ronnbergia morreniana* Linden & André from Colombia and Ecuador. Cruz et al. (2017) note that multiple accessions for the petiolate species *Cryptanthus beuckeri* were not recovered as monophyletic in their phylogenetic analyses, suggesting that petiolate leaves may have arisen several times within that genus and underscoring the intriguingly recurrent nature of this trait in the bromeliads. Leaf shape is intimately associated with foliar venation architecture, and therefore varies in tandem with leaf hydraulic properties and responses to water deficit (Males, 2017a). The more xeromorphic leaf morphoanatomy displayed by *Fosterella* spp. relative to most *Pitcairnia* spp. was not associated with any major difference in hydrological habitat position, although it is accepted that most *Fosterella* spp. tend to occupy very exposed microsites with free drainage (Wagner et al., 2013a). Even the two lowland Amazonian species are restricted to rocky bluffs and fluvial boulders (Wagner et al., 2013a). As in some

Pitcairnia spp., endurance of extreme seasonality in *Fosterella* spp. is often associated with deciduousness (Benzing, 2000).

Succulence and CAM evolved independently in the Hechtioideae, Xeric Clade Pitcairnioideae, Puyoideae and Bromelioideae (Givnish et al., 2011; Crayn et al., 2015; Males, 2016), and each of these lineages extend into regions of climate space characterised by lower total moisture and stronger precipitation seasonality than is observed for any but the most xeromorphic of C₃ or C₃-CAM species. This lends strong quantitative support to the notion that origins of CAM have allowed different bromeliad lineages to adapt to continuously or seasonally water-limited environments (Griffiths and Smith, 1983; Smith et al., 1986; Martin, 1994; Benzing, 2000; Males, 2016). CAM species with hydrological habitat position scores corresponding to particularly arid conditions were typically thick- and narrow-leaved (e.g. *Deinacanthon urbanianum* (Mez) Mez, *Neoglaziovia variegata* (Arruda) Mez). However, miniaturisation was also associated with environmental extremity in *Deuterocohnia*, which is perhaps analogous to the combination of neoteny and xeromorphy that occurs in the atmospheric epiphytes of the genus *Tillandsia*. Meanwhile, CAM species occurring in less arid conditions were sometimes relatively thin-leaved (e.g. *Hechtia lundelliorum* L.B.Sm. and *H. tillandsioides* (André) L.B.Sm.). In the case of *Hechtia*, new phylogenetic analyses are needed to determine if thin-leaved species are early-diverging within the genus and are representative of the intermediate form between a C₃ mesic terrestrial ancestor and the highly xeromorphic succulent CAM species of the crown radiation of *Hechtia*. It is interesting to note that in the Crayn et al. (2015) $\delta^{13}\text{C}$ dataset, while still clearly strong CAM plants, *H. lundelliorum* and the morphologically similar *H. caerulea* (Matuda) L.B.Sm. display two of the most negative values of all *Hechtia* species (-15.2 ‰ and -15.8 ‰ respectively).

In genera with both C₃ and CAM (and sometimes C₃-CAM) species, there was limited evidence for hydrological habitat differentiation between species of contrasting photosynthetic pathways. This was perhaps true of *Cryptanthus*, where the only species for which CAM appears to be absent, *C. schwackeanus* Mez (Crayn et al., 2015), showed higher mean values for total moisture variables than definite CAM species. In *Puya*, C₃ species generally occupied the area of climate space associated with higher moisture and lower seasonality, with the regions occupied by C₃-CAM and CAM species being broadly congruent and shifted further towards lower moisture and higher seasonality.

Some C₃ succulent terrestrial bromeliads occur well into the temperate zone of South America, with *Fascicularia bicolor* (Ruiz & Pav.) Mez and *Ochagavia* spp. recorded as far south as Chiloé (Zizka et al., 2009). Adaptation to subtropical precipitation (and temperature) regimes explains why several of these species (e.g. *F. bicolor*, *O. carnea* (Beer) L.B.Sm. & Looser) have become naturalised and even

invasive at high latitudes in northwest Europe (Nelson and Zizka, 1997; Morais et al., 2017). The success of *Ochagavia* species in considerably more seasonal environments than their close relative *F. bicolor* could be explained by the development of more extensive hydrenchyma in *Ochagavia* species, especially when compared with *F. bicolor* ssp. *canaliculata*, which is largely restricted to Valdivian temperate rainforest (Zizka et al., 2009). Other *Ochagavia* species for which sufficient distributional data were not available, *O. andina* (Phil.) Zizka, Trumpler & Zöllner and *O. elegans* Phil., occur as saxicoles in drier Andean habitats and on the exposed coastal cliffs of the Juan Fernández Islands respectively; both situations where high hydraulic capacitance could be particularly advantageous (Zizka et al., 2009). The extent to which such considerations might apply to *Greigia* spp. is unclear, since availability of comparative morphological, anatomical, and ecophysiological data for these plants is currently very limited. The physiological and bioclimatic significance of structural variation in other large genera such as *Puya* also requires further investigation.

Variation in hydrological habitat position for *Orthophytum* species provided quantitative support for the suggestion of Louzada et al. (2010) that species in the derived polyploid lineage occur in more xeric environments than earlier-diverging diploid species. Polyploidisation can have dramatic effects on plant-environment interactions (Levin 1983; Baker et al. 2017; Donkpegan et al. 2017), and Paule et al. (2017) have recently demonstrated that its occurrence in *Fosterella* was associated with a shift in temperature niche. It is possible that changes in ploidy could also impact on plant water relations via cell size effects, thereby altering hydrological niche position and/or width (Males 2016). It is notable that polyploidy also occurs in other terrestrial bromeliads with both extreme hydrological habitat positions, such as the Xeric Clade Pitcairnioideae, and very wide hydrological habitat ranges, including the early-diverging Bromelioideae (Gitaí et al. 2014).

Some of the unexplained interspecific variation in hydrological habitat position could relate to differences in germination requirement and seedling ecophysiology. Müller et al. (2016) describe germination as a key 'bottleneck' in the determination of species' distributions. The literature on bromeliad germination biology is quite extensive relative to pollination and dispersal, and seedling mortality in bromeliads has been studied quite intensively in the epiphytic Tillandsioideae subfamily (particularly the genera *Tillandsia* and *Vriesea*; Hietz et al., 2002; Winkler et al., 2005; Bader et al., 2009; Montes-Recinas et al., 2012; Toledo-Aceves et al., 2012). The applicability of the results from these groups to terrestrial species is not clear, but the general consensus arising from work on bromeliad regeneration niches is that they are strongly influenced by microenvironmental conditions (Winkler et al., 2007; Wagner et al., 2013b). More research is needed to determine the

extent to which the sensitivities of the earliest stages of plant development impact on bromeliad distributions and habitat occupancy.

2.4.2 Diversity and drivers of hydrological habitat range

Hydrological habitat range varied extensively among the species considered here. It is important to note that at least some of this variation could be a by-product of bias in the number of presence points available for different species. Underestimation of hydrological habitat range could result from under-recording of species distributions. This is a recognised limitation of GBIF data, and limited botanical sampling effort may be especially characteristic of some of the remote and challenging Neotropical environments to which many bromeliads are native (Yesson et al., 2007; Beck et al., 2014). However, in many cases, a low number of recorded presence points may well reflect genuine narrow geographical and bioclimatic endemism, which is an accepted feature of bromeliad evolution (Benzing, 2000; Givnish et al., 2014). This is particularly true of certain early-diverging groups (e.g. Lindmanioideae), representatives of which have been included in assessments of climate-change-induced extinction risk due to their narrow bioclimatic ranges (e.g. Safont et al., 2012). While some caution should be exercised in the interpretation of the results of hydrological habitat range analysis, they could provide meaningful and important insights into the evolution of plant-environment interactions in the Bromeliaceae.

Variation in the width of hydrological habitat ranges of C₃ mesic terrestrials tended to align more closely with loadings for variables related to overall moisture, indicating the pre-eminent importance of adequate, often high water supply for these species. The apparently greater importance of P_{dry} in limiting hydrological habitat range in C₃ succulent and CAM terrestrial bromeliads is consistent with the hypothesis that succulent plants tend to occur within relatively narrow ranges of dry season precipitation (Ellenberg, 1981; Ogburn and Edwards, 2010; Males, 2017b).

Particularly broad hydrological habitat ranges occurred in certain taxonomic groups. This was true of *Ananas* spp., for which a broad hydrological niche could partly explain why the pineapple, *Ananas comosus* (L.) Merr., can be grown successfully in so many tropical and subtropical regions of the world (Bartholomew et al., 2002). Broad hydrological habitat range in species of other early-diverging CAM Bromelioideae (e.g. *Bromelia* spp., *Neoglaziovia variegata*) provides good evidence of the flexibility of CAM and the physiological advantages it confers under a wide range of environmental conditions (Lüttge, 2010). In several genera, some of the broadest hydrological habitat ranges occurred in miniaturised species (e.g. *Deuterocohnia strobilifera* Mez, *Lindmania subsimplex* L.B.Sm., *Navia duidae* L.B.Sm.). There are various possible explanations for this

observation. Small plant size could be associated with enhanced environmental tolerances in some cases (e.g. due to reduced surface area: volume ratio), but could also increase the importance of microclimatic factors that may not be well represented in the bioclimatic datasets used here.

Other specialised growth-forms occur in the terrestrial bromeliads, notably the tank growth-form in *Brocchinia* spp. including *B. hechtioides* and *B. tatei* L.B.Sm. Both of these species showed broader habitat ranges than their congeners, presumably due to the provision of external hydraulic capacitance and nutrient acquisition strategies by the tank (Givnish et al., 1997; Benzing, 2000; Males, 2016). Meanwhile one of the broadest hydrological habitat ranges in the genus *Pitcairnia* occurred in *P. heterophylla* (Lindl.) Beer, which is well-known for its drought-deciduousness, using a tuberous rhizome to survive periods of environmental adversity (Benzing, 2000). Other instances of apparently broad hydrological habitat ranges can be explained by polymorphism within species. For example, *Fascicularia bicolor* includes two subspecies (ssp. *bicolor* and ssp. *canaliculata*) that differ in their investment in hydrenchyma and occupy distinct ecological zones. Each subspecies may in fact be relatively narrowly specialised, making this a promising system in which to study the structural-functional basis of environmental adaptation.

Narrow hydrological habitat ranges appeared to be driven by specific bioclimatic variables in a species-dependent manner. In some cases, variables related to total moisture (MAP, AI, AET/PET) appeared to be more limiting, while in other cases factors related to precipitation seasonality (P_{dry} , P_{seas}) seemed to be more limiting. It was difficult to identify morphological factors that could explain this distinction or that were associated with narrow hydrological habitat range in general. However, some preliminary inferences could be drawn. Long, grass-like foliage in certain Brocchinioideae, Lindmanioideae and Navioideae was associated with narrow hydrological habitat range, perhaps because of the potential for high rates of water loss and hydraulic dysfunction in this high-conductance, low-capacitance arrangement. Similarly, long, thin, strap-like leaves in *Greigia alborosea* (Griseb.) Mez could limit its internal water-storage capacity and restrict it to high-moisture environments in its native Venezuela (Morillo et al., 2009). Interestingly, several of the *Orthophytum* species with narrow habitat range were caulescent rather than rosette-forming, suggesting that the caulescent growth form could be associated with greater environmental specialisation.

Morphologically-convergent succulent xerophytes (e.g. Xeric Clade Pitcairnioideae, *Puya* spp.) showed a considerable amount of variation in both hydrological habitat position and range in spite of their apparent structural similarity. While cryptic variation in internal anatomy could confer contrasting physiological characteristics and thereby promote ecological diversity among these groups, as will be addressed in subsequent chapters, it seems likely that to a large extent the

apparent segregation of environmental niches among these plants is the product of dispersal limitation or biotic interactions leading to spatial structuring.

As with hydrological habitat position, variation in habitat range is clearly strongly influenced by plant traits. Several traits that have evolved convergently in different bromeliad lineages appear to be frequently associated with a shift in habitat position towards more arid, seasonal environments, and/or increased environmental specialisation (i.e. narrower habitat range). These include succulence, CAM, and deciduousness (Fig. 2.4.1). Convergent evolution of traits such as petiolate leaf morphology can likewise be linked with invasion of more humid, aseasonal habitats. Instances of unique (rather than convergent) innovations of bioclimatic relevance are less easily identifiable, but include the origin of root xylem vessels in the genus *Pitcairnia*. The combination of convergent and divergent trait evolution has been shown to have been important in shaping the evolution of climatic niches in other plant groups (e.g. Evans *et al.* 2008), and is consistent with a complex mixture of adaptive constraints and opportunities (Losos 2011).

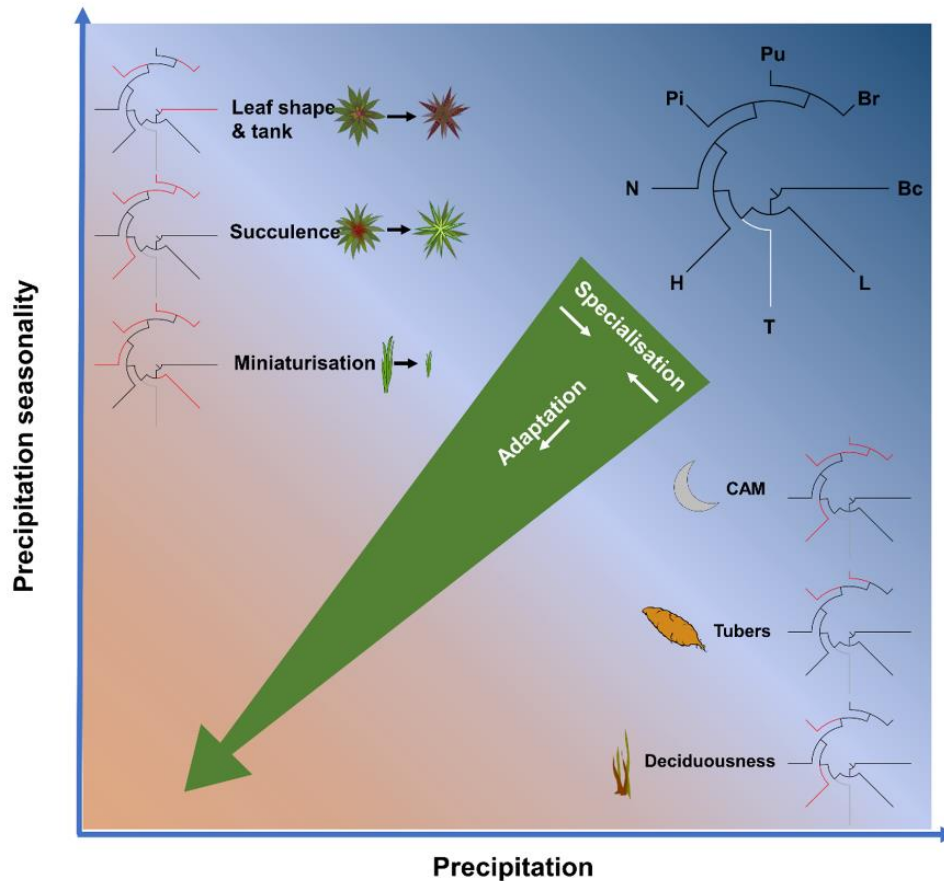


Figure 2.4.1. Major examples of divergent and convergent trait evolution associated with adaptation to more arid and/or seasonal habitats and stronger environmental specialisation (narrower habitat ranges). Reference cladogram (top-right) shows distribution of subfamilies: Bc = Brocchiniaceae; L = Lindmaniaceae; T = Tillandsiaceae (not represented in this investigation); H = Hechtioideae; N = Navioideae; Pi = Pitcairnioideae; Pu = Puyoideae; Br = Bromelioideae. Red lines on cladograms adjacent to trait labels denote presence of the trait. For simplicity, only transitions towards occupancy of more arid and/or seasonal habitats and increasing environmental specialisation are depicted.

Of potentially profound significance for niche width and therefore for habitat range are intraspecific phenotypic variation and the capacity for phenotypic plasticity (Sultan, 2001; González and Gianoli, 2004; Miner et al., 2005; Sides et al., 2014; cf. Valladares et al., 2007). Neither of these phenomena are well-characterised in the bromeliads, and require further investigation.

2.4.3 Relationships between hydrological habitat position and range

The only hydrological habitat indicator variable for which there was a strong, consistent relationship between species' mean scores and ranges was P_{dry} . One interpretation of this correlation is that adaptation to lower precipitation levels during the driest part of the year involves more

specialisation, perhaps because particular structural or physiological traits associated with water scavenging or retention are optimally operative under particular sets of conditions. Weaker correlations between mean and range for MAP and AI are consistent with this hypothesis, but the fact that the relationships are not stronger implies that species of contrasting levels of specialisation can occur under the same environmental conditions, with important implications for bromeliad evolution and community ecology. However, the weak negative relationship between mean and range for AET/PET for some groups was in all cases driven by a cluster of very low range values at the highest mean values. This suggests that species adapted to the lowest levels of moisture deficit are highly specialised and perhaps restricted to narrow geographical ranges. The relationship was particularly strong in Navioideae, where it could relate to the narrow endemism of certain species in high-rainfall environments on the Guiana Shield (Givnish et al., 2011).

2.4.4 *Relevance to bromeliad ecological and species diversity*

The strong correlation between diversity of hydrological habitat position and range across genera highlights the degree of coordination in the evolution of plant-environment interactions in the terrestrial Bromeliaceae. Those genera which have radiated into diverse areas of hydrological habitat space tend to include species with the broadest range of hydrological habitat ranges. The accumulation of high levels of ecological diversity therefore seems to depend on the admixture of both hydrological generalists and specialists. Studies of other groups of organisms have demonstrated similar results, with climate niche lability being a good predictor of clade diversity (e.g. Martínez-Cabrera et al., 2012; Koch et al., 2017), whereas in other cases the phylogenetic niche conservatism paradigm has been invoked (e.g. Skeels and Cardillo, 2017). The extent of univariate hydrological habitat overlap in terms of AI and P_{seas} was found to vary considerably between taxonomic groups, even when controlling for diversity in mean species-level bioclimatic ranges. Extensive hydrological habitat overlap occurs in the Xeric Clade Pitcairnioideae, which, when considered alongside their relatively uniform vegetative morpho-anatomy and life-history, suggests that that pronounced species-level hydrological niche segregation is not a feature of this group. By contrast, in lineages such as the C_3 early-diverging Bromelioideae genera and the Hechtioideae, where there is less extensive overlap and much greater vegetative diversity, segregation in hydrological niche may have been more important in the generation of species diversity. That abiotic niche specialisation should be stronger in these higher-latitude groups is consistent with the hypothesis that the relative importance of abiotic to biotic niche segregation as a driver of species diversity increases further from the equator (Hulshof et al., 2013). The Navioideae represent an interesting case, since they showed very different levels of overlap depending on which bioclimatic

variable (AI or P_{seas}) was considered. The comparatively low levels of habitat overlap in P_{seas} suggest that species diversification in Navioideae may have been contingent on adaptation to contrasting levels of P_{seas} while overall environmental moisture requirements have remained evolutionarily conserved.

Hydrological habitat range was strongly correlated with species' geographical range size across all functional types in the terrestrial bromeliads, as has been observed in other taxonomic groups (Morin and Chuine 2006; Essl et al. 2009; Slayter et al. 2013). This scaling relationship is probably driven strongly by environmental tolerance, but trade-offs between environmental specialisation and dispersal ability could also be relevant (Jocque et al. 2010). For example, species that are strongly adapted in their vegetative structure and function to sites characterised by very low water availability may allocate fewer resources to seed dispersal mechanisms. Meanwhile, the robust positive correlations between species richness and the ranges of hydrological habitat position scores and ranges across genera is consistent with the hypothesis that ecological diversification associated with differentiation in hydrological niche could have been an important factor in the generation of species diversity in the terrestrial bromeliads. The data therefore cast new light on the observation made by Givnish et al. (2014) that bromeliad clades with large geographical ranges are more species rich (see also Ricklefs and Renner, 1994; Dodd et al., 1999; Davies et al., 2004; Jansson and Davies, 2008; Givnish, 2010). Moreover, these relationships are tentatively consistent with the adaptive radiation paradigm. Convincing demonstration of the operation of adaptive radiation within individual taxonomic groups will be dependent on comprehensive ecophysiological characterisation of relevant species and the identification of trait divergences that can be linked to bioclimatic differentiation (Givnish, 2015).

Testing the general applicability of the results found for terrestrial bromeliads to epiphytic species would require extensive and intensive *in situ* quantitative characterisation of epiphyte hydrological habitat occupancy. Different groups of epiphytes probably match the patterns for terrestrial species to differing degrees. For example, tank-less, non-atmospheric epiphytes (e.g. *Acanthostachys* spp.) might prove relatively comparable to xeromorphic terrestrials, whereas innovations such as the impounding tank and the atmospheric growth-form are likely to have remodelled the relationships between plant traits and environmental interactions (see **Chapter 3**; Males and Griffiths, 2017a). Moreover, while there is very little evidence for taxonomic phorophyte specificity among bromeliads and other vascular epiphytes preferences for particular phorophyte bark traits and architectures are often cited as determinants of epiphyte distributions (Wagner et al., 2015; Chaves et al., 2016).

For both terrestrial and epiphytic bromeliads, a range of other factors have been shown to be involved in the generation and maintenance of species diversity, and could constrain species' habitat occupancy to a small subspace of the suitable habitat predicted by trait-based fundamental hydrological niches. Notable examples include dispersal barriers and limitations (Linares-Palomino and Kessler, 2009; Jabaily and Sytsma, 2013; Givnish et al., 2014), and specialisation in biotic interactions and mating systems (Krömer et al., 2008; Matallana et al., 2010; Palma-Silva et al., 2011; Christianini et al., 2013; Givnish et al., 2014 cf. Piacentini and Varassin, 2007; Wendt et al., 2008). Little is known about other potentially important contributing factors to the overall environmental niche, such as sensitivity to soil composition and topographically- or vegetationally-determined differences in light regimes (Benzing, 2000). Likewise, interspecific competition and facilitation effects (with bromeliads and non-bromeliads) could curtail or extend habitat occupancy, but are little-studied (Miller and Silander, 1991; Scarano, 2002). The realised habitat occupancy of bromeliad species may also be limited by disturbance phenomena, including human activity, hurricanes, and fire (Miller and Silander, 1991; Benzing, 2000). The corollary of this is that species with narrower niches and habitat ranges are likely to be more vulnerable to disturbance and global change (Thuiller et al., 2005; Broennimann et al., 2006). Despite the fact that the hydrological component of the species niche is only one piece of the jigsaw, when the evidence presented here is considered alongside the acknowledged proliferation in the Bromeliaceae of innovations associated with water-use strategies, it is clear that specialisation and differentiation in the hydrological habitat occupancy has been a central theme in bromeliad evolution (Males, 2016). Further examination of bromeliad hydrological habitat occupancy could in future incorporate consideration of variation in water availability at a range of temporal scales to cast further light on the relevance of temporal variability for species diversity (Chesson et al., 2004; Reineking et al., 2006; Schwinning and Sala, 2007; Reyer et al., 2013).

2.5 Conclusions

Hydrological habitat position and range vary systematically between taxonomic and functional groups within the terrestrial bromeliads. Differentiation in hydrological habitat occupancy has probably been an important aspect of the generation of species diversity in the Bromeliaceae, with different genera being characterised by varying patterns of specialisation and degrees of lability in hydrological habitat properties. Hydrological habitat range can be limited by different aspects of water availability, depending on the species, and terrestrial bromeliads' geographical range sizes are closely linked with the range of hydrological habitats in which they occur. Interspecific variation in hydrological habitat position and range can partly be explained with reference to existing knowledge

of life-history, morphological and ecophysiological traits, which collectively define the hydrological niche. However, explanations for the residual variation must be sought in as yet unexplored aspects of internal anatomy and physiology. Subsequent chapters will demonstrate how structure-function relationships link plant form and environmental responses in bromeliads of contrasting phylogenetic identity and functional type.

3. Integration of hydraulic and anatomical traits in the leaf economics of the Bromeliaceae

Content from this chapter is published in Males and Griffiths (2017a) and Males and Griffiths (2017c)

3.1 Introduction

In this chapter, the second question posed in the **General Introduction** is addressed:

How does diversity in leaf structural and functional traits relate to divergences in water-use strategies between bromeliads of contrasting functional types?

Further to the observations of the extensive hydrological habitat diversity reported in **Chapter 2**, the overall aim of the strand of research presented in this chapter was to determine the extent and significance of interspecific variation in leaf hydraulic and anatomical traits, and specifically those which are putatively involved in drought resistance, for ecological specialisation among the major functional types in the Bromeliaceae. This investigation was performed in the context of intense contemporary interest in the extent and strength of coordination in the evolution of economic and hydraulic leaf traits, and makes important contributions to that discourse (Males and Griffiths, 2017a,c).

3.1.1 Trait-mediated niche differentiation

There is increasing interest in the use of functional traits to define the environmental niches of plants (Violle and Jiang, 2009; Kearney et al., 2010), and the relevance of functional trait variation for community assembly is much-studied (Kraft et al., 2008; Kraft and Ackerly, 2010; Lebrija-Trejos et al., 2010; Sterck et al., 2011; Loranger et al., 2016). While the morphological and ecological distinctiveness of the functional types of bromeliads are well known, the range of variation in key anatomical and physiological traits within and between functional types remains unexplored. Since functional trait variation has the potential to be an important mode of ecological niche segregation and diversification, it was hypothesised that the divergent ecophysiological syndromes of the bromeliad functional types would be at least partly underpinned by differences in key leaf traits.

3.1.2 Relationships among leaf economic and hydraulic traits

Both within the bromeliads and across the angiosperms in general, variation in leaf traits is finite and highly structured. Leaf economic theory states that fundamental developmental and physiological constraints limit the region of leaf trait hyperspace occupied during plant evolution (Donovan et al., 2011; Vasseur et al., 2012; Díaz et al., 2016). The core leaf economic traits, photosynthetic capacity

(A_{max}), respiration rate (R), leaf nitrogen content (N_{leaf}), leaf phosphorus content (P_{leaf}), leaf mass per unit area (LMA), and leaf lifespan (LL), show strong coordination across the plant kingdom (Reich et al., 1997, 1999; Wright et al., 2004, 2005). Although leaf economic theory has focussed principally on covariation in a limited set of core leaf traits, efforts have been made to extend the approach to consider other traits, including leaf hydraulics (e.g. Niinemets et al., 2007; Sack et al., 2013). The relationships between gas exchange, hydraulic conductance and hydraulic vulnerability are of particular interest because of their importance for plant survival or mortality under environmental stress (Choat et al., 2012; Mitchell et al., 2013). Hydraulic conductance and hydraulic vulnerability may trade off in plant stems, supporting the so-called safety vs. efficiency hypothesis (Pockman and Sperry, 2000; Maherali et al., 2004; Wheeler et al., 2005; Hacke and Sperry, 2006; Jacobsen et al., 2007; Gleason et al., 2016). However, such relationships are less well defined in leaves, despite a growing recognition of the relative importance of leaf venation and extra-xylary compartments in regulating transpiration fluxes (Cochard et al., 2004; Buckley et al., 2015; Scoffoni et al., 2017). It is generally accepted that leaf hydraulics and photosynthetic capacity are closely coupled (Scoffoni et al., 2016a), although some gas exchange parameters may be decoupled from hydraulic parameters in pulse-driven systems or in the presence of carbon-concentrating mechanisms (Blackman et al., 2010; Ocheltree et al., 2016). Despite their importance in natural vegetation and in agriculture, integrative analyses of leaf economic and hydraulic properties in radiations of herbaceous plants are still surprisingly scarce (Dunbar-Co et al., 2009; Muir et al., 2014; Mason and Donovan, 2015; Nolf et al., 2016). The bromeliads have attracted some recent attention from plant hydraulic researchers (e.g. North et al., 2013, 2015), and offer an excellent opportunity to study the coordination of leaf economic and hydraulic traits in an ecophysiologically diverse herbaceous clade displaying a broad range of water-use strategies (Males, 2016).

3.1.3 Drought resistance traits in ecological specialisation

Several of the leaf traits interacting via LES-type coordination are likely to contribute to drought resistance, which is a complex, multifactorial character. One key marker of drought resistance is the turgor loss point (TLP), at which the cellular water potential (Ψ_w) equals osmotic potential ($\pi_{t_{lp}}$). TLP is typically the point at which carbon metabolism is limited by leaf water status (McDowell, 2011), and turgor loss in mesophyll cells may also be an important contributor to leaf hydraulic dysfunction by reducing extra-xylary hydraulic conductance (see **Chapter 4**; Brodribb and Holbrook, 2004a; Scoffoni et al., 2014; Trifilò et al., 2016). Leaves reaching the TLP at a low relative water content demonstrate *drought tolerance*: the ability to endure reductions in leaf water potential. In contrast, *drought avoidance* is considered to involve the deployment of mechanisms to prevent reductions in

leaf water potential in desiccating environments. It is already well-established that many bromeliads can survive extreme reductions in relative water content, but that since this is largely restricted to changes in hydrenchyma water content, bromeliads qualify as drought avoiders (Martin and Adams, 1987; Stiles and Martin, 1996; Nowak and Martin, 1997; Zotz and Andrade, 1998; Benzing, 2000). However, as outlined in the **General Introduction**, almost no comparative data are available for Ψ_{tip} , and the relevance of any interspecific variation in this parameter for ecological differentiation is therefore unknown. The rapidly-quantifiable bulk osmotic potential of fully hydrated leaves (π_o) has recently been shown to be robustly proportional to π_{tip} (Bartlett et al., 2012a), and therefore π_o can be used as a convenient surrogate for π_{tip} in analyses of drought resistance.

Another trait that has sometimes been identified as a surrogate for drought resistance is leaf mass per unit area (LMA). High LMA is frequently associated with drought tolerance, particularly in Mediterranean biome species, because it is often anatomically manifested through denser, thicker cell walls that limit deformation-induced damage caused by declining leaf water potential (Cunningham et al., 1999; Niinemets, 2001; Lamont et al., 2002; Knight and Ackerly, 2003; Poorter et al., 2009). However, in tropical floras there are numerous examples of species that display high LMA yet are comparatively intolerant of drought (Bartlett et al., 2012b), suggesting that the utility of LMA as a drought resistance index may be rather context-dependent. Among the bromeliads most variation in LMA is probably associated with differential investment in water-storage tissue, and we speculate that it may therefore be more closely aligned with drought avoidance.

Leaf water storage, or capacitance, is an important contributor to drought resistance (e.g. Graham and Nobel, 1999). In leaf-succulent species with specialised non-photosynthetic water-storage tissue (hydrenchyma), stored water can be remobilised during drought to maintain near-constant high (less negative) water potentials in photosynthetic tissues (Schmidt and Kaiser, 1987; Herrera et al., 2000). One measure of succulence is saturated water content (SWC), which is defined as the quotient of area-specific water storage and area-specific dry mass (Ogburn and 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. WMA therefore complements SWC by providing an area-specific measure of water storage.

3.1.4 Approach

Until now, no quantitative analyses have explored how differences in leaf economic and hydraulic traits, and the architecture of the conceptual network of correlations that connects them (hereafter 'trait-network'), relate to ecological differentiation in the Bromeliaceae. Nor have any wide-ranging

systematic surveys of drought resistance traits been performed. In this investigation, variation and coordination in a range of anatomical and physiological leaf traits in 50 bromeliad species representing each of the functional types was studied. Four key drought resistance traits (LMA, SWC, WMA and π_o) and basic anatomical parameters were also quantified in an extended set of 376 species to test the hypotheses that convergent origins of succulence (associated with high SWC) and tank-epiphytism would have relaxed the selective pressure for drought tolerance (represented by π_o) by increasing either internal or external hydraulic capacitance. For 308 of these species, drought resistance traits were compared with bioclimatic data to determine the extent to which these traits were associated with differences in bioclimatic habitat.

3.2 Methods

3.2.1 Plant material and growth conditions

For leaf economic and hydraulic trait quantification, 50 bromeliad species were selected to represent the ecological and phylogenetic diversity of the family. The species set included 10 C₃ terrestrials, 14 C₃ tank-epiphytes, 7 CAM terrestrials, 10 CAM tank-epiphytes, and 9 CAM atmospheric epiphytes. A full list of species is provided in **Appendix 3.1**. Plants were grown at Cambridge University Botanic Garden, UK (52.1938° N, 0.1279° E). Species native to the humid tropics were grown under partial shade in a tropical glasshouse with daytime temperatures of 25-30 °C, night-time temperatures of 18-24 °C, and relative humidity of approximately 80%. Species native to subtropical/temperate zones were grown in a cool temperate house with daytime temperatures of 10-25 °C, night-time temperatures above 0 °C, and relative humidity of approximately 60%. As a diagnostic screen for CAM, $\delta^{13}\text{C}$ was quantified from 1 mg of dried, powdered material sampled from the centre of leaf blades and leaf tissue, using a Thermo Finnigan MAT 253 mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) fitted with a Costech elemental analyser (Costech Analytical Technologies, Valencia, California, USA) at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge. Values of $\delta^{13}\text{C}$ were similar to existing reports for most species (Crayn et al., 2015), however, they suggested that all of the *Puya* Molina species ($n = 5$) were primarily performing C₃ photosynthesis under well-watered conditions.

The expanded set of 376 species used in the survey of drought resistance traits covered a broad taxonomic range, including representatives of seven out of the eight subfamilies in the Bromeliaceae. All plant specimens used were in cultivation at one of four institutions: Cambridge University Botanic Garden (UK), Royal Botanic Gardens Kew (UK), Royal Botanic Gardens Edinburgh (UK), and the Marie Selby Botanical Gardens (FL, USA). Depending on origin, plants were grown

either in glasshouses, outdoors under shade, or outdoors without shade. Glasshouse conditions were similar at each site. A full list of species is included in **Appendix 3.1**. For all measurements, fully-expanded, non-senescent leaves were selected, generally corresponding to the central layer of leaves in rosette-forming species. 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.

3.2.2 Bioclimatic and habitat data

Variation in drought resistance traits in the expanded species set was compared with variation in bioclimatic indices. The methodology for acquisition of bioclimatic data was identical to that used in hydrological habitat occupancy analysis in **Chapter 2**, and sufficient data were available for 308/376 species. As discussed in **Chapter 2**, the use of global climate datasets for the analysis of the distributions of epiphytic species must be undertaken with caution. Since the aim of this strand of work was primarily to obtain a qualitative impression of whether variation in drought resistance traits was associated with bioclimatic differentiation, it was deemed that the approach was acceptable. Two bioclimatic indices were selected for use in analyses here: precipitation seasonality (P_{seas} , the coefficient of variation (%) in monthly precipitation), because of the supposed relationship between precipitation seasonality and succulence (Ellenberg, 1981), and aridity index (AI, mm mm^{-1}), as a proxy for potential water stress. For each species, the mean value of each climatic variable across all reliable presence points was used in analyses. Linear regression was then performed in R to identify relationships between these values and π_o , SWC, WMA, and LMA.

The primary habitat of each species was assigned by interrogating the eMonocot portal (<http://www.e-monocot.org>). Data were unavailable for two species. The following habitat categories were used: desert and xeric scrubland; Mediterranean forest and scrub; tropical dry forest; tropical moist forest; temperate mixed forest; and Andean tundra.

3.2.3 Anatomical parameters

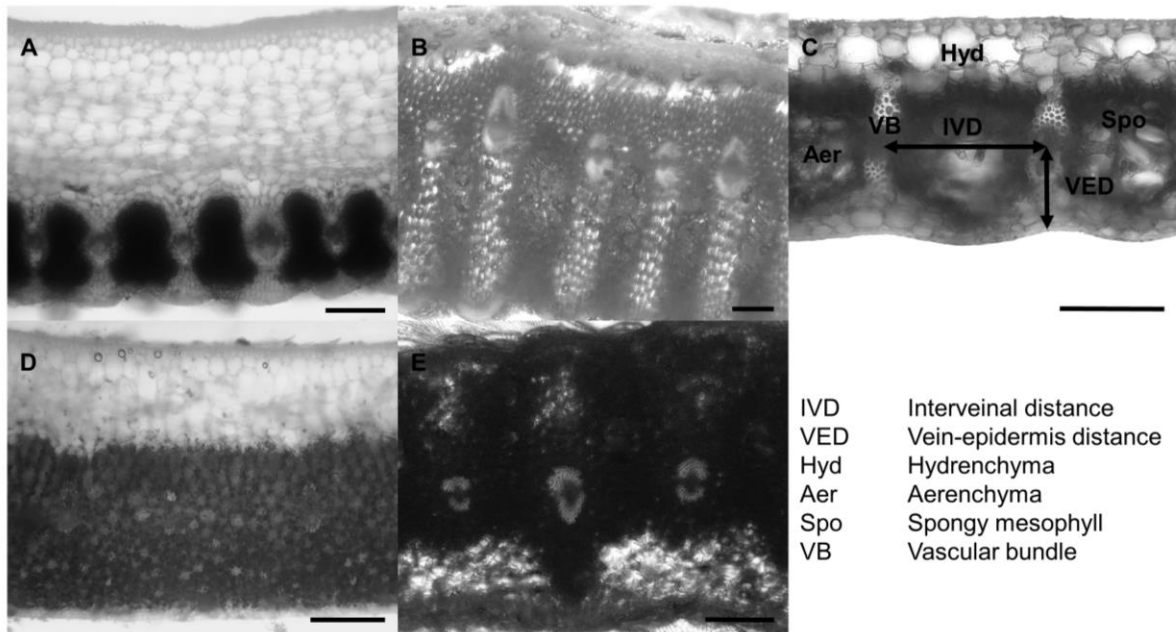


Figure 3.2.1. Examples of transverse leaf cross sections from species of different functional types showing key tissues and anatomical parameters. a) *Puya chilensis* (C_3 succulent terrestrial); b) *Bromelia humilis* (CAM terrestrial); c) *Lutheria splendens* (C_3 tank-epiphyte); d) *Aechmea fasciata* (CAM tank-epiphyte); e) *Tillandsia caput-medusae* (CAM atmospheric). Bars = 200 μm .

Examples of leaf anatomical variation among species used in this investigation are depicted in Fig. 3.2.1, which highlights key anatomical traits characterised for all 376 species in the expanded set. In the bromeliads, absorptive trichomes may occur on either epidermis (or both), and differ strongly in density and absorptive capacity between functional types (Benzing, 2000). Vascular bundles occur in a single plane and alternate with longitudinal air lacunae, which may be continuous with substomatal cavities. In the species set used here, the photosynthetic mesophyll was differentiated into spongy and palisade layers only in *Aechmea nudicaulis* (L.) Griseb. The adaxial water-storage tissue varies widely in thickness and cell dimensions. All species used in this investigation were hypostomatous, with the exception of *Catopsis berteroniana* (Schult. & Schult.f.) Mez. Vein-epidermis distance (VED) and interveinal distance (IVD) were identified as focal anatomical traits, due to their relationships with leaf hydraulic capacity and extra-xylary hydraulic path-length, respectively. At least ten replicate leaves were sampled from each of five individuals, and transverse sections were hand-cut from the central portion of leaf blades and viewed under a light microscope to quantify these parameters. For the amphistomatous *C. berteroniana*, VED was not significantly different between adaxial and abaxial surfaces (data not shown). Stomatal density (SD) was measured by microscopic imaging of epidermal impressions of the central portion of the leaf blade

microscope. Stomata were then counted on an area-normalised basis using ImageJ (NIH, Bethesda, MD, USA). Twenty replicate leaves drawn from at least five plants per species were used for all anatomical measurements.

3.2.4 Leaf mass per unit area and saturated water content

For all 376 species, discs were bored from four locations along the leaf axis, and dried to constant mass. Leaf mass per unit area (LMA) was calculated as mean dry disc mass/disc area (g m^{-2}), using four replicate sets of discs from five individuals per species.

SWC was quantified for all species as a proxy for succulence (Ogburn and 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 according to Equation 3.2.1.

Eq. 3.2.1.
$$SWC = \frac{(\text{turgid mass} - \text{dry mass})}{\text{dry mass}}$$

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

3.2.5 Intercellular air space

The intercellular air space fraction (IAS) was quantified for the core 50 species using the vacuum infiltration technique (Unger, 1854; Smith and Heuer, 1981). A PMS pressure chamber (PMS, Albany, Oregon, USA) was used to measure native leaf water potential (Ψ_{leaf}) in freshly-cut leaves. The plants had been well watered and Ψ_{leaf} was high in all cases (> -0.1 MPa). Leaves were cut into 3 mm transverse slices, weighed and transferred into a beaker of isotonic mannitol solution. The beaker was placed inside a vacuum chamber, which was evacuated until the solution was almost boiling. The leaf slices were removed and blotted dry before reweighing. This process was repeated until constant weight of infiltrated samples was achieved (typically 2-3 repeats). IAS was calculated as the change in sample mass following infiltration divided by the final post-infiltration mass and expressed as a percentage. Twenty replicate measurements were made using leaves from at least five plants per species.

3.2.6 Gas exchange

All gas exchange measurements were performed on at least ten leaves drawn from at least five plants for each of the core 50 species. The maximum rate of photosynthetic assimilation (A_{\max}) at ambient CO_2 (~ 400 ppm) and maximum stomatal conductance ($g_{s\max}$) were measured by gas exchange using a Li-6400XT portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA). Leaves of well-watered plants ($\Psi_{\text{leaf}} > -0.1$ MPa) were maintained with a constant leaf temperature (22 °C) and relative humidity (85%), and a saturating PAR level determined from preliminary light-curves (usually $\sim 300 \mu\text{mol m}^{-2} \text{s}^{-1}$). Leaves equilibrated for at least 20 min before four data points were logged at 15 s intervals and parameters averaged. For CAM species, measurements were made in darkness during the peak assimilation period of Phase I. The instantaneous water-use efficiency (iWUE) was calculated as the quotient of A_{\max} and $g_{s\max}$.

For C_3 species, measurements of dark respiration (R_D) were performed by allowing the leaf to acclimatise to darkness in the chamber for 3 min (to avoid the post-illumination respiratory burst) before four points were logged at 15 s intervals and averaged. Respiration in the light (R_L) was calculated from coupled gas exchange and fluorescence measurements (Bellasio et al. 2016), using a Li-Cor fluorometer chamber. For CAM species, R_D measurements were made in the same manner during Phase IV (direct RuBisCO-mediated CO_2 fixation through open stomata in late afternoon) where possible. In species not displaying Phase IV fixation, R_D was measured at the end of the dark period when the mesophyll acid pool was full (Wagner and Larcher, 1981). For CAM species displaying Phase IV fixation, R_L was measured using the same method as for the C_3 species. For other species, R_L was measured by cutting slices of light-acclimated leaves to allow respiratory CO_2 to escape from air spaces, and placing these in the Li-Cor chamber. Although this destructive method had the potential to introduce substantial wound-induced artefacts, the values measured were comparable to those obtained for other species using different methods, and were therefore retained for analysis.

The leaf water potential at 50% stomatal closure (P_{50s}) was obtained by measuring gas exchange and Ψ_{leaf} during gradual plant dehydration (over a period of up to 2 months for the most drought resistant species). P_{50s} was identified by using non-linear curve-fitting to predict the value of Ψ_{leaf} by which g_s had declined by 50% relative to the value measured at $g_{s\max}$ in fully hydrated plants.

3.2.7 Carbon, nitrogen and phosphorus assays

Carbon-to-nitrogen ratio (C: N) and leaf nitrogen content (N_{leaf}) were determined alongside $\delta^{13}\text{C}$ (see 3.2.1 above) for the core 50 species. Photosynthetic nitrogen-use efficiency (PNUE) was calculated as

the quotient of mass-normalised A_{\max} and N_{leaf} . Leaf phosphorus content (P_{leaf}) was determined by sulphuric acid digestion and spectrophotometric assay (Buyarski et al. 2013), using six leaf tissue samples from at least three different plants.

3.2.8 Pressure-volume curves

For the core 50 species, leaves were detached from plants and immersed in water until full hydration (determined by constant mass). Rehydration times varied between 12 h and 2 d. Images of fresh leaves were used for leaf area measurement using ImageJ (NIH, Bethesda, Maryland, USA). Hydrated leaves were gently wiped dry and allowed to reach equilibrium in a plastic bag before Ψ_{leaf} was measured using a pressure chamber (PMS Instruments, Albany, OR, USA). Leaves were immediately weighed and then air-dried. At regular, species-dependent intervals, coupled measurements of Ψ_{leaf} and leaf mass were made. After the final measurements, leaves were oven dried to constant dry mass. The resulting values of Ψ_{leaf} and leaf mass were analysed using the pressure-volume (PV) curve analysis spreadsheet prepared by Sack et al. (2011). Estimates of the leaf water potential at turgor loss point (Ψ_{TLP}), the bulk modulus of elasticity (ϵ), and area-specific capacitance (C_{FT}) were averaged across six replicate PV curves per species, using leaves drawn from at least three plants.

3.2.9 Psychrometric determination of π_o

For each species in the full set of 376 that did not feature in the core set of 50 species, at least six replicate leaves sampled from a minimum of three plants 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 prominent midrib in *Pitcairnia* species. Leaf discs 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.

3.2.10 Leaf hydraulic conductance

Leaf hydraulic conductance (K_{leaf}) was measured for the core 50 species using the evaporative flux method (Sack and Scoffoni, 2012). Leaves were detached from plants at dawn and allowed to reach

full hydration in water before being re-cut underwater and connected to the evaporative flux apparatus. 15 mM KCl in degassed reverse-osmosis water was supplied in the reservoir. The leaf was carefully inserted in a custom-built glass cuvette fitted with fans to minimise boundary layer resistance. A water jacket connected to a water bath was used to control cuvette air temperature, and humidified air was fed into the chamber to achieve a specific dew point. Leaf temperature was tracked with a thermocouple, and relative humidity was recorded inside the cuvette. Relative humidity was maintained at 85% by varying the dew point. The system was illuminated by a halogen lamp to provide $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf-level for C_3 species, while measurements on CAM species were performed during the night in darkness. Leaves were allowed to transpire under constant conditions for at least 30 min. The leaf was then removed from the system and placed in a plastic bag to equilibrate before the measurement of final Ψ_{leaf} using the pressure chamber. Leaf area was measured using ImageJ. Area-specific K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was calculated as the molar quantity of water drawn from the balance reservoir (mmol) divided by the product of leaf area (m^2), duration (s) and the driving force (MPa), as shown in Equation 3.2.2.

Eq. 3.2.2.
$$K_{\text{leaf}} = \frac{\text{mmol } H_2O}{\text{m}^2 \times \text{s} \times \text{MPa}}$$

3.2.11 Leaf hydraulic vulnerability curves

Hydraulic vulnerability curves were constructed using at least six leaves sampled from at least three plants for the core 50 species by measuring K_{leaf} (see 3.2.10 above) at intervals of Ψ_{leaf} . Preliminary comparison was made of vulnerability curves constructed using the standard bench-drying methodology and using overpressure. In the former, initially fully-hydrated leaves were allowed to dry on the bench between regular measurements of K_{leaf} . In the latter, leaves were dehydrated by very gradually applied overpressure in the pressure chamber. Leaves were pressurised until the extrusion of solution from the cut end of the xylem ceased, and K_{leaf} was then measured. This was performed at evenly-spaced pressure increments, using separate five replicate leaves from different plants for measurements at each interval. Comparison of the overpressure and bench-drying methodologies showed that in these bromeliad species they produced equivalent results, despite the artefacts that could be introduced by damage to tissue structure in the overpressure method (comparative data are shown in **Appendix 3.2**). The overpressure method was considerably faster than bench drying (hours rather than days or weeks, depending on the species), and was therefore used for subsequent repeat measurements for all species. P_{50L} was determined as the value of Ψ_{leaf}

on a sigmoidal curve fitted to the data where K_{leaf} had declined to 50% of its maximum value in fully hydrated leaves (K_{leafmax}).

3.2.12 Statistical analysis

All statistical analyses were performed in R (R Development Core Team, 2008). Linear or non-linear regression was used on a pairwise basis to identify trait relationships, and analysis of variance (ANOVA) was used to identify differences in trait values between functional types. In general, r^2 values > 0.25 were interpreted as suggesting a mechanistic relationship between traits (Poorter et al., 2014). It was not possible to perform phylogenetic analysis of trait data due to the current lack of resolution in the phylogeny of Bromeliaceae. Principal components analysis (PCA) was performed in R to identify major axes of variation among functional traits and clustering of species in trait space.

Decisions about which groups of functional types could be meaningfully compared for consideration of evolutionary trends were based on the phylogenetic analyses of Givnish et al. (2011, 2014).

Inspection of the phylogenetic patterning of functional types reveals a series of major transitions in functional type, displayed in Table 3.2.1.

| 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 |

Table 3.2.1. Major transitions in functional type in the Bromeliaceae inferred from phylogenetic distribution of functional types according to topologies resolved by Givnish et al. (2011, 2014).

3.3 Results

3.3.1 Variation in leaf traits

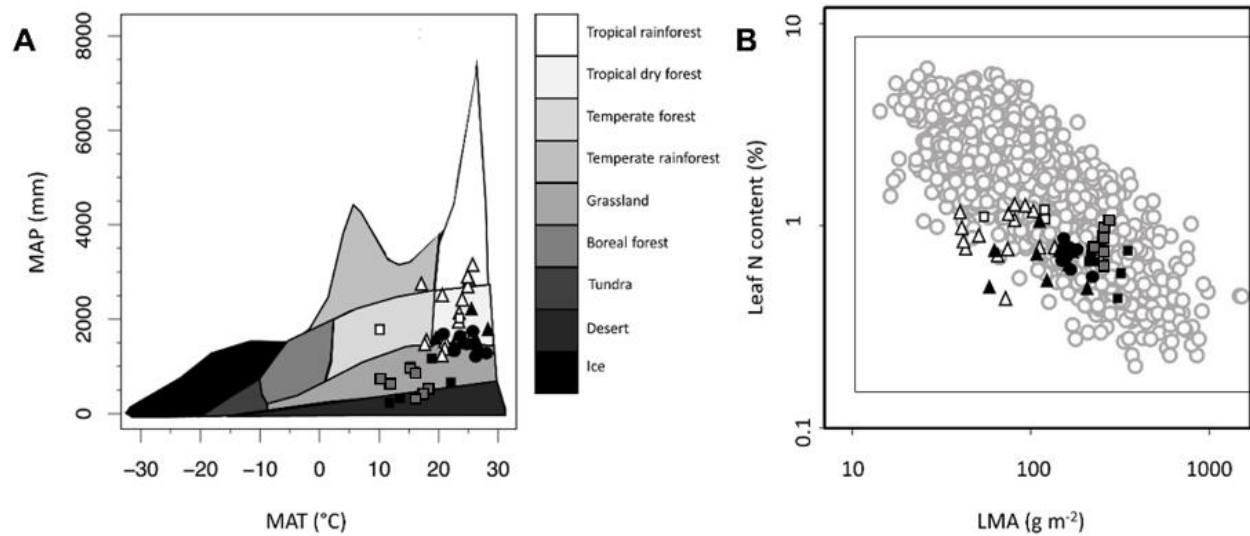


Figure 3.3.1. a) Bioclimatic distribution of 46 of the 50 bromeliad species for which all traits were quantified across the space defined by mean annual precipitation (MAP) and mean annual temperature (MAT; Whittaker, 1975). White squares: C₃ mesic terrestrials; grey squares: C₃ succulent terrestrials; black squares: CAM terrestrials; white triangles: C₃ tank-epiphytes; black triangles: CAM tank-epiphytes; black circles: CAM atmospheric epiphytes. b) Location of all 50 bromeliad species in the leaf economic spectrum (LES) trait space defined by leaf mass per unit area (LMA; g m⁻²) versus leaf N content (%; Wright et al., 2004). Background points (grey circles) represent non-bromeliad angiosperm data sampled from the Global Plant Trait Network (GLOPNET) Database, with bromeliad species plotted by functional type as in (a).

The bromeliads occur in a wide variety of biomes, habitats and microhabitats throughout the Neotropics and into adjacent subtropical and temperate zones in both hemispheres (Benzing, 2000). This diversity in habitat occupancy was explored from a hydrological perspective in **Chapter 2**, and the data presented in this chapter provide insights into how diverse environmental niches could be underpinned by radiation into diverse areas of trait-space (Fig. 3.3.1). Values for many physiological rates were an order of magnitude lower relative to many other angiosperm groups, including for A_{\max} (0.70-6.81 $\mu\text{mol m}^{-2} \text{s}^{-1}$), g_{smax} (0.005-0.243 $\text{mol m}^{-2} \text{s}^{-1}$), K_{leafmax} (0.01-5.84 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) R_{L} (0.18-0.46 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and R_{D} (0.25-0.71 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and values of N_{leaf} (0.45-1.71%) and P_{leaf} (0.002-0.024%) were also low. There was considerable variation in IAS (3.88-19.42%), iWUE (15.2-328.0 $\mu\text{mol mol}^{-1}$), LMA (38.3-380.0 g m⁻²), and PNUE (0.26-11.11). All values of Ψ_{tip} (-1.57 - -0.63 MPa) and P_{sol} (-1.74 - -0.60 MPa) were rather high, suggesting that bromeliads are relatively drought-sensitive at the cellular level. C_{FT} was high (2.38-14.05 $\text{mol m}^{-2} \text{MPa}^{-1}$), particularly among

species with thick adaxial layers of water-storage tissue. ϵ occasionally reached extremely low values (1.60-21.91 MPa). Intervenal distances (IVD) were comparable towards their lower limit to C_3 grasses (190.0-426.7 μm ; Griffiths et al., 2013), while vein-epidermis distance (VED) was highly variable (90.0-495.0 μm).

Pressure-volume curve construction by bench-drying took up to 4 w for the most succulent, xeromorphic species, indicative of strong stomatal closure and low cuticular conductances. No values of Ψ_{tip} and P_{50L} were recorded below -1.74 MPa, suggesting that bromeliads are relatively drought-sensitive at the cellular level. C_{FT} was high, particularly among species with thick adaxial layers of hydrenchyma. In one species, ϵ reached the extremely low value of 1.6 MPa. Intervenal distances (IVD) were comparable towards their lower limit to C_3 grasses (Griffiths et al., 2013), while vein-epidermis distance (VED) was highly variable. Stomatal responses to Ψ_{leaf} were quantified as P_{50S} (Fig. 3.3.2), which was not correlated with other leaf traits and consistently less negative than P_{50L} . Examples of leaf hydraulic vulnerability curves and g_s - Ψ_{leaf} curves used to derive P_{50L} and P_{50S} are shown in Fig. 3.3.3. In all species, K_{leaf} declined sigmoidally as Ψ_{leaf} became more negative, whereas g_s decayed exponentially.

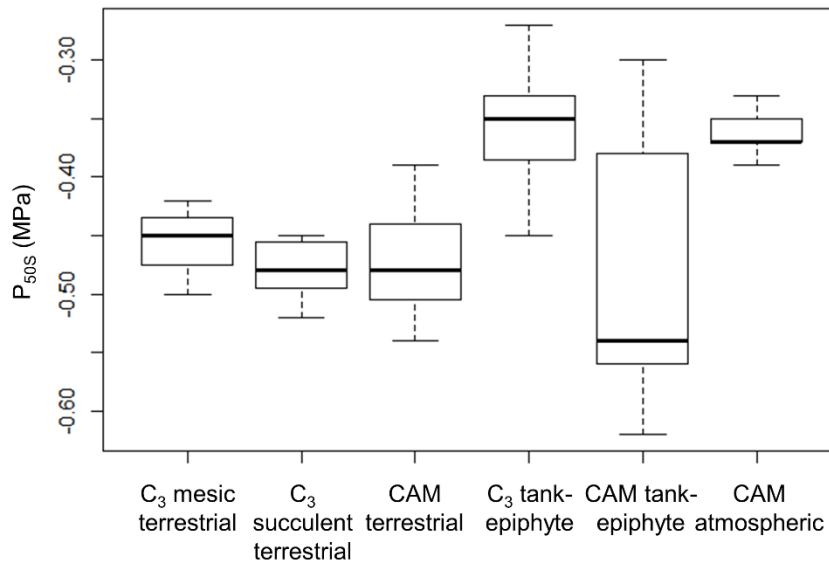


Figure 3.3.2. Leaf water potential at 50% stomatal closure (P_{50S}) by functional type ($n = 50$). Boxes show median values and interquartile range (IQR), with whiskers of length (1.5 x IQR).

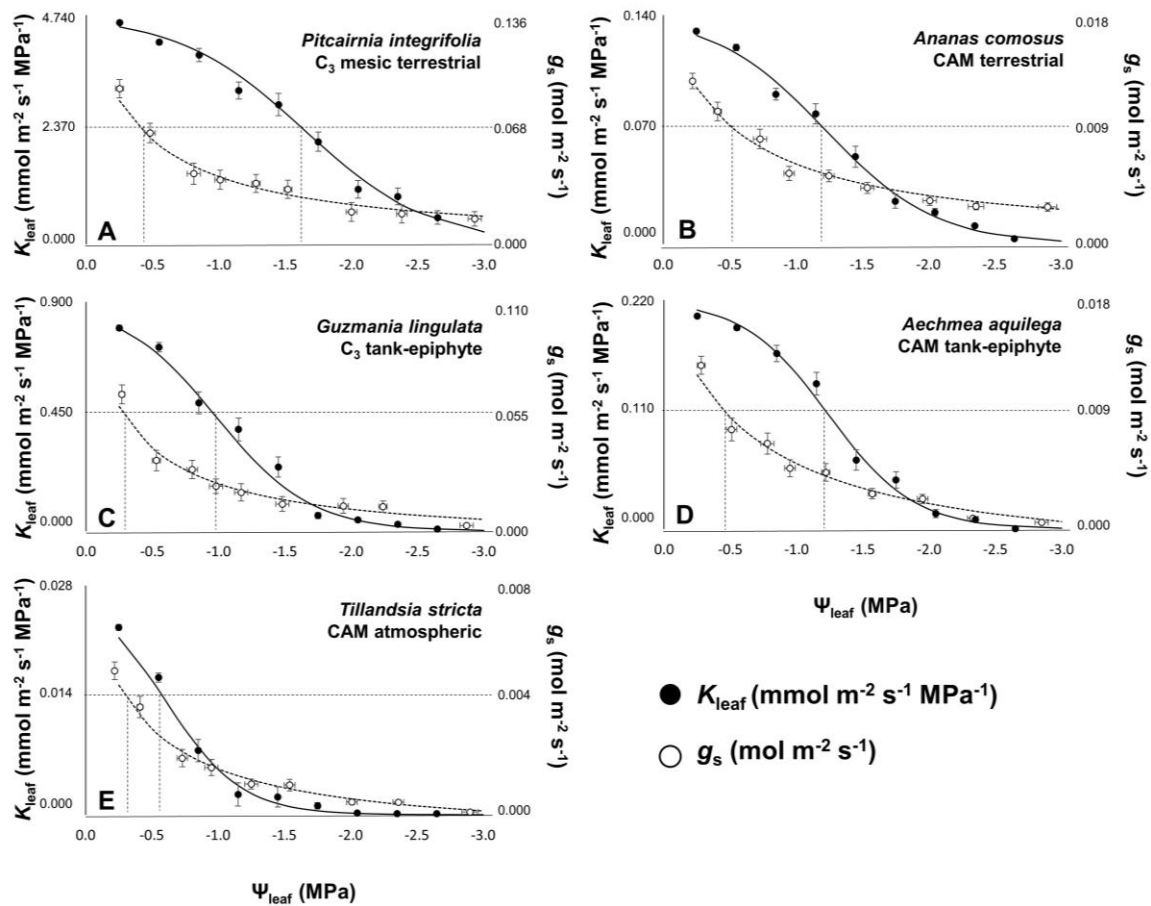
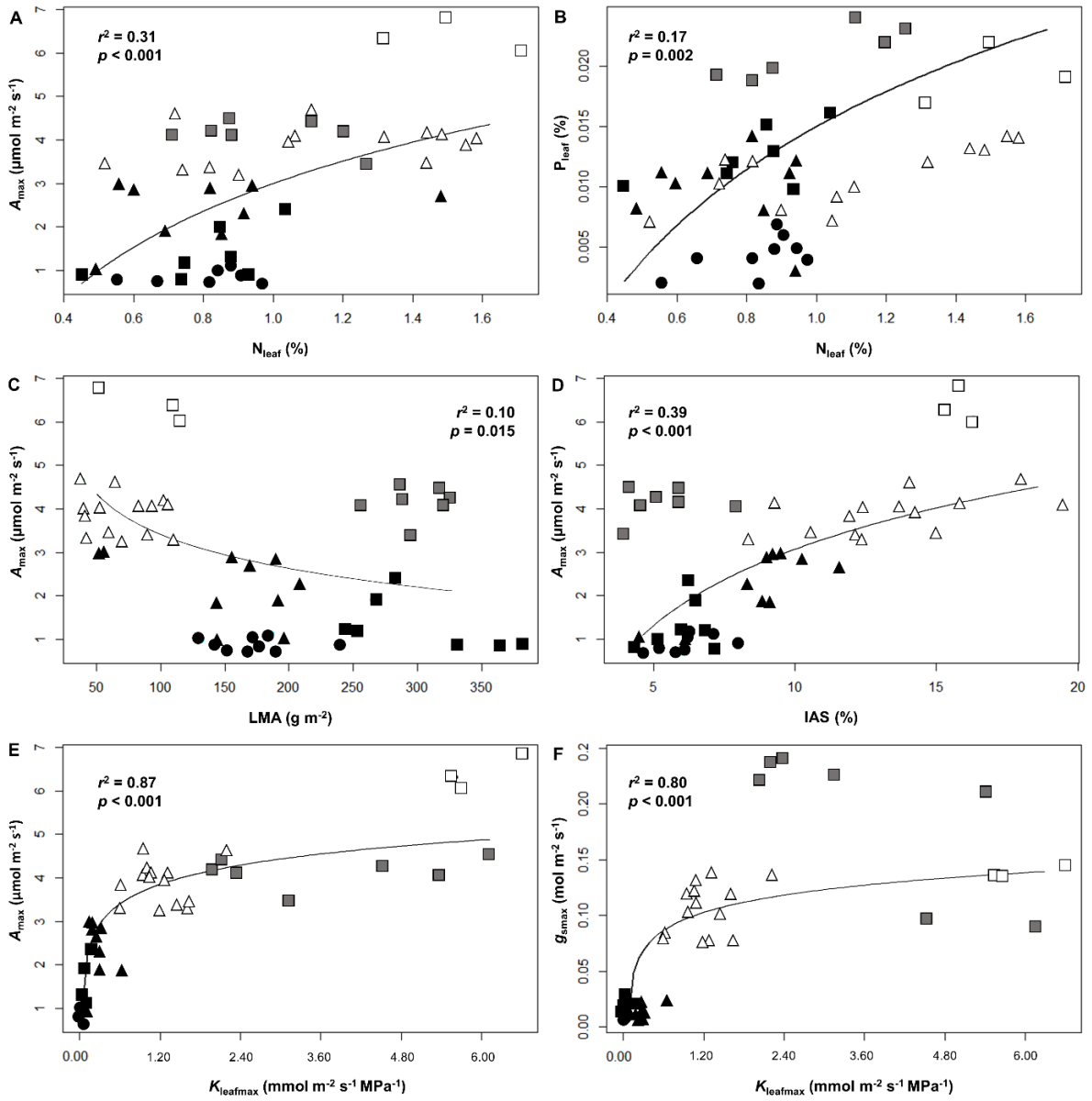


Figure 3.3.3. Leaf hydraulic conductance (K_{leaf}) and stomatal conductance (g_s) as functions of declining leaf water potential (Ψ_{leaf}) for six representative bromeliad species of different functional types: a) *Pitcairnia integrifolia*, C₃ mesic terrestrial; b) *Puya alpestris*, C₃ succulent terrestrial; c) *Ananas comosus*, CAM terrestrial; d) *Guzmania lingulata*, C₃ tank-epiphyte; e) *Aechmea aquilega*, CAM tank-epiphyte; f) *Tillandsia stricta*, CAM atmospheric. Black circles show mean values of K_{leaf} ; white circles show mean values of g_s . Means are based on five biological replicates per species; error bars indicate \pm standard error of the mean. Solid lines show fitted curves for K_{leaf} ; short dashed lines show fitted curves for g_s . Intersections of the fitted curves with the straight line at PLC = 50% represent leaf water potential at 50% loss hydraulic conductance (P_{50L}) and 50% stomatal closure (P_{50S}).

3.3.2 Coordination among leaf traits

Pairwise regression analysis of all continuous variables yielded correlations that supported most of the core relationships of leaf economic theory. Key relationships and underlying data are displayed in Fig. 3.3.4, with the statistically significant correlations being detailed in Table 3.3.1. The full trait dataset is available in **Appendix 3.3**.



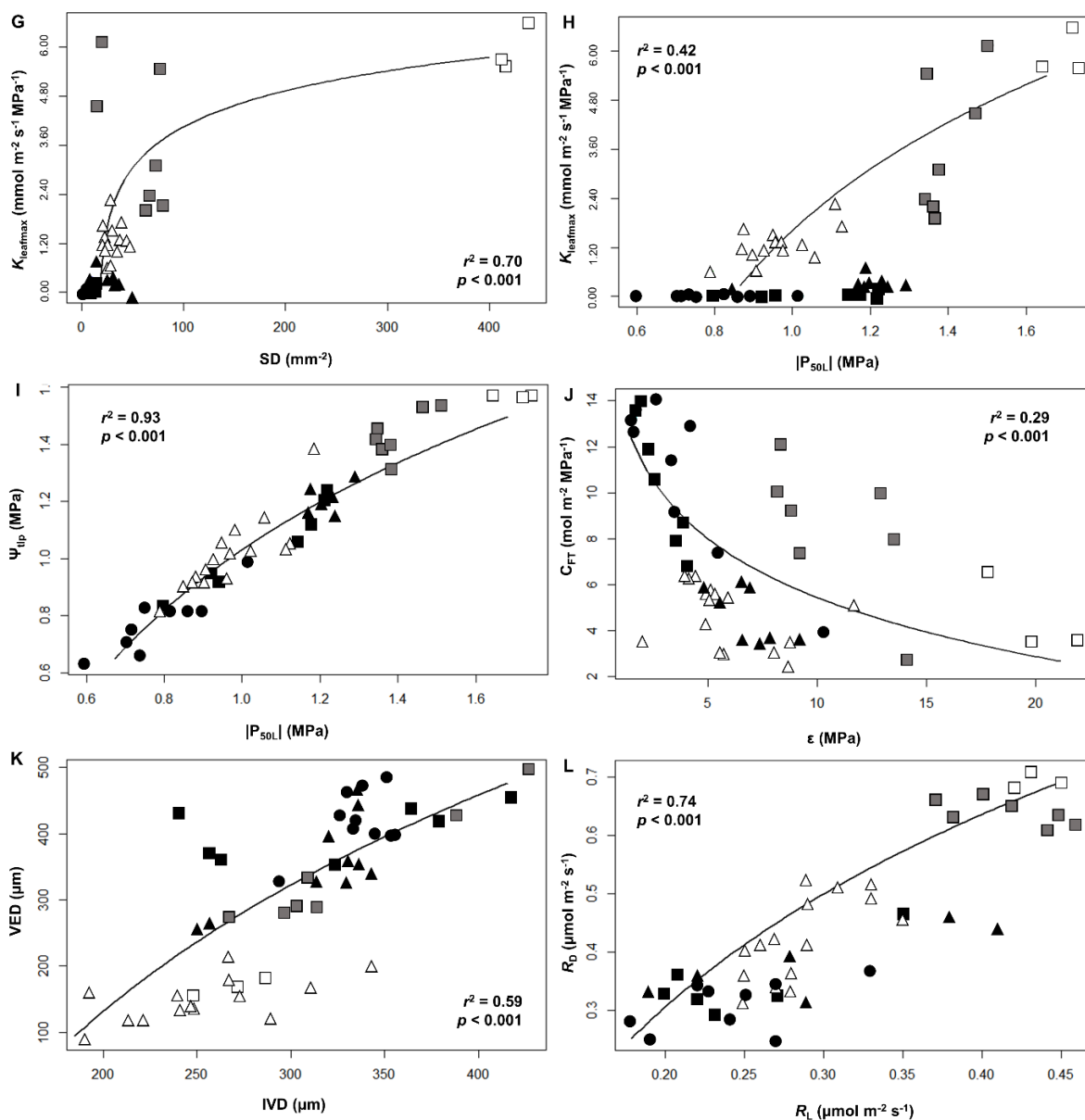


Figure 3.3.4. Leaf economic, hydraulic and anatomical trait relationships in the bromeliads showing raw data with non-linear regression lines. a) A_{\max} vs. N_{leaf} ; b) P_{leaf} vs. N_{leaf} ; c) A_{\max} vs. LMA; d) A_{\max} vs. IAS; e) A_{\max} vs. K_{leafmax} ; f) g_{smax} vs. K_{leafmax} ; g) K_{leafmax} vs. SD; h) K_{leafmax} vs. absolute P_{50L} ; i) Ψ_{tip} vs. absolute P_{50L} ; j) C_{FT} vs. ϵ ; k) VED vs. IVD; l) R_{D} vs. R_{L} . White squares: C_3 mesic terrestrials; grey squares: C_3 succulent terrestrials; black squares: CAM terrestrials; white triangles: C_3 tank-epiphytes; black triangles: CAM tank-epiphytes; black circles: CAM atmospheric epiphytes. Correlations are presented in Table 3.3.1. Trait key: A_{\max} = photosynthetic capacity; N_{leaf} = leaf nitrogen content; P_{leaf} = leaf phosphorus content; LMA = leaf mass per unit area; IAS = leaf internal air space fraction; K_{leafmax} = maximal leaf hydraulic conductance; g_{smax} = maximal stomatal conductance; SD = stomatal density; P_{50L} = leaf water potential at 50% loss hydraulic conductance; Ψ_{tip} = leaf water potential at turgor loss point; C_{FT} = leaf capacitance at full turgor; ϵ = leaf bulk elastic modulus; VED = vein-epidermis distance; IVD = interveinal distance; R_{D} = dark respiration; R_{L} = respiration in the light.

| Functional types | Trait 1 | Trait 2 | Sign of correlation | r^2 | p |
|----------------------|----------------------|---------|---------------------|---------|----------------------|
| A_{\max} | N_{leaf} | + | 0.31 | < 0.001 | A_{\max} |
| A_{\max} | P_{leaf} | + | 0.49 | < 0.001 | A_{\max} |
| A_{\max} | LMA | - | 0.10 | 0.015 | A_{\max} |
| N_{leaf} | P_{leaf} | + | 0.17 | 0.002 | N_{leaf} |
| A_{\max} | R_D | + | 0.63 | < 0.001 | A_{\max} |
| A_{\max} | R_L | + | 0.48 | < 0.001 | A_{\max} |
| R_L | R_D | + | 0.74 | < 0.001 | R_L |
| P_{leaf} | R_D | + | 0.69 | < 0.001 | P_{leaf} |
| P_{leaf} | R_L | + | 0.51 | < 0.001 | P_{leaf} |
| LMA | VED | + | 0.39 | < 0.001 | LMA |
| LMA | IAS | - | 0.56 | < 0.001 | LMA |
| VED | IAS | - | 0.63 | < 0.001 | VED |
| IVD | VED | + | 0.59 | < 0.001 | IVD |
| A_{\max} | SD | + | 0.44 | < 0.001 | A_{\max} |
| K_{leafmax} | SD | + | 0.70 | < 0.001 | K_{leafmax} |
| R_D | SD | + | 0.38 | < 0.001 | R_D |
| R_L | SD | + | 0.26 | < 0.001 | R_L |
| ϵ | SD | + | 0.62 | < 0.001 | ϵ |
| P_{50L} | SD | - | 0.42 | < 0.001 | P_{50L} |
| Ψ_{tip} | SD | - | 0.31 | < 0.001 | Ψ_{tip} |
| A_{\max} | $g_{s\max}$ | + | 0.57 | < 0.001 | A_{\max} |
| A_{\max} | IAS | + | 0.39 | < 0.001 | A_{\max} |
| A_{\max} | K_{leafmax} | + | 0.87 | < 0.001 | A_{\max} |
| K_{leafmax} | $g_{s\max}$ | + | 0.80 | < 0.001 | K_{leafmax} |
| K_{leafmax} | P_{50L} | - | 0.42 | < 0.001 | K_{leafmax} |
| P_{50L} | Ψ_{tip} | + | 0.93 | < 0.001 | P_{50L} |
| C_{FT} | ϵ | - | 0.29 | < 0.001 | C_{FT} |

Table 3.3.1. Significant correlations between species-mean leaf trait values in different functional groups of bromeliad species. See trait key in caption to Figure 3.3.4 above.

Species-specific absolute values of K_{leaf} and g_s at $\Psi_{\text{leaf}} = P_{50S}$ and $\Psi_{\text{leaf}} = P_{50L}$ predicted by curve-fitting were compared. When values were log-transformed, there was a statistically significant positive relationship between g_s and K_{leaf} at P_{50S} ($r^2 = 0.85$, $p < 0.001$; Fig. 3.3.5a) and at P_{50L} ($r^2 = 0.60$, $p < 0.001$; Fig. 3.3.5b), with the highest values occurring among C_3 terrestrials, followed by C_3 tank-epiphytes. Those species able to maintain higher absolute K_{leaf} at more negative Ψ_{leaf} could therefore support higher absolute g_s . A robust positive correlation between log-transformed values of P_{50L} and the absolute value of K_{leaf} at P_{50L} ($r^2 = 0.42$, $p < 0.001$; Fig. 3.3.5c) suggests that species that are more resistant to dehydration maintain higher absolute as well as proportional hydraulic conductance at more negative Ψ_{leaf} than less resistant species. Furthermore, across all species, there was a strong negative relationship between K_{leafmax} and P_{50L} ($r^2 = 0.52$, $p < 0.001$; Fig. 3.3.5d), apparently contradicting the hydraulic safety-efficiency hypothesis.

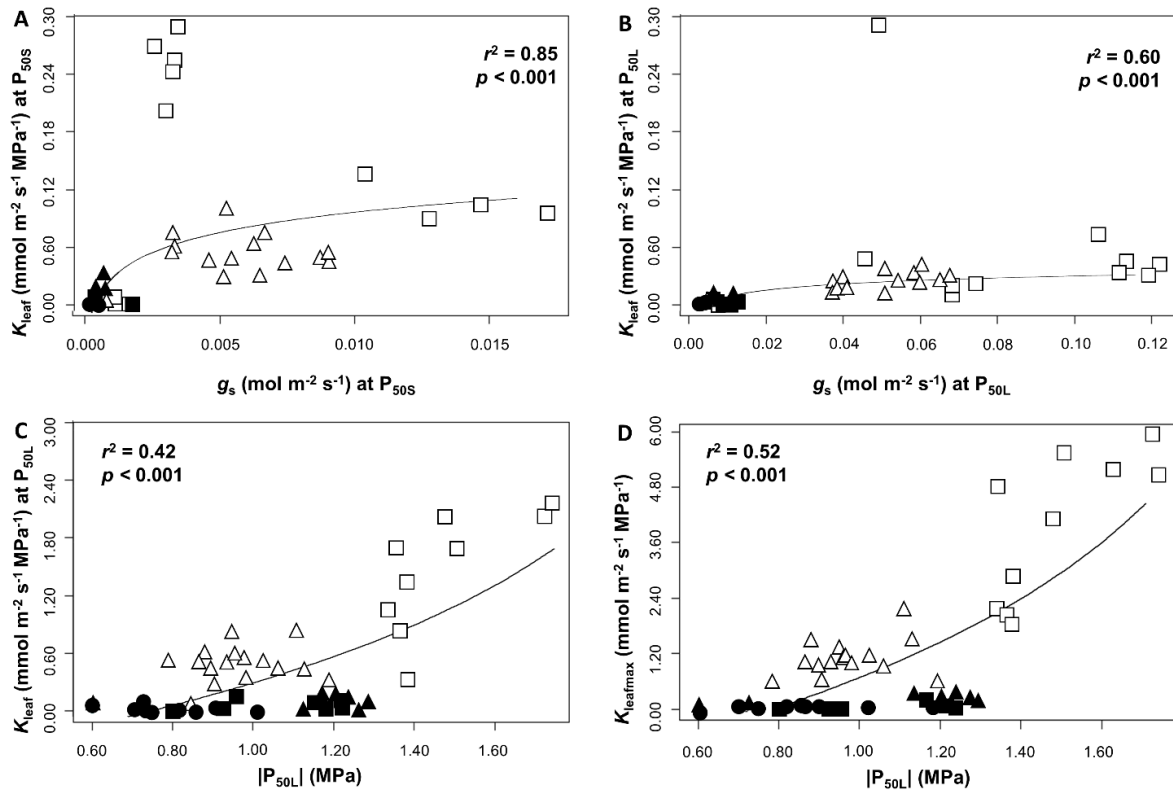


Figure 3.3.5. a) Relationship between absolute values of leaf hydraulic conductance (K_{leaf}) and stomatal conductance (g_s) at the leaf water potential at 50% loss hydraulic conductance (P_{50L}); b) relationship between absolute values of K_{leaf} and g_s at the leaf water potential at 50% stomatal closure (P_{50S}); c) relationship between absolute P_{50L} and absolute value of K_{leaf} at P_{50L} ; d) relationship between maximal leaf hydraulic conductance (K_{leafmax}) and P_{50L} . Main plots show values calculated from K_{leaf} -leaf water potential (Ψ_{leaf}) and g_s - Ψ_{leaf} curve-fitting, insets show log-transformed values with linear regression lines. Open squares: C_3 terrestrials; filled squares: CAM terrestrials; open triangles: C_3 tank-epiphytes; filled triangles: CAM tank-epiphytes; filled circles: CAM atmospheric epiphytes.

A_{max} and K_{leafmax} were decoupled in CAM atmospheric epiphytes, and A_{max} , g_{smax} and IAS were decoupled in CAM terrestrials and atmospheric epiphytes (see **Appendix 3.4** for non-significant relationships described here). Interestingly, K_{leafmax} was not correlated with IVD ($r^2 = -0.02$, $p = 0.693$), implying either that interspecific differences are related to xylem structural properties that were not measured, or that extra-xylary factors are more important in determining overall leaf hydraulic conductance. The strong positive correlation between P_{50L} and Ψ_{tip} (Table 3.3.1) is suggestive of the importance of extra-xylary vulnerability to embolism.

Some correlations were apparent or considerably stronger in specific functional types. C_{FT} and ϵ were negatively correlated in all CAM functional types epiphytes, suggestive of the importance of

cell wall flexibility for the recharge of succulent tissues in all of these groups. The absence of this relationship in C₃ functional types could relate to structural differences and a reduced reliance on internal hydraulic capacitance.

3.3.3 *Principal components analysis*

Log-transformed data for all continuous variables were used to perform principal component analyses (PCA) for the core species set ($n = 50$ spp.). Full results are available in **Appendix 3.5**. For the core leaf economic traits, loadings displayed approximately the expected directionality (Fig. 3.3.6a). The first two principal components explained 48.3% and 18.7% of the variance respectively. The eigenvectors of traits related to vascular geometry, tissue density and water storage showed strong alignment, while a second, looser grouping of trait eigenvectors included most gas exchange, hydraulic and nutrient-related traits. The presence of these two axes implies a certain degree of independence in variation in some structural and functional traits. The eigenvector for P₅₀₅ did not align with that of any other trait. Functional groups segregated quite clearly in the morphospace defined by the first two principal components (Fig. 3.3.6b), showing that there is a clear physiological and anatomical basis for the differentiation of ecophysiological functional types in the Bromeliaceae. All C₃ terrestrials clustered towards the high-productivity end of the functional axis, with mesic and succulent functional types segregating along the structural axis. Meanwhile C₃ tank-epiphytes were distinctively positioned towards the end of the structural axis of variation defined by low tissue density and high PNUE. C₃ functional types tended to show greater variation along the axis associated with leaf structure, whereas the CAM functional types varied primarily along the axis associated with physiological functions.

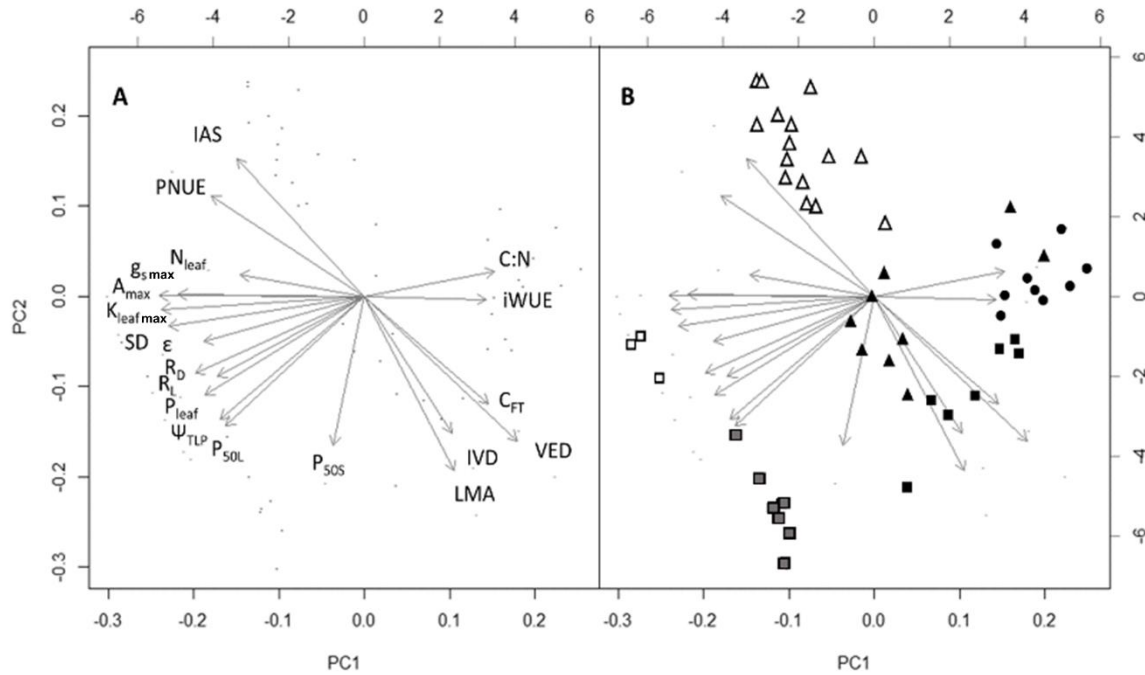


Figure 3.3.6. Biplots of first two principal components in an analysis of variation in 20 continuous traits across 50 bromeliad species showing a) trait loadings and b) species loadings plotted by functional types. White squares: C_3 mesic terrestrials; grey squares: C_3 succulent terrestrials; black squares: CAM terrestrials; white triangles: C_3 tank-epiphytes; black triangles: CAM tank-epiphytes; black circles: CAM atmospheric epiphytes. Trait key: IAS = internal air space fraction; PNUE = photosynthetic nitrogen use efficiency; N_{leaf} = leaf nitrogen content; $g_{s\text{max}}$ = maximal stomatal conductance; A_{max} = photosynthetic capacity; K_{leafmax} = maximal leaf hydraulic conductance; SD = stomatal density, ϵ = leaf bulk elastic modulus; R_D = dark respiration; R_L = respiration in the light; P_{leaf} = leaf phosphorus content; Ψ_{tlp} = leaf water potential at turgor loss point; P_{50L} = leaf water potential at 50% loss hydraulic conductance; P_{50S} = leaf water potential at 50% stomatal closure; LMA = leaf mass per unit area; IVD = interveinal distance; VED = vein-epidermis distance; C_{FT} = leaf capacitance at full turgor, iWUE = instantaneous water-use efficiency; C: N = ratio of leaf carbon to nitrogen content.

3.3.4 Comparison of trait values between functional types

Values of all traits differed significantly between all functional types, but more structured insights were obtained by considering the differences in trait values between species of contrasting functional types. Mean values of functional traits by functional type are displayed in Table 3.3.2.

| Trait | C ₃ terrestrials (n = 10) | CAM terrestrials (n = 7) | C ₃ tank- epiphytes (n = 14) | CAM tank- epiphytes (n = 10) | CAM atmospheric epiphytes (n = 9) |
|--|---|--------------------------------|---|------------------------------------|--|
| A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 4.84 ± 0.36 | 1.47 ± 0.30 | 3.71 ± 0.18 | 2.19 ± 0.27 | 0.86 ± 0.05 |
| N_{leaf} (%) | 1.14 ± 0.10 | 0.80 ± 0.07 | 1.10 ± 0.09 | 0.83 ± 0.09 | 0.83 ± 0.04 |
| PNUE ($A_{\text{max}}/N_{\text{leaf}}$) | 2.66 ± 0.74 | 0.58 ± 0.09 | 5.91 ± 0.92 | 3.45 ± 0.98 | 0.63 ± 0.04 |
| LMA (g m^{-2}) | 236.34 ± 32.39 | 302.96 ± 20.95 | 75.86 ± 8.36 | 151.52 ± 18.75 | 173.20 ± 10.60 |
| g_{smax} ($\text{mol m}^{-2} \text{s}^{-1}$) | 0.175 ± 0.019 | 0.018 ± 0.002 | 0.099 ± 0.008 | 0.012 ± 0.001 | 0.007 ± 0.001 |
| iWUE (A_{max}/g_s) | 32.04 ± 4.75 | 85.11 ± 21.13 | 40.89 ± 3.44 | 200.18 ± 27.55 | 130.79 ± 11.69 |
| P_{leaf} (%) | 0.021 ± 0.001 | 0.012 ± 0.001 | 0.011 ± 0.001 | 0.010 ± 0.001 | 0.004 ± 0.001 |
| C: N | 35.23 ± 3.83 | 56.28 ± 7.39 | 44.06 ± 3.77 | 56.51 ± 5.95 | 53.65 ± 3.95 |
| R_L ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 0.42 ± 0.01 | 0.25 ± 0.02 | 0.28 ± 0.01 | 0.28 ± 0.02 | 0.24 ± 0.02 |
| R_D ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 0.66 ± 0.01 | 0.35 ± 0.02 | 0.42 ± 0.02 | 0.37 ± 0.02 | 0.31 ± 0.02 |
| δ¹³C (‰) | -26.13 ± 0.78 | -15.07 ± 0.57 | -25.62 ± 0.41 | -16.19 ± 0.75 | -15.18 ± 0.27 |
| VED (μm) | 289.08 ± 34.61 | 404.21 ± 15.49 | 154.74 ± 11.45 | 362.50 ± 19.72 | 421.39 ± 16.27 |
| IVD (μm) | 311.06 ± 17.45 | 320.86 ± 26.14 | 253.09 ± 11.09 | 322.71 ± 8.36 | 336.63 ± 6.41 |
| K_{leafmax} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) | 3.64 ± 0.47 | 0.05 ± 0.02 | 0.98 ± 0.10 | 0.18 ± 0.03 | 0.02 ± 0.002 |
| Ψ_{tip} (MPa) | -1.47 ± 0.03 | -1.07 ± 0.06 | -1.01 ± 0.04 | -1.07 ± 0.08 | -0.86 ± 0.04 |
| E (MPa) | 13.45 ± 1.58 | 2.86 ± 0.35 | 6.35 ± 0.65 | 5.96 ± 0.44 | 3.86 ± 0.93 |
| C_{FT} ($\text{mol m}^{-2} \text{MPa}^{-1}$) | 7.32 ± 1.01 | 10.50 ± 1.06 | 4.30 ± 0.34 | 5.09 ± 0.39 | 10.90 ± 1.13 |
| P_{50s} (MPa) | -0.47 ± 0.01 | -0.47 ± 0.02 | -0.36 ± 0.01 | -0.49 ± 0.04 | -0.37 ± 0.006 |
| P_{50L} (MPa) | -1.49 ± 0.05 | -1.07 ± 0.07 | -0.98 ± 0.03 | -1.10 ± 0.08 | -0.87 ± 0.05 |
| SD (mm^{-2}) | 165.74 ± 56.46 | 11.24 ± 1.34 | 30.15 ± 2.52 | 23.40 ± 4.15 | 3.75 ± 0.16 |
| IAS (%) | 8.47 ± 1.64 | 6.03 ± 0.36 | 13.08 ± 0.85 | 8.63 ± 0.67 | 6.02 ± 0.33 |

Table 3.3.2. Mean values (± standard error of the mean) for leaf traits by functional type. See trait key in caption to Fig. 3.3.6 above.

Relative to C₃ terrestrials, CAM terrestrials showed significantly lower A_{max} ($F = 45.40$, $p < 0.001$), g_{smax} ($F = 48.30$, $p < 0.001$), K_{leafmax} ($F = 40.12$, $p < 0.001$), SD ($F = 5.14$, $p = 0.039$), R_L ($F = 78.35$, $p < 0.001$), R_D ($F = 215.50$, $p < 0.001$), N_{leaf} ($F = 6.08$, $p = 0.026$), P_{leaf} ($F = 52.80$, $p < 0.001$), $PNUE$ ($F = 5.37$,

$p = 0.035$), ϵ ($F = 30.01$, $p < 0.001$), less negative P_{50L} ($F = 26.23$, $p < 0.001$), Ψ_{tip} ($F = 40.81$, $p < 0.001$), and higher C: N ($F = 7.57$, $p = 0.015$), iWUE ($F = 8.37$, $p = 0.011$), and VED ($F = 6.95$, $p = 0.019$). These differences are generally reflective of slower-growth strategies and more conservative water use, consistent with the bioclimatic relations of C_3 and CAM bromeliads.

C_3 tank-epiphytism, relative to the C_3 terrestrial habit, was associated with reduced A_{max} ($F = 5.05$, $p = 0.016$), g_{smax} ($F = 9.59$, $p = 0.001$), $K_{leafmax}$ ($F = 29.52$, $p < 0.001$), SD ($F = 4.88$, $p = 0.018$), R_L ($F = 40.49$, $p < 0.001$), and R_D ($F = 41.26$, $p < 0.001$). C_3 tank-epiphytes also showed significantly lower C_{FT} ($F = 5.88$, $p = 0.009$), suggesting that investment in external capacitance (the tank) reduces the requirement for internal capacitance (in succulent water-storage tissue). However, less negative values of P_{50L} ($F = 47.07$, $p < 0.001$), P_{50S} ($F = 18.70$, $p < 0.001$) and Ψ_{tip} ($F = 44.82$, $p < 0.001$) also occur in C_3 tank-epiphytes. Lower LMA ($F = 17.99$, $p < 0.001$), P_{leaf} ($F = 48.58$, $p < 0.001$) and VED ($F = 8.96$, $p = 0.001$) in C_3 tank-epiphytes may reflect adaptation to resource limitation and maximisation of canopy area under these conditions. Higher IAS ($F = 3.94$, $p = 0.035$) could also reduce construction costs, as well as providing ventilation to submerged tissues.

CAM tank-epiphytes in the Bromelioideae subfamily showed similar differences from terrestrial CAM species, including reduced C_{FT} ($F = 27.20$, $p < 0.001$), g_{smax} ($F = 10.94$, $p = 0.005$), LMA ($F = 27.16$, $p < 0.001$), and increased IAS ($F = 8.92$, $p = 0.010$) and PNUE ($F = 5.82$, $p = 0.030$). However, they displayed higher $K_{leafmax}$ ($F = 14.12$, $p = 0.002$), SD ($F = 5.60$, $p = 0.033$) and iWUE ($F = 9.18$, $p = 0.009$) than their terrestrial counterparts, suggesting that the combination of tank and absorptive trichomes may facilitate enhanced productivity. Unlike in the case of the C_3 lineages, IVD, P_{leaf} , P_{50L} , P_{50S} , R_D , R_L and Ψ_{tip} did not differ significantly between CAM terrestrials and CAM tank-epiphytes ($p > 0.05$). This reflects the fact that terrestrial CAM species already engage in highly conservative water-use strategies, which may have made the epiphytic habit easier to evolve.

Relative to the C_3 tank-epiphytes of the Tillandsioideae from which they evolved, CAM atmospheric epiphytes showed significantly lower A_{max} ($F = 146.80$, $p < 0.001$), $K_{leafmax}$ ($F = 61.84$, $p < 0.001$), g_{smax} ($F = 78.78$, $p < 0.001$), SD ($F = 69.35$, $p < 0.001$) and iWUE ($F = 82.21$, $p < 0.001$). These reductions, in combination with lower R_L ($F = 5.08$, $p = 0.035$) and R_D ($F = 14.30$, $p = 0.001$), are associated with lower productivity compared with C_3 tank-epiphytes. Less negative Ψ_{tip} ($F = 8.15$, $p = 0.009$) corresponds to an increased investment in drought resistance over drought tolerance. In terms of leaf structure, lower IAS ($F = 40.92$, $p < 0.001$) and higher C_{FT} ($F = 47.09$, $p < 0.001$) in atmospheric epiphytes are the result of dense tissue packing. Reduced N_{leaf} ($F = 5.16$, $p = 0.033$) and P_{leaf} ($F = 46.88$, $p < 0.001$) might reflect the adaptation of atmospheric epiphytes to low mineral nutrient supply.

3.3.5 Drought resistance trait survey

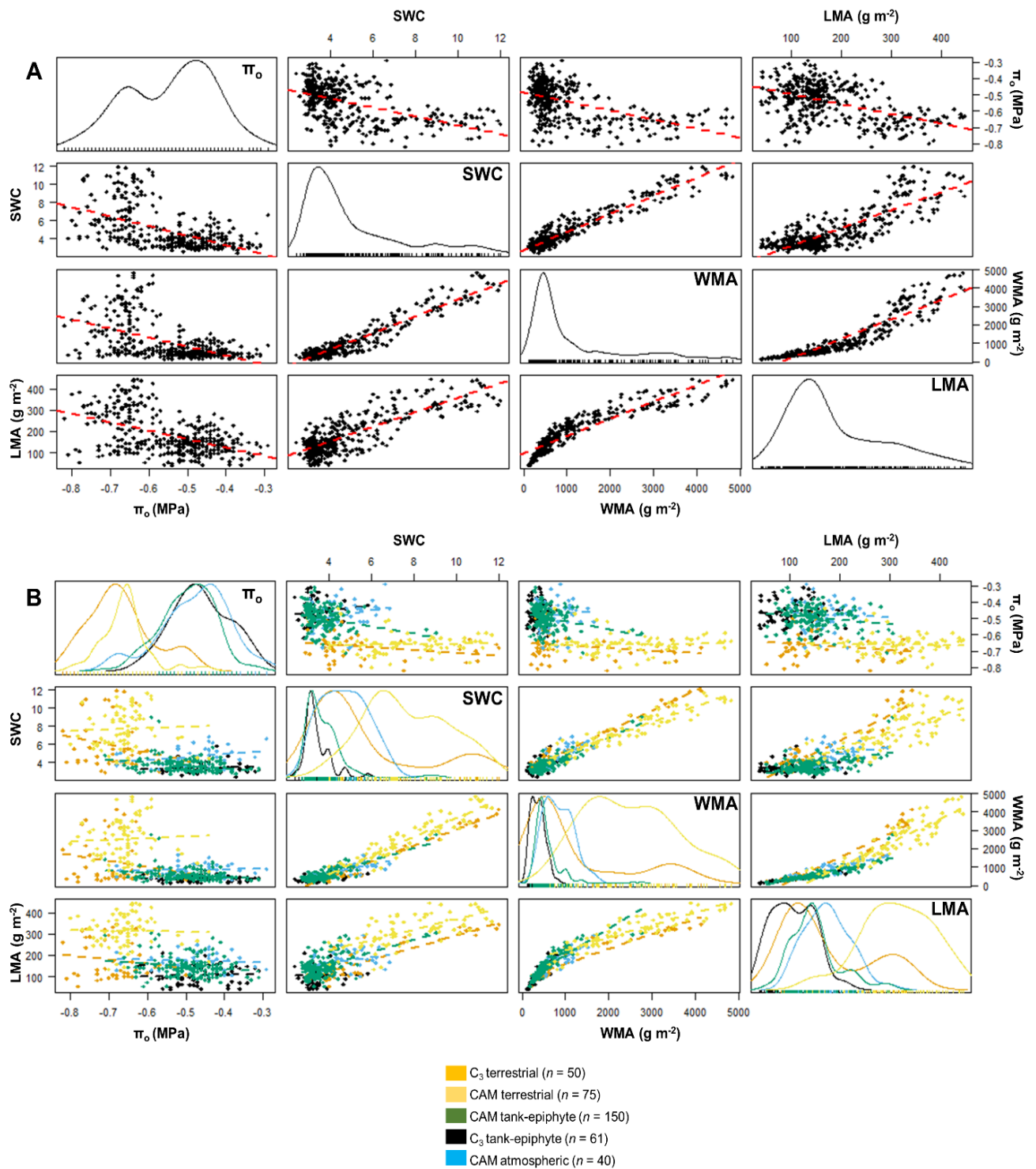


Figure 3.3.7. Variation in leaf drought resistance traits (osmotic potential at full turgor, π_o ; saturated water content, SWC; water mass per unit area, WMA; and leaf mass per unit area, LMA). a) Results for all species ($n = 376$). Central diagonal panels show density plots for each trait; red dashed 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.

The variation in drought resistance traits for all 376 species is displayed in density plots and bivariate plots in Fig. 3.3.7a-b. Values of π_0 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 (C₃ 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 (C₃ tank-epiphyte) to 11.98 in *Puya humilis* Mez (C₃ terrestrial), while WMA values ranged between 117.72 g m⁻² in *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 *Guzmania monostachia* (C₃-CAM tank-epiphyte) and 446.94 g m⁻² in *Neoglaziovia variegata* (Arruda) Mez (CAM terrestrial). The complete dataset is available in **Appendix 3.3**.

3.3.6 Relationships between drought resistance traits

| | SWC | WMA (g m ⁻²) | LMA (g m ⁻²) |
|--------------------------|----------------------------|----------------------------------|----------------------------|
| π_0 (MPa) | -, $r^2 = 0.29, p < 0.001$ | -, $r^2 = 0.27, p < 0.001$ | -, $r^2 = 0.25, p < 0.001$ |
| LMA (g m ⁻²) | +, $r^2 = 0.67, p < 0.001$ | +, $r^2 = 0.90, p < 0.001$ (log) | - |
| WMA (g m ⁻²) | +, $r^2 = 0.91, p < 0.001$ | - | - |

Table 3.3.3. Correlations between drought-resistance traits across 376 bromeliad species, based on linear regression except where stated.

Pairwise relationships between the four drought resistance traits are displayed in Table 3.3.3. 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_0 and SWC and between π_0 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_0 and LMA. LMA and SWC showed a very strong positive correlation, consistent with the possibility 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 lifespan in the harsh, often oligotrophic environments to which many of these species are native (Benzing, 2000; Wright et al., 2002, 2004; Poorter et al., 2009). It is notable 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.

Inspection of the bivariate plots with linear regression fitted by functional type (Fig. 3.3.7b) 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_0 , suggesting that interspecific variation in this trait within functional types may be decoupled from variation in the remaining three drought resistance traits.

3.3.7 Variation in drought resistance traits between functional types

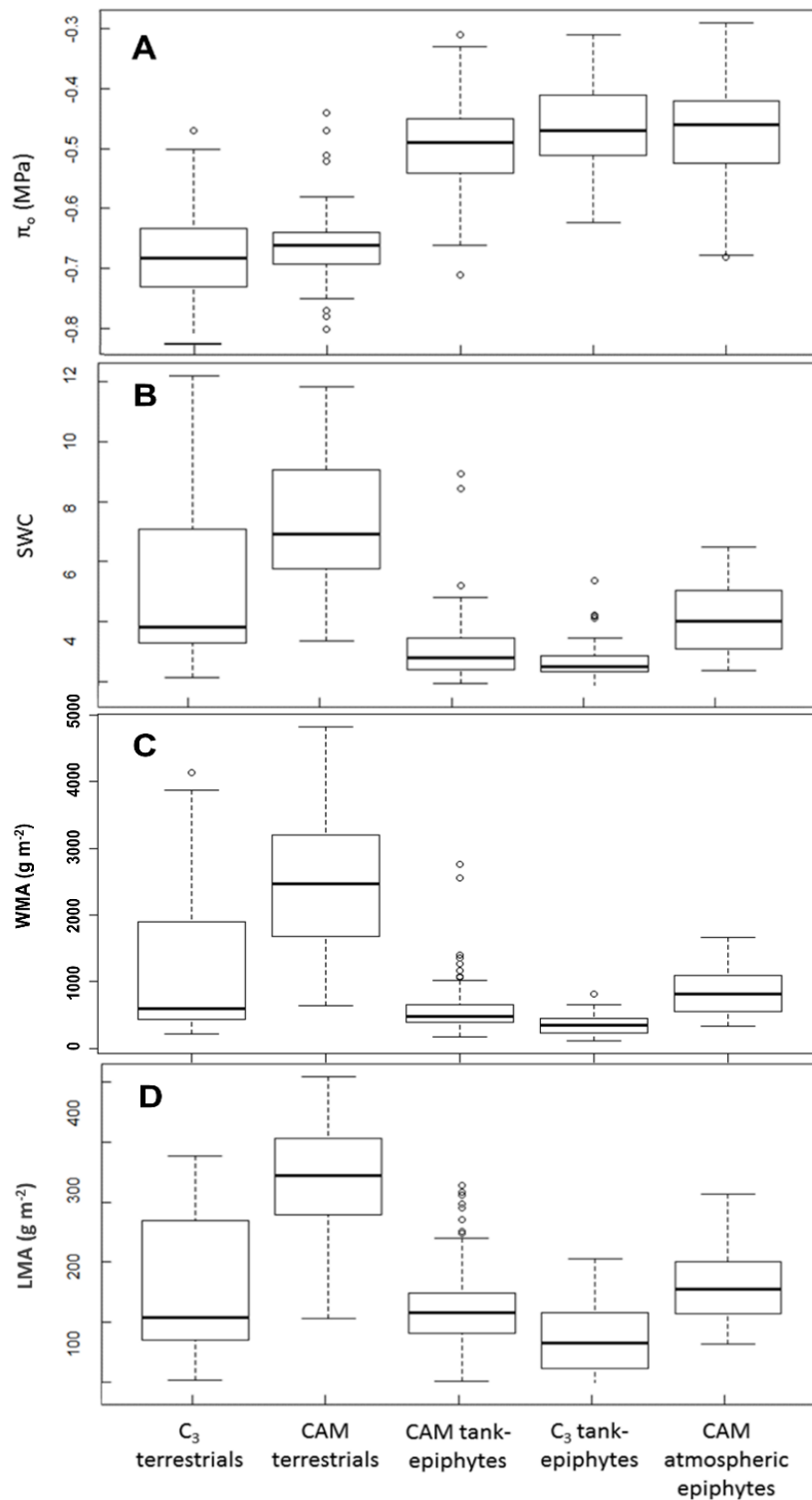


Figure 3.3.8. Drought resistance traits by functional type: a) osmotic potential at full turgor, π_0 ; b) saturated water content, SWC; c) water mass per unit area, WMA; d) leaf mass per unit area, LMA. Sample sizes: C₃ terrestrials, $n = 50$; CAM terrestrials, $n = 75$; CAM tank-epiphytes, $n = 150$; C₃ tank-epiphytes, $n = 61$; CAM atmospheric epiphytes, $n = 40$. Boxes show median values and interquartile range (IQR), with whiskers of length (1.5 x IQR) and outliers plotted as individual points.

All four drought resistance traits differed significantly between functional types (Fig. 3.3.8). Epiphytic functional types showed less negative values of π_0 (Fig. 3.3.8a), while CAM terrestrials were most distinctive in terms of SWC (Fig. 3.3.8b), WMA (Fig. 3.3.8c), and LMA (Fig. 3.3.8d), with the highest median values. Variation within functional types was particularly high for π_0 in the CAM atmospheric epiphytes and for LMA, SWC and WMA in the C_3 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 C_3 terrestrials, CAM terrestrials did not differ in π_0 ($F = 1.06$, $p = 0.306$), but showed higher SWC ($F = 23.20$, $p < 0.001$), WMA ($F = 40.50$, $p < 0.001$), and LMA ($F = 86.49$, $p < 0.001$). 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_0 than CAM terrestrials ($F = 82.09$, $p < 0.001$), as well as lower SWC ($F = 146.70$, $p < 0.001$), WMA ($F = 412.20$, $p < 0.001$), and LMA ($F = 136.40$, $p < 0.001$). C_3 tank-epiphytes also displayed significantly less negative π_0 than C_3 terrestrials ($F = 45.64$, $p < 0.001$), as well as lower SWC ($F = 17.58$, $p < 0.001$), WMA ($F = 29.70$, $p < 0.001$), and LMA ($F = 16.16$, $p < 0.001$). Tank-epiphytism in both C_3 and CAM lineages therefore appeared to be associated with high π_0 and low SWC, WMA and LMA, consistent with our hypothesis that tank-based external capacitance 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 ($F = 0.094$, $p = 0.759$), but, as we had hypothesised, the atmospheric displayed significantly higher SWC ($F = 51.99$, $p < 0.001$) and WMA ($F = 91.78$, $p < 0.001$) as well as LMA ($F = 60.98$, $p < 0.001$). 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.

3.3.8 Relationships between drought resistance traits and bioclimatic factors

| Bioclimatic variable | π_0 (MPa) | SWC | LMA (g m^{-2}) |
|----------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| AI (mm mm^{-1}) | + , $r^2 = 0.26$, $p < 0.001$ | - , $r^2 = 0.20$, $p < 0.01$ | - , $r^2 = 0.18$, $p < 0.01$ |
| P_{seas} (%) | - , $r^2 = 0.13$, $p < 0.001$ | + , $r^2 = 0.20$, $p < 0.001$ | + , $r^2 = 0.12$, $p < 0.001$ |

Table 3.3.4. Correlation matrix for bioclimatic variables and drought tolerance traits for 308 bromeliads species. Relationships with $r^2 \geq 0.15$ are presented in bold typeface. AI = aridity index; P_{seas} = precipitation seasonality; π_0 = osmotic potential at full turgor; SWC = saturated water content; LMA = leaf mass per unit area.

The relationships between measured drought resistance traits and bioclimatic occupancy (AI and P_{seas}) are presented in Table 3.3.4 and illustrated for the whole species set for which bioclimatic data were available ($n = 308$) and individual functional types in Fig. 3.3.9. Relationships between variables with $r^2 \geq 0.15$ were considered relatively strong (Poorter et al., 2014). Across all species, there was a positive relationship between π_o 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_o . There was a weaker negative relationship between π_o 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, $r^2 = 0.23$, $p < 0.001$), WMA (log, $r^2 = 0.22$, $p < 0.001$), and LMA (linear, $r^2 = 0.18$, $p < 0.001$), 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 ($r^2 = 0.20$, $p < 0.001$) and WMA ($r^2 = 0.17$, $p < 0.001$) than with LMA ($r^2 = 0.12$, $p < 0.001$), suggesting that higher capacity for internal water-storage becomes more advantageous when water availability is concentrated in limited temporal windows.

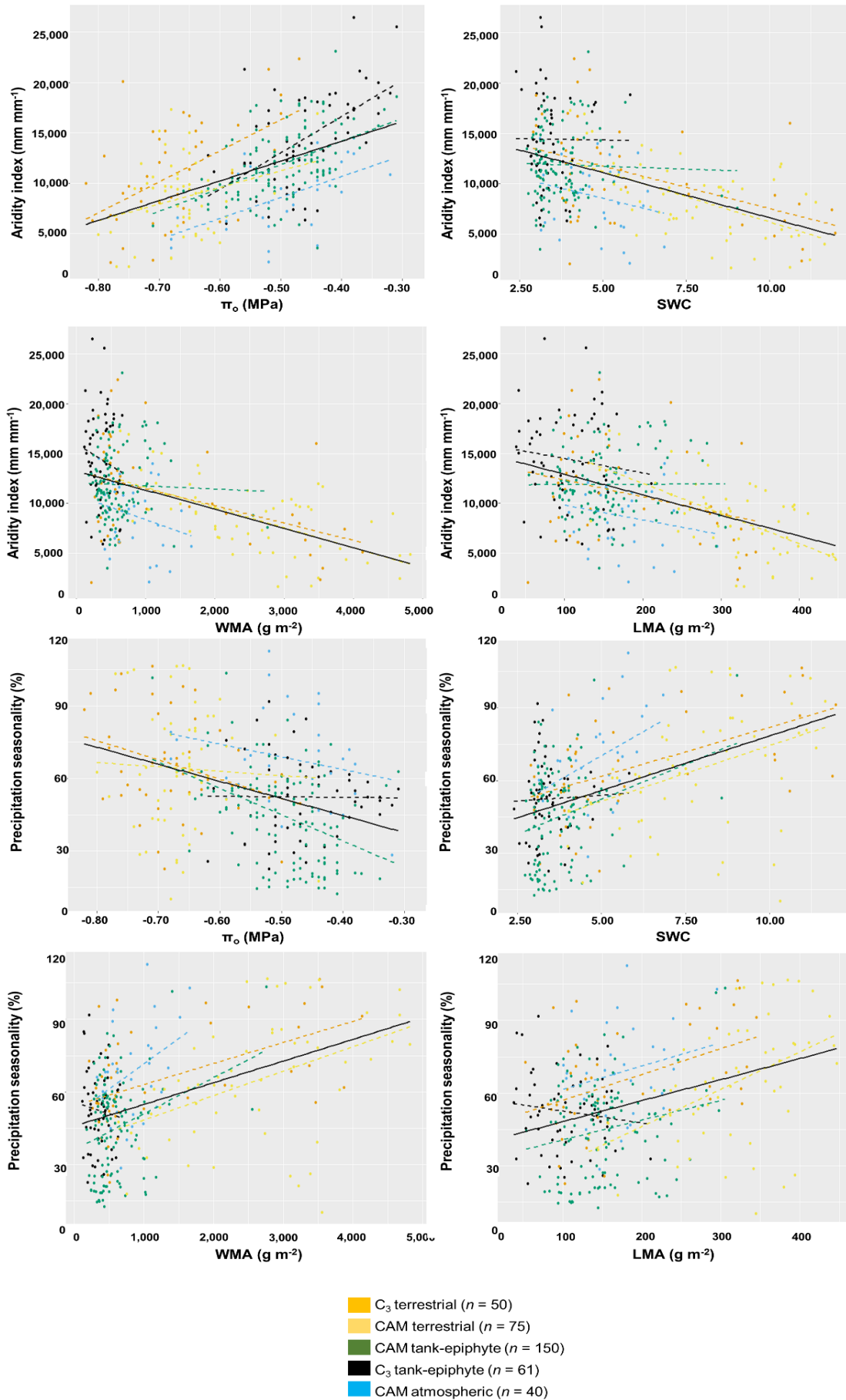


Figure 3.3.9 (overleaf). a-h) Relationships between species' mean values for drought resistance traits (osmotic potential at full turgor, π_0 ; saturated water content, SWC; water mass per unit area, WMA; leaf mass per unit area, LMA) and bioclimatic factors (aridity index and precipitation seasonality) across the full species set ($n = 376$). Dashed lines show linear regression by group; solid line shows linear regression across all species.

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. 3.3.10a-d). Across all species, aridity index scores showed a strong negative correlation with PC1 scores ($r^2 = 0.26$, $p < 0.001$; Fig. 3.3.10a), but only a very weak positive correlation with PC2 scores ($r^2 = 0.06$, $p < 0.001$; Fig. 3.3.10b). Similarly, precipitation seasonality scores displayed a positive correlation with PC1 scores ($r^2 = 0.18$, $p < 0.001$; Fig. 3.3.10c), and a statistically significant but extremely weak correlation with PC2 scores ($r^2 = 0.01$, $p = 0.045$; Fig. 3.3.10d). Overall, species' positions along the PC1 axis therefore explained an important proportion of variation in bioclimatic affinities.

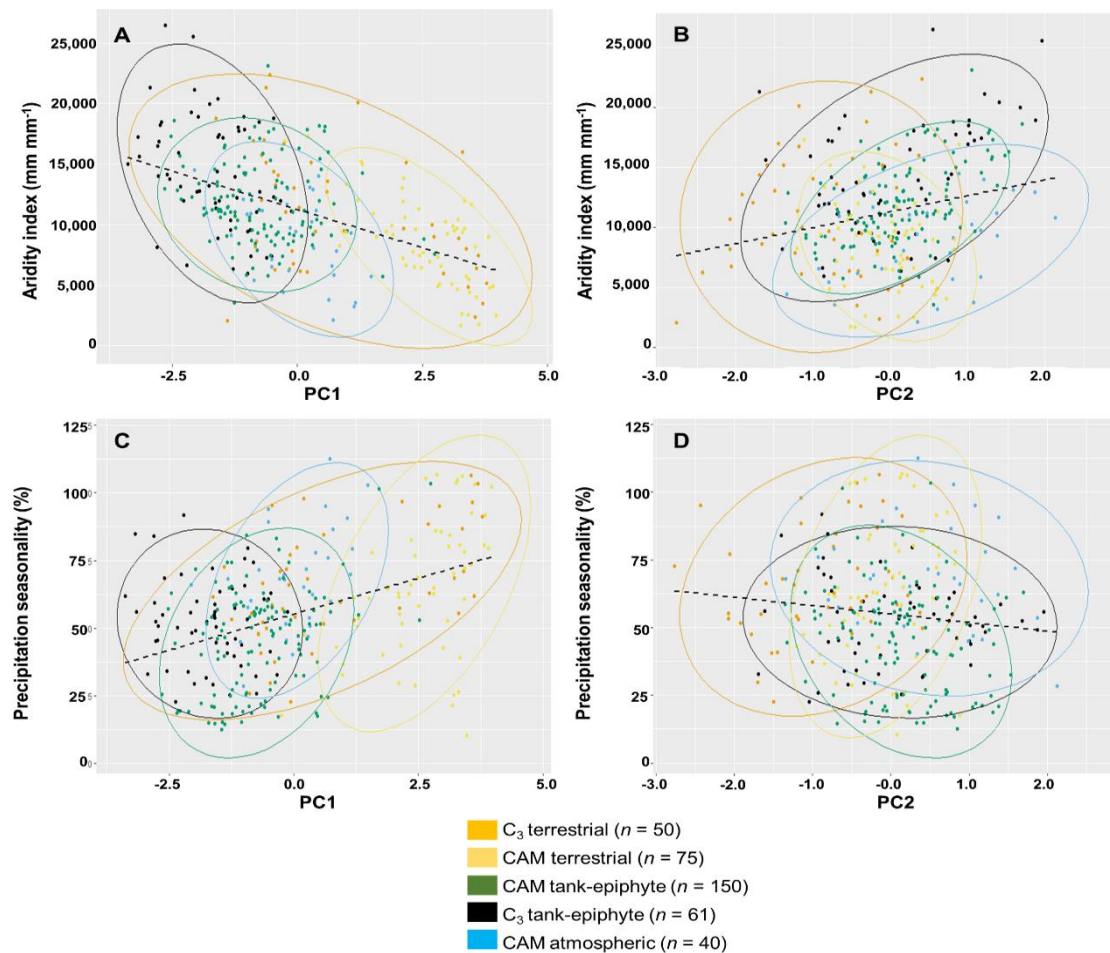


Figure 3.3.10. a-d) Relationships between species' principal component scores (PC1 and PC2) and bioclimatic scores (aridity index and precipitation seasonality). Species are plotted by functional type, with black dashed lines showing linear regression across all species ($n = 376$).

3.4 Discussion

3.4.1 Overview

While extensive consideration has previously been given to the relationships between key innovations, functional types and species diversification (Givnish et al., 2014; Silvestro et al., 2014), the interactions between trait-network architecture and functional type differentiation in the Bromeliaceae have remained little explored until now. This is despite the fact that leaf trait values are readily measured, and that trait variation has presumably been important in promoting ecological divergences both within and between functional types. The data presented here provide crucial insights into the nature of trait divergences between functional types, and place variation among bromeliad species into a wider context by positioning them on the leaf economic spectrum. They also shed light on critical general questions in contemporary leaf hydraulics, and represent a

significant contribution towards ameliorating the underrepresentation of herbaceous species in trait-based ecophysiology.

3.4.2 *Functional trait divergence and ecological diversity in the bromeliads*

The ecological differentiation of bromeliads into distinctive functional types was critical to their successful radiation into numerous highly stressful habitats (Benzing, 2000). Until now, the variation in functional traits underpinning this differentiation had never been systematically investigated. The multi-stranded investigation presented here demonstrates that the ecological divergence of functional types has a complex basis in anatomical and physiological trait change and trait-network rewiring (Fig. 3.4.1). In the analysis of core leaf trait variation, structural and functional traits aligned on orthogonal axes, suggesting that while individual pairs of structural and functional traits may be mechanistically linked and evolutionarily coordinated, there is considerable flexibility in the combination of functionalities in different structural backgrounds. Moreover, functional types segregated in the multivariate trait space almost completely, suggesting that differences in the traits measured here can account for a large proportion of the ecophysiological distinctiveness of each functional type.

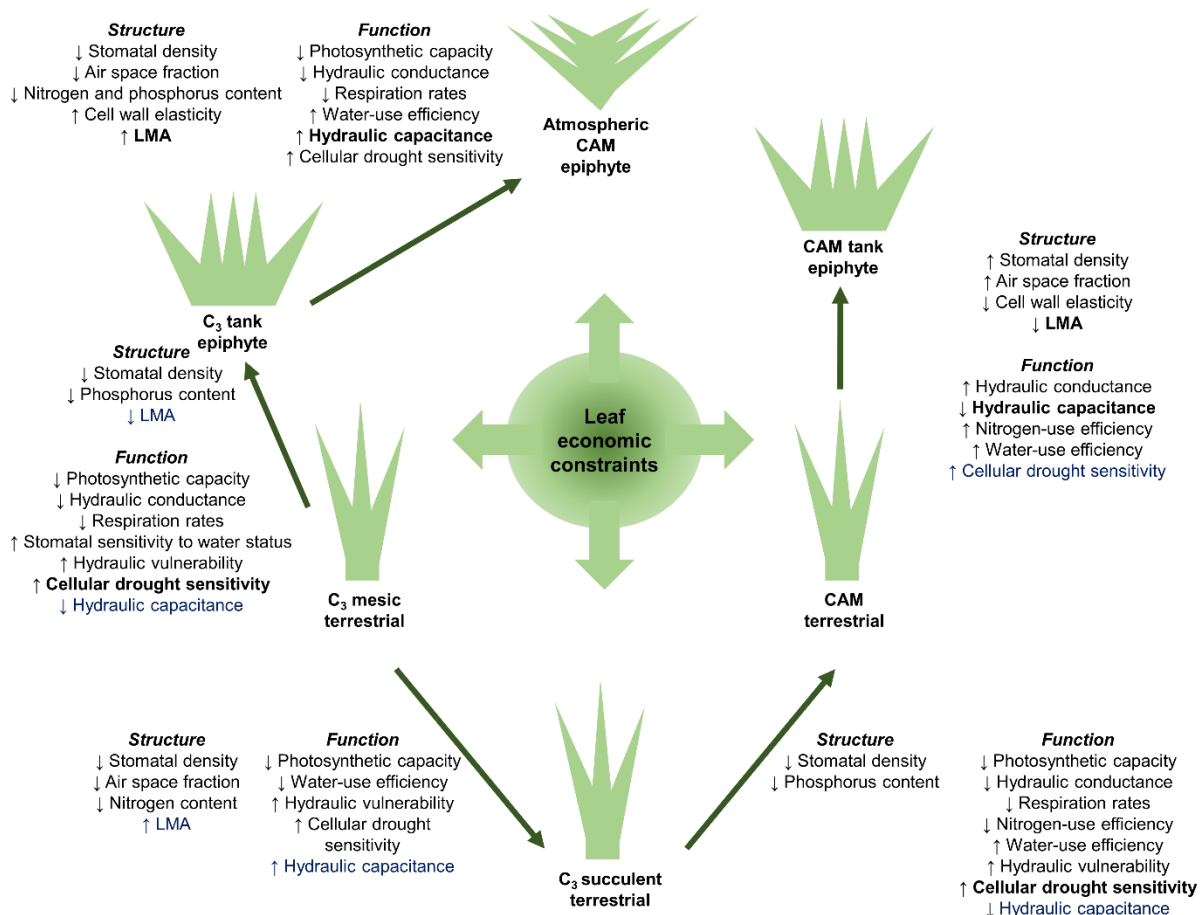


Figure 3.4.1. Summary of divergences in ecophysiological traits associated with transitions between functional types. Bold typeface indicates changes corroborated by data from both core leaf trait and drought resistance trait surveys; blue typeface indicates changes suggested by drought resistance trait survey only. Directionality of transitions between functional types based on Givnish et al. (2014).

The most basic condition in the Bromeliaceae is the combination of the C₃ photosynthetic pathway with a relatively mesic terrestrial habit (Benzing, 2000; Givnish et al., 2011, 2014), although there is considerable trait variation and climate relations in this functional type, often associated with differences in leaf morphology (Males, 2017). C₃ mesic terrestrials show comparatively high levels of physiological activity and low-cost leaves which enables them to exploit long- or short-term periods of high water availability. This relatively high growth potential is presumably more critical for terrestrial bromeliads occupying mesic sites than for xerophytes and epiphytes, which are likely to encounter considerably less interspecific competition and can therefore afford to be slower-growing but more stress-tolerant (Benzing, 2000). Pronounced leaf-succulence has evolved in some C₃ terrestrial lineages, entailing much structural and functional reorganisation. The increase in internal hydraulic capacitance associated with this transition simultaneously enhanced drought avoidance

and relaxed selection for drought tolerance, facilitating the invasion of habitats with lower water availability and higher precipitation seasonality. However, it also involved an increase in LMA and leaf size, and therefore presumably higher leaf construction costs and slower growth.

Perhaps building on pre-existing cell-level succulence (Heyduk et al., 2016), CAM has evolved convergently in multiple bromeliad lineages (Crayn et al., 2015). In the dataset presented here, the differences between C₃ succulent and CAM terrestrial species in terms of carbon and water relations were clear, and suggest that CAM can act as a downward gear-change in physiological productivity that can nevertheless provide a competitive advantage in highly stressful environments. The radiations of CAM terrestrial bromeliad lineages such as *Hechtia* and the Xeric Clade Pitcairnioideae (*Deuterocohnia*, *Dyckia* and *Encholirium*) provide good examples of the ability of CAM plants to survive high levels of water deficit (**Chapter 2**). In the drought resistance trait survey, while SWC was, as expected, higher in the C₃ succulent terrestrials than in the C₃ mesic terrestrials, it was significantly lower in CAM terrestrials than C₃ succulent terrestrials. Despite the impressive thickness and morphological succulence of the leaves of some CAM terrestrial bromeliads, the ratio of hydrenchyma to chlorenchyma in these leaves is actually often rather low (Benzing, 2000), particularly when compared with C₃ succulent terrestrials. All else being equal, where CAM has evolved, there may be less demand for hydrenchymatous capacitance, whereas there may be rather strong selection for increased chlorenchyma thickness and density to reduce CAM leakiness and increase organic acid storage capacity (Nelson et al., 2005; Nelson and Sage, 2008). The coupling of cell wall elasticity and hydraulic capacitance associated with the evolutionary transition between C₃ succulent terrestrial and CAM terrestrial functional types could explain could be due to the increased relative importance of the chlorenchyma in water storage in CAM species, perhaps alongside a reduction in wall thickness for the larger chlorenchyma cells. As shown in **Chapter 2**, flexible C₃-CAM systems in *Puya* spp. appear to underpin broad environmental niches (Herrera et al., 2010; Quezada et al., 2014), but it is unclear why photosynthetic systems that are apparently so advantageous should exist in *Puya* but not among comparable groups of terrestrial xerophytes spanning C₃ and CAM elements (e.g. Pitcairnioideae, early-diverging Bromelioideae).

Origins of tank-epiphytism in C₃ (Tillandsioideae) and CAM (Bromelioideae) lineages appear to have involved both shared and contrasting trait changes. It is well-established that changes in foliar trichome morphology, density, and uptake efficiency were key to these transitions (Benzing and Burt, 1970; Benzing, 1976, 2000; Givnish et al., 2014), and indeed less specialised foliar water uptake capacity is an important component of the water balance of many vascular epiphytes (Darby et al., 2016). However, divergences in other structural and functional traits may have been equally important, and are comparable with the concerted trait changes associated with origins of

epiphytism in other angiosperm groups (e.g. Zhang et al., 2015). Leaves became thinner and less dense in both cases, probably reducing leaf construction costs and improving leaf optics in light-limited canopy microenvironments (Benzing, 2000). Whereas tank-less vascular epiphytes (e.g. orchids, cacti) often display rather high internal hydraulic capacitance (Nobel, 2006; Yang et al., 2016), in both C₃ and CAM tank bromeliads there was a reduction in internal leaf hydraulic capacitance relative to terrestrial species, presumably due to the provision of external hydraulic capacitance by the tank. A relatively reliable source of tank water may have relaxed the selective pressure against hydraulic vulnerability and cellular drought sensitivity in both cases. This result is consistent with the observations of Gotsch et al. (2015), who found in a survey of a taxonomically-diverse set of tropical montane cloud forest vascular epiphytes that there was a trade-off between investment in drought tolerance and drought avoidance traits. How the 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 et al., 2002; Winkler et al., 2005). The parallel transition in CAM bromeliads seems to have involved an increase in physiological function and efficiency. While the ecophysiological strategies of C₃ and CAM tank-epiphyte bromeliads may appear rather similar, the evolutionary pathways that gave rise to them may have been quite different. The existence of highly conservative water use in CAM terrestrials may have facilitated survival in water-limited arboreal environments during the early stages of the evolutionary transition to tank-epiphytism (Zotz and Hietz, 2001; Crayn et al., 2004; Givnish et al., 2014; Silvestro et al., 2014).

Since the CAM atmospheric epiphytes of the genus *Tillandsia* represent the ultimate expression of extreme epiphytism (Benzing and Ott, 1981), it is not surprising that they show very low physiological activity and nutrient content. Increased SWC, WMA and LMA and reduced stomatal density were probably important components of the increase in water-use efficiency and highly effective drought avoidance associated with the evolution of this functional type, in combination with enhanced stomatal sensitivity (see **Chapter 5**). Despite previous suggestions that the dense indumentum of absorptive foliar trichomes in these species should negate the need for axial water transport through veins (Benzing, 2000), the data presented here demonstrate the potential for vascular water distribution at low fluxes (see **Chapter 4**). Neotenic development is probably the driver of strongly reduced internal air spaces and vasculature in these species, and may have been an important evolutionary route towards increased succulence and CAM function (Till, 1992; Benzing, 2000; Males, in review).

3.4.3 Leaf hydraulic trait interactions in the bromeliads

Strong correlations between A_{\max} and K_{leafmax} across all species suggest that leaf gas exchange and hydraulic conductance have evolved in a coordinated manner in the bromeliads. This is consistent with a growing corpus of data covering many, primarily woody, plant groups (Brodrribb et al., 2002, 2005, 2007; Scoffoni et al., 2016a). The negative relationship between K_{leafmax} and P_{50L} in the bromeliads does not support the classical hydraulic safety vs. efficiency hypothesis, and is instead consistent with concerted convergence of hydraulic safety and efficiency in terrestrial species. Blackman et al. (2010) had suggested that the likelihood of finding evidence for the safety vs. efficiency trade-off in leaves was low because P_{50L} and K_{leafmax} should relate to different aspects of leaf anatomy. Specifically, these authors expected P_{50L} to correlate with xylem conduit structure, and K_{leafmax} to be determined by venation density, mesophyll architecture, and aquaporin regulation. Scoffoni et al. (2016b) have recently demonstrated that xylem structure is an important determinant of leaf hydraulic vulnerability in other angiosperms. While this may also be true for the bromeliads, the data presented here are consistent with the possibility that both K_{leafmax} and P_{50L} are strongly influenced by extra-xylary factors (see **Chapter 4**). The strong correlation between Ψ_{tip} and P_{50L} across all species is in accord with reports from other plant groups (Blackman et al., 2010; Scoffoni et al., 2011, 2012; Villagra et al., 2013; Nardini and Luglio, 2014), and the fact that P_{50L} approximately equals Ψ_{tip} could support the contention that hydraulic resistance and vulnerability resides predominantly in the extra-xylary compartment (Cochard et al., 2004; Blackman et al., 2010; Scoffoni et al., 2014, 2017).

Loss of turgor in mesophyll cells, perhaps causing changes in the conformation or continuity of transcellular and apoplastic pathways of extra-xylary water transport, could be an important means by which overall leaf hydraulic conductance declines during dehydration. P_{50L} was also negatively correlated with ϵ in C_3 species, consistent with the idea that inflexible cell walls confer improved drought resistance because they facilitate large changes in water potential in response to small changes in relative water content (Niinemets, 2001; Blackman et al., 2010). The role of aquaporins in variable leaf hydraulic conductance in the bromeliads is unknown, but warrants further study.

Following the observation that IVD was apparently not related to K_{leafmax} , more detailed investigation of the functional significance of variation in bromeliad vascular properties is presented in **Chapter 4**.

Stomatal density is an important predictor of hydraulic and photosynthetic capacity across the bromeliads, but the independence of variation in P_{50S} from variation in all other traits, considered alongside the fact that P_{50S} was consistently and substantially less negative than P_{50L} , is intriguing. It implies that the stomatal behaviour of bromeliads has evolved along a unique trajectory and yet is critical to the control of leaf water balance. Further investigation of the relationships between

diverse stomatal complex morphologies and different aspects of stomatal function is presented in **Chapter 5**.

The role of hydraulic capacitance in vascular plant leaf hydraulics is increasingly well-studied. Understanding how water is moved into storage and subsequently metered out to sustain transpiration under limited soil water availability is both an interesting question in evolutionary physiology (Blackman and Brodribb, 2011; Griffiths, 2013), and could be critical in applied contexts such as the improvement of crop drought resistance. In the bromeliads, the negative relationship between C_{FT} and A_{max} and positive relationship between C_{FT} and LMA show that more succulent species tend to be situated at the slower-growing end of the leaf economic spectrum. In the expanded drought resistance trait survey, the evolution of SWC and LMA appeared to have been tightly coordinated across the bromeliad family, suggesting 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). 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 and Lamont, 1991; Niinemets, 1999; Wilson et al., 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, determined by differences in cell wall properties, also makes an important contribution to variation in water storage, as is the case in many succulent plant groups (Nobel et al., 1992). This could provide an additional link between SWC and LMA, but will require further investigation. 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). However, C_3 succulent terrestrials (particularly *Puya* spp.) supported relatively high photosynthetic capacity, perhaps allowing them to make considerable carbon gains during pulses of water availability before reducing gas exchange rates and relying on stored water during drought periods. Nevertheless, the negative relationship between C_{FT} and A_{max} in CAM bromeliads is suggestive of a trade-off between degree of succulence and photosynthetic capacity, perhaps due to diffusion constraints (Maxwell et al., 1997).

3.4.4 Interactions between leaf traits and bioclimatic factors

The correlations between π_o , SWC, WMA and LMA with AI and P_{seas} scores also support the hypothesis that variation in functional traits explains some of the adaptation to bioclimatic habitats that could not be accounted for in **Chapter 2**. Some of the residual unexplained variance (particularly within functional types) in these relationships can be attributed to interactions between π_o , SWC, WMA, LMA and other drought resistance traits, and the fact that these analyses were performed on plants grown under glasshouse conditions. Like in other plant groups, the interactions between leaf structural and functional traits and water relations, phylogenetic background and environmental specialisation are complex (Liu and Osborne, 2014), and differences in the interplay between these factors in different bromeliad taxa could reduce the apparent strength of relationships across large species sets.

The relationships between drought resistance traits and bioclimatic scores were also 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 group. 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 and Sanderson, 2015), quantitative traits have likely also played a role in the generation of species-level diversity through ecological differentiation.

3.5 Conclusions

Trait variation in the Bromeliaceae can be accommodated in the existing framework of the leaf economic spectrum, with allowances made for trait-network rewiring associated with functional type differentiation driven by innovations such as CAM, tank-epiphytism and neoteny. Relatively weak overall coupling between structural and functional traits hints at extensive flexibility in leaf design, which may have been crucial for the rapid evolution of ecological novelty. Trait-level adaptation to contrasting habits and growth-strategies is reflected by the clear segregation of functional types in a multivariate space defined by key anatomical and physiological traits. The data presented here are also consistent with the possibility that across all bromeliads, leaf hydraulics and gas exchange are coupled, but the hydraulic safety vs. efficiency hypothesis is not supported, and hydraulic sensitivity to declining leaf water potential resides primarily at the stomatal and extra-xylary levels. The expanded dataset shows that four leaf-level drought resistance traits are correlated across a diverse range of species, 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.

4. Structure-function relationships in bromeliad leaf hydraulics and photosynthesis

4.1 Introduction

In **Chapter 2** it was shown that the bromeliads occupy a broad region of hydrological habitat space, and in **Chapter 3** it was then demonstrated that divergences in bromeliad leaf traits and their coordination have been associated with the evolutionary differentiation of ecologically distinctive functional types. In this chapter, the focus is shifted to specific leaf trait complexes that are hypothesised to be of special importance in defining species' overall water-use strategies, with the aim of addressing the following question posed in the **General Introduction**:

How do specific structure-function relationships influence plant-environment interactions?

With this overarching aim, two lines of enquiry are pursued, summarised by the following questions:

- A) How do vein placement, venation architecture, and vascular anatomy affect leaf hydraulic function?
- B) How does extra-xylary anatomy affect leaf hydraulic design?

Variation in vein placement was analysed across the same 376 species as in the survey of drought resistance traits in **Chapter 3**, and compared with bioclimatic data to explore the functional significance of this variation in vein placement among a subset of species. Venation architecture was characterised in the same 50 species as used in the leaf economic and hydraulic investigation in **Chapter 3**. The results were compared with hydraulic traits and other leaf anatomical parameters, as well as the results of rehydration kinetics measurements for a subset of 19 of these species, to determine the contribution of vascular and extra-xylary anatomy to leaf hydraulic design.

The species selection for each strand of the investigation was largely constrained by access to living collections and the availability of plant material, as well as the time required to perform certain types of measurement. The sampling strategy employed nevertheless ensured that as much diversity as possible was represented in each analysis, with the best available coverage of different taxonomic groups and functional types.

The investigations performed around each of the questions addressed in this chapter are covered in separate sections (A/B) including an Introduction, Methods, and Results. The chapter's Discussion section then draws together insights from each strand of investigation in considering how these different structure-function relationships come together to affect the ecological differentiation of functional types in the Bromeliaceae.

A- Vascular hydraulics: vein placement, venation architecture, and vascular anatomy

4A.1 Introduction to strand A

The arrangement of veins in the three-dimensional space of vascular plant leaves interacts via transpiration with micro-environmental factors to affect the homogeneity of water potential distribution across the lamina. Since homogeneous lamina water potential will tend to promote maximal leaf-level productivity, these structure-function relationships could have important consequences for whole-plant growth and viability (Zwieniecki and Boyce, 2014). Experimentation with artificial biomimetic leaves by Noblin et al. (2008) suggested that the optimal arrangement for hydraulic efficiency should be achieved by the equalisation of the interveinal distance (IVD) and the distance between the vascular plane and the stomatiferous epidermis (VED). Zwieniecki and Boyce (2014) demonstrated a shift towards near-equal values of IVD and VED during angiosperm evolution, which may have been a key factor (alongside others such as the origin of xylem vessels) in the increased hydraulic efficiency of the angiosperms and their proliferation into diverse ecological niches (Boyce et al., 2009).

While most angiosperm species examined by Zwieniecki and Boyce (2014) fell close to the $IVD = VED$ line, there were exceptions among some early-diverging monocots, where $IVD \gg VED$. The authors described these species as 'underinvesting' in veins, which are situated relatively close to the epidermis and widely horizontally spaced through the lamina. This syndrome was hypothesised by Zwieniecki and Boyce to be physiologically permissible only in humid, shaded environments where heterogeneous epidermal water potential is unlikely to be propagated and impose excessive costs on plant carbon balance. If, as has been postulated, there exists a developmental link between leaf width and IVD (Dengler and Kang, 2001), then high IVD might additionally be associated with the broadening of leaf-blades for light or water interception, while low VED could reduce area-specific leaf construction costs. Some bromeliads, notably several ecologically important radiations of C_3 tank-epiphytes, are restricted to very moist, low-light microclimates (Benzing, 2000), and might therefore be good candidates for underinvestment. Meanwhile, vascular 'overinvestment' ($IVD < VED$) was not observed by Zwieniecki and Boyce (2014) in their species set, and they considered that this arrangement would generally be physiologically disadvantageous since it would involve the replacement of photosynthetic mesophyll cells with hydraulically redundant vascular bundles. However, while overinvestment in veins might not be beneficial in the context of 'normal' water fluxes through the transpiration stream, it might be expected to occur in succulent tissues where veins are involved in the rapid recharge of hydraulic capacitance during short pulses of water availability (Griffiths, 2013). High VED relative to IVD could simultaneously increase the resistance to transpirational water loss by increasing the hydraulic path length in the extra-xylary compartment

(Noblin et al., 2008). Seasonal water limitation is characteristic of the environments in which many xerophytic bromeliads with storage-succulence occur (see **Chapter 2**; Benzing, 2000), making these species contenders for vascular overinvestment.

Previous studies have also identified a close relationship between leaf hydraulic function and hierarchical venation architecture, which has been explored in other plant groups through combined modelling and empirical studies (McKown et al., 2010). While the principle of division of labour has thus been invoked to explain the evolution of specialised hydraulic roles for veins of different size orders in angiosperms, the functional significance of the range of hierarchical venation patterns that occur in the Bromeliaceae has not hitherto been examined (Tomlinson, 1969).

4A.2 Methods for strand A

4A.2.1 Anatomical and morphological measurements and habitat assignment

For the survey of vein placement ($n = 376$ spp.), transverse leaf cross sections were hand-cut and inspected by light microscopy. Interveinal distance (IVD) and vein-epidermis distance (VED) were quantified as described in **Chapter 3**. For C_3 succulent terrestrial and CAM terrestrial species, images were captured using a light-microscope-mounted camera and ImageJ (NIH, Bethesda MD, USA) was used to trace the shortest apoplastic route from bundle sheath cells to the abaxial epidermis. This provided a measure of the extra-xylary apoplastic hydraulic path-length to test for the strength of the relationship between this quantity and VED in succulent species. For C_3 tank-epiphytes, leaf width and length were measured to test for relationships with vein placement. For all anatomical and morphological measurements in this survey, mean trait values were recorded across at least 20 replicate leaf sections per species. Habitat type (DXS = desert and xeric scrubland; MFS = Mediterranean forest and scrub; TDF = tropical dry forest; TMF = tropical moist forest; TMX = temperate mixed forest; TRA = Andean tundra) was assigned for all 376 species on the basis of the classification given in the eMonocot portal (e-monocot.org).

In the survey of hierarchical venation architecture carried out in a smaller subset of species ($n = 50$ spp.), transverse leaf sections were hand-cut and imaged using a light-microscope-mounted camera. ImageJ was used for measurements of IVD by vein order and VED. The thickness-to-span ratio, t/b^2 (where t = double-wall thickness and b = diameter of xylem lumen), was also measured for the largest xylem conduits in 1° veins for each species as an index of xylem reinforcement against implosion. For characterisation of venation architecture, the pattern of veins of alternating size order was determined. Mean vessel and/or tracheid number and radius (r) were determined for primary and secondary conduits in each vein order and each species. According to the Hagen-

Poiseuille equation, the conductance (K^*) of each xylem conduit was deemed to be proportional to πr^4 . The sum of K^* components in each vein order (ΣK^*) was calculated, which was assumed to be proportional to the conductance of a vein of that order. For interspecific comparisons, these values were then scaled up using IVD data to represent the combined ΣK^* of each vein order to theoretical K_x across a normalised leaf width of 1 m ($\Sigma \Sigma K^*_{norm}$). The partial contributions of each vein order to $\Sigma \Sigma K^*_{norm}$ were calculated. The normalisation approach could not be used to assess the contribution of the midrib in *Pitcairnia* spp. because the midrib can by definition only occur once across the horizontal width of the leaf blade. Instead, the total number of vascular bundles across the mean leaf width was calculated by dividing leaf width by IVD, and the sum of ΣK^* components, including ΣK^*_{midrib} across this idealised leaf ($\Sigma \Sigma K^*_{leaf}$) was calculated. The contribution of ΣK^*_{midrib} was then calculated as a percentage of $\Sigma \Sigma K^*_{leaf}$. In this survey, a minimum of 20 replicate leaf sections was analysed per species.

4A.3 Results for strand A

4A.3.1 Vein placement

Comparison of the values and ratio of interveinal distance (IVD) and vein-epidermis distance (VED) for the 376 bromeliad species measured in this investigation with those reported for other plant groups by Zwieniecki and Boyce (2014) reveals that the bromeliads occupy an extremely broad swathe of IVD: VED morphospace (Fig. 4A.3.1). The absolute values of IVD and VED were generally comparable with other angiosperms, although VED reached the upper limit of the values reported by Zwieniecki and Boyce (2014). While many bromeliad species clustered roughly along the IVD=VED line, like most other angiosperms, a considerable proportion were placed far into the regions of the morphospace associated with either underinvestment or overinvestment in veins. There was a near 30-fold variation in IVD: VED, from 0.16 in the succulent CAM terrestrial *Hechtia purpusii* Brandegee to 4.50 in the unusually petiolate CAM terrestrial *Bromelia scarlatina* (Henriq. ex Linden) E.Morren ex C.H.Morren. Full data are in **Appendix 4.1**.

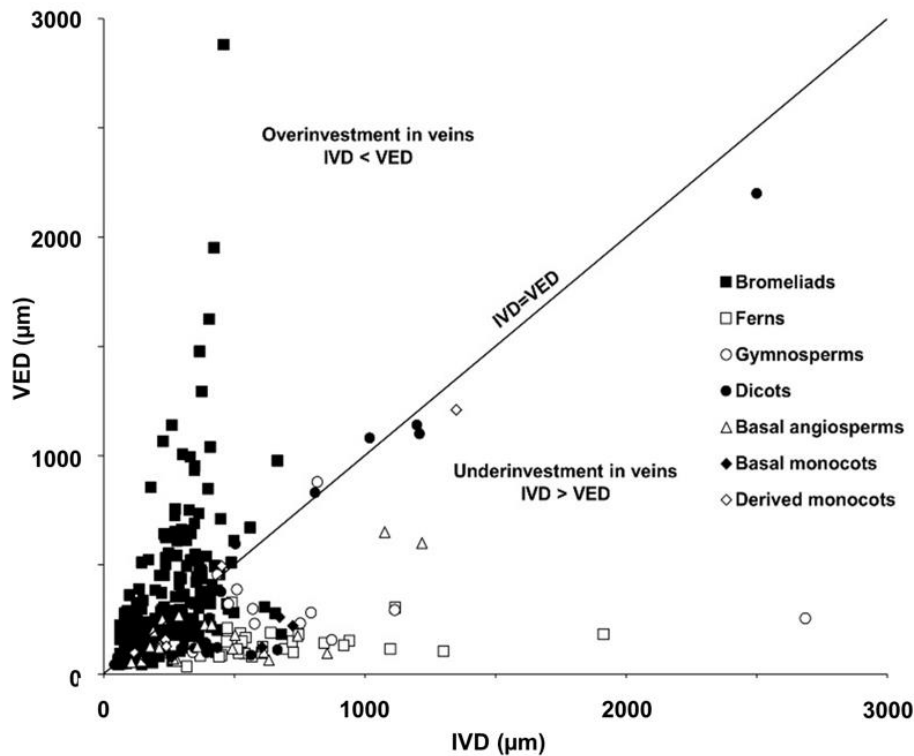


Figure 4A.3.1. Location of 376 bromeliad species in the morphospace defined by interveinal distance (IVD) and vein-epidermis distance (VED), shown with data for other plant groups compiled by Zwieniecki and Boyce (2014). Solid line shows $IVD=VED$, separating morphospace regions associated with underinvestment or overinvestment in veins. Key to plant groups: closed squares- bromeliads; open squares- ferns; open circle- gymnosperms; closed circles- dicots; open triangles- basal angiosperms; closed diamonds- basal monocots; open diamonds- derived monocots.

One-way ANOVA showed that IVD: VED varied significantly between functional types ($F = 21.83$, $p < 0.001$; Fig. 4A.3.2a). The mean value of IVD: VED was greater than 1 in C_3 mesic terrestrials (1.44 ± 0.13), C_3 succulent terrestrials (1.60 ± 0.80) and CAM tank-epiphytes (1.15 ± 0.04), but was highest in C_3 tank-epiphytes (1.71 ± 0.08). Meanwhile the mean value of IVD: VED was below 1 in both CAM terrestrials (0.73 ± 0.61) and CAM atmospheric epiphytes (0.84 ± 0.06), suggesting the prevalence of vascular overinvestment in these functional types.

The range of mean IVD: VED values within functional types was high in all cases, and was associated with differences in species' morphology and ecology. Among C_3 mesic terrestrials, an exceptionally low value occurred in the borderline succulent Guiana Shield endemic *Navia arida* L.B.Sm. & Steyererm. (0.30), followed by some of the more xeromorphic *Fosterella* and *Pitcairnia* species. Meanwhile the highest values occurred in mesophytic *Pitcairnia* species. Notably, the highest value (4.50) was recorded in a petiolate species with broad leaf-blades, *Pitcairnia undulata* Scheidw.

Among the CAM terrestrials, the lowest value of IVD: VED (0.16) occurred in *Hechtia purpusii*, a strongly xeromorphic species, closely followed by *Deuterocohnia* spp. with similar morphology (0.21-0.23). The highest value occurred again occurred in a petiolate species with broad leaf-blades, *Bromelia scarlatina* (4.50), which is native to moist Amazonian forests, followed by *Cryptanthus beuckeri* E.Morren (2.39), another petiolate species from the Atlantic Forest. Very low IVD: VED values occurred in some of the more xeromorphic CAM tank-epiphytes, including *Hohenbergia catingae* Ule (0.29), a secondarily terrestrial species of the dry Brazilian Caatinga, and *Aechmea disjuncta* (L.B.Sm.) Leme & J.A. Siqueira (0.34), an exposure-demanding epiphyte of the Atlantic Forest. Meanwhile the highest values were measured in thin-leaved species such as the Venezuelan cloud forest endemic *Aechmea filicaulis* (Griseb.) Mez (3.80) and Atlantic Forest *Lymania* spp. (2.31-2.55). The lowest IVD: VED values in the C₃ tank-epiphytes were confined to a few large epiphytic or epilithic species of *Alcantarea* and *Mezobromelia*, including *A. simplicisticha* Leme & A.P.Fontana (0.78) and *M. pleiosticha* (Griseb.) Utley & H.Luther (0.78). The highest value of IVD: VED occurred in the understory/lower-canopy species *Catopsis floribunda* L.B.Sm. (4.42), followed by the understory species *Tillandsia leiboldiana* Schldtl. (3.80) and *Racinaea dyeriana* (André) Barfuss & W.Till (3.67). Finally, among the CAM atmospheric species the lowest value occurred in *T. tenuifolia* L. (0.36), while the highest occurred in *T. stricta* Sol. ex Ker Gawl. (1.94). Both of these species are geographically and climatically widespread.

IVD: VED also differed significantly between species associated with different habitats ($F = 7.36$, $p < 0.001$; Fig. 4A.3.2b). The highest mean values of IVD: VED occurred among species from tropical moist forest (1.28 ± 0.04 , $n = 280$), followed closely by temperate mixed forest (1.15 ± 0 , $n = 1$) and Andean alpine tundra (1.14 ± 0 , $n = 1$). A slightly lower mean value of IVD: VED occurred in Mediterranean forest and scrubland species (1.08 ± 0.14 , $n = 32$), while the lowest values occurred in species from tropical dry forest (0.71 ± 0.09 , $n = 56$) and desert and xeric scrubland (0.67 ± 0.33 , $n = 4$).

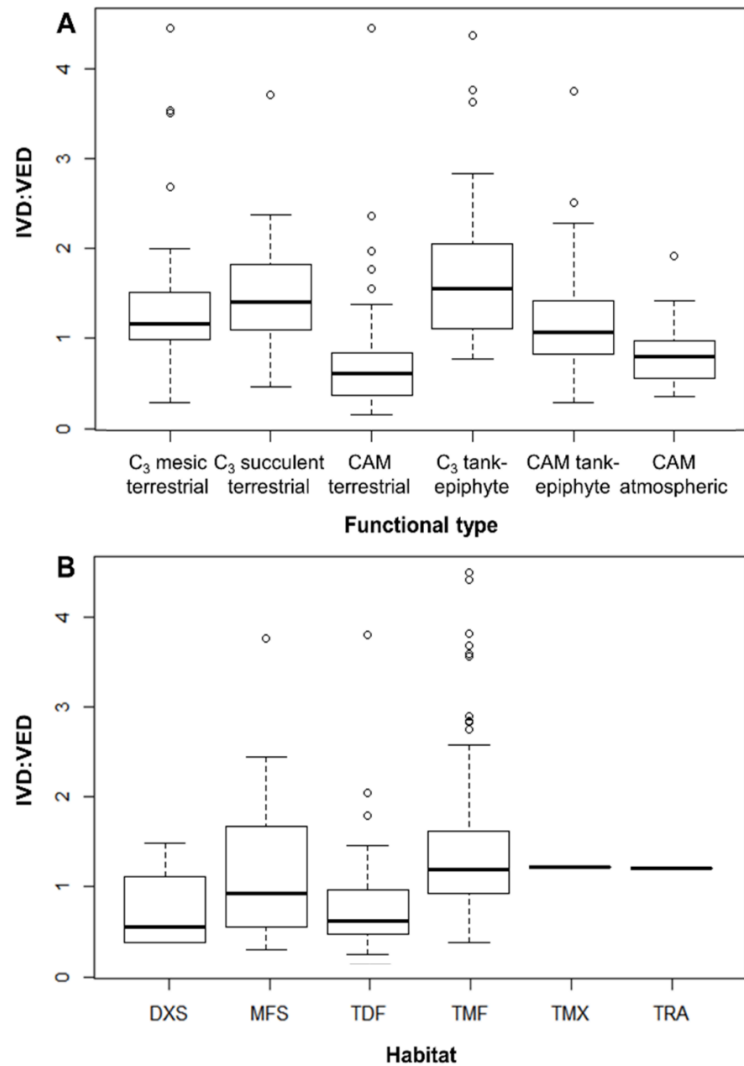


Figure 4A.3.2. Ecological covariates of the ratio of intervein distance (IVD) to vein-epidermis distance (VED). a) IVD: VED by functional type; b) IVD: VED by habitat. Boxes show median values and interquartile range (IQR), with whiskers of length $(1.5 \times \text{IQR})$ and outliers plotted as individual points. Key to habitats in (b): DXS = desert and xeric scrubland; MFS = Mediterranean forest and scrub; TDF = tropical dry forest; TMF = tropical moist forest; TMX = temperate mixed forest; TRA = Andean alpine tundra.

Among the pooled C₃ succulent and CAM terrestrials ($n = 90$), there was a very strong positive correlation between anatomically-estimated extra-xylary apoplastic hydraulic path length and VED ($r^2 = 0.99$, $p < 0.001$; Fig. 4A.3.3). Linear regression showed that across these functional types, apoplastic hydraulic path length was approximately 1.1 x higher than VED. While many other apoplastic and symplastic factors will be involved in determining overall extra-xylary hydraulic resistance, this result highlights the importance of variation in VED in driving a major component of that resistance.

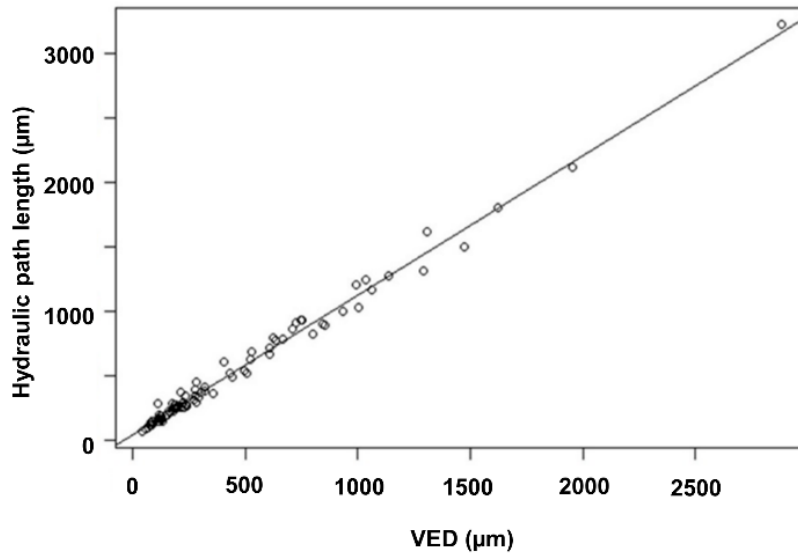


Figure 4A.3.3. Relationship between VED and extra-xylary apoplastic hydraulic path length in C_3 succulent and CAM terrestrial bromeliads ($n = 90$).

As hypothesised, in the C_3 tank-epiphytes ($n = 61$), there was a weak positive relationship between the ratio of leaf-blade width to leaf length ($W_{\text{leaf}}: L_{\text{leaf}}$) and IVD ($r^2 = 0.24$, $p < 0.001$; Fig. 4A.3.4a), This suggests that veins tend to be more widely spaced in shorter, broader leaf-blades. A stronger positive relationship was identified between $W_{\text{leaf}}: L_{\text{leaf}}$ and IVD: VED ($r^2 = 0.77$, $p < 0.001$; Fig. 4A.3.4b), because high values of $W_{\text{leaf}}: L_{\text{leaf}}$ only occurred in species with low VED. Leaf mass per unit area (LMA) was positively correlated with VED among the C_3 tank-epiphytes ($r^2 = 0.26$, $p < 0.001$; Fig. 4A.3.4c), suggesting that minimisation of VED reduces the area-specific biomass investment in leaf tissue, although there was a considerable amount of unexplained variance.

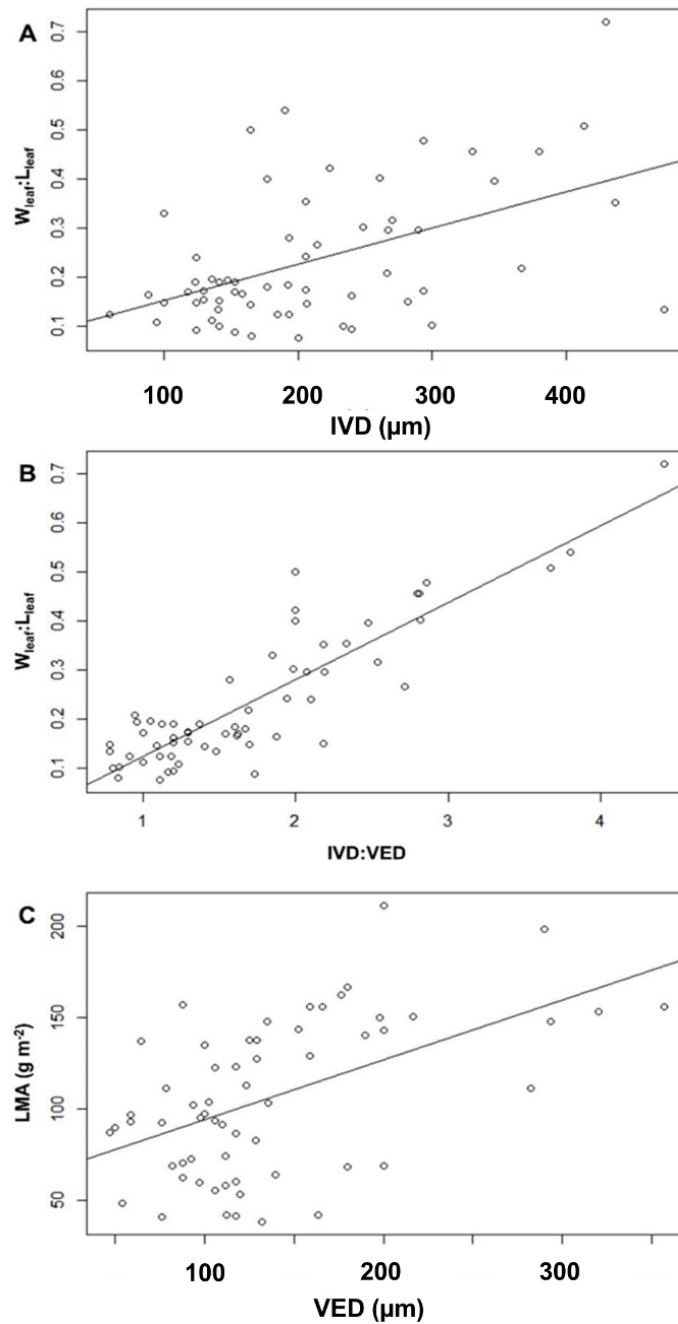


Figure 4A.3.4. a) Relationship between interveinal distance (IVD) and ratio of leaf-blade width to leaf-blade length ($W_{\text{leaf}}:L_{\text{leaf}}$) in C_3 tank-epiphyte bromeliads ($n = 61$). b) Relationship between ratio of interveinal distance to vein-epidermis distance (IVD: VED) and ratio of leaf width to leaf length ($W_{\text{leaf}}:L_{\text{leaf}}$) in C_3 tank-epiphyte bromeliads ($n = 61$). c) Relationship between vein-epidermis distance (VED) and leaf mass per unit area (LMA, g m^{-2}) in C_3 tank-epiphyte bromeliads ($n = 61$).

4A.3.2 Hierarchical venation patterns

Among the subset of 50 bromeliad species investigated for leaf venation architecture, five distinct hierarchical venation arrangements were identified (Fig. 4A.3.5). These venation patterns can be

described on the basis of the repeating units of first- (1°), second- (2°) and third- (3°) order veins: A) $\{1^\circ, 2^\circ\}$; B) $\{1^\circ, 2^\circ, 2^\circ\}$; C) $\{1^\circ, 2^\circ, 2^\circ, 2^\circ\}$; D) $\{1^\circ, 3^\circ, 2^\circ, 3^\circ\}$; E) $\{1^\circ, 3^\circ, 2^\circ, 3^\circ\} + \text{midrib}$.

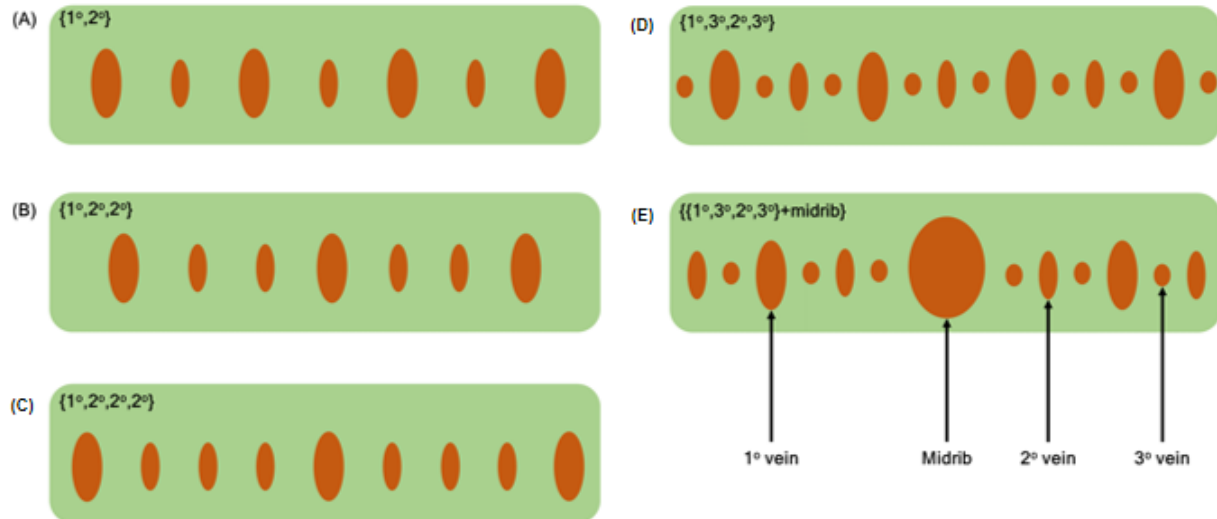


Figure 4A.3.5. Hierarchical patterns in venation architecture. Each venation pattern (A-E) is described with reference to the repeating units of first- (1°), second- (2°) and third- (3°) order veins and (where present) the midrib.

There were clear divergences in venation pattern between functional types. C_3 mesic terrestrial *Pitcairnia* species showed the most complex pattern, $\{1^\circ, 3^\circ, 2^\circ, 3^\circ\} + \text{midrib}$, termed venation pattern E. C_3 succulent terrestrials displayed either $\{1^\circ, 2^\circ, 2^\circ, 2^\circ\}$ (*Puya*; venation pattern C) or $\{1^\circ, 3^\circ, 2^\circ, 3^\circ\}$ (*Fascicularia*, *Ochagavia*; venation pattern D), whereas CAM terrestrials showed either $\{1^\circ, 2^\circ\}$ (*Ananas*, *Cryptanthus*; venation pattern A) or $\{1^\circ, 2^\circ, 2^\circ\}$ (*Bromelia*, *Deuterocohnia*, *Dyckia*; venation pattern B) venation. C_3 tank-epiphytes displayed venation pattern C, except for the two C_3 *Tillandsia* species, which instead showed venation pattern A. All CAM tank-epiphytes showed venation pattern A, as did the CAM atmospheric.

4A.3.2.1 Xylem composition and anatomy

Leaf xylem vessels were absent in venation pattern D C_3 succulent terrestrials (*Fascicularia* and *Ochagavia*), some venation pattern A and B CAM terrestrials (*Ananas*, *Deuterocohnia*, *Dyckia*), venation pattern C C_3 tank-epiphyte *Guzmania* spp., all venation pattern A CAM tank-epiphytes (*Aechmea*, *Nidularium*) and all venation pattern A CAM atmospheric *Tillandsia* species. Phylogenetic patterning of the presence or absence of vessels was therefore not entirely congruent with the

distribution of venation patterns. Furthermore, the largest tracheids in the 1° veins of many species in these genera displayed similar radii to vessels in other species. By far the largest vessels occurred in the midribs of the venation pattern E *Pitcairnia* species ($d = 87.6 \mu\text{m}$ in *P. imbricata*), while variation in the diameter of primary conduits in 1° veins spanned from a mean value of $7.26 \mu\text{m}$ for the CAM atmospheric to $45.71 \mu\text{m}$ for the C₃ mesic terrestrial *Pitcairnia* species. Full data for all species are available in **Appendix 4.1**.

The mean diameter of the primary conduits in 1° veins was positively and linearly correlated with the mean diameter of the secondary conduits in 1° veins across all species with venation pattern A-D ($r^2 = 0.63$, $p < 0.001$) and although there were only three venation pattern E *Pitcairnia* species, these also appeared to show an equivalent relationship with a shallower gradient. Meanwhile there was a significant positive linear correlation between the diameter of primary conduits in 1° veins and 2° veins across species of all venation patterns ($r^2 = 0.94$, $p < 0.001$).

4A.3.2.2 Relationships between venation pattern and order-specific interveinal distances

IVD for 1° veins (IVD₁) was highest in species with venation pattern D ($1626.80 \mu\text{m}$), suggesting that the production of 2° and 3° veins comes at the cost of reduced density of 1° veins. However, the mean value of IVD₁ for venation pattern E *Pitcairnia* species, which also have both 2° and 3° veins, was not so high ($1076.27 \mu\text{m}$). Differences in IVD₂ between venation patterns were as expected. IVD₂ was highest in the venation pattern D and E species where 2° veins were interspersed with 3° veins, being especially high in venation pattern D species ($1626.80 \mu\text{m}$). Meanwhile IVD₂ was lowest in venation pattern C species ($363.38 \mu\text{m}$). Between the two venation patterns where 3° veins were present, IVD₃ was lower in the venation pattern E C₃ mesic terrestrial *Pitcairnia* species than in the venation pattern D C₃ terrestrial succulents. There were no statistically significant correlations between interveinal distances and xylem conduit diameters.

4A.3.2.3 Relationships between venation pattern, K_x and K_{ox}

Consistent with the hypothesis that hierarchical venation architecture should influence leaf hydraulic function, there were significant differences in K_x between species of different venation patterns ($F = 34.8$, $p < 0.001$; Table 4A.3.3). Mean K_x was highest in midrib-equipped venation pattern E species, followed by venation pattern D species. Although the mean value for species with venation pattern C venation was lower, this group also showed the greatest variance. Mean K_x was very low for venation patterns A and B. Equivalent patterns were observed for K_{ox} . Full data are presented in **Appendix 4.1**.

| Venation pattern | K_x (mmol m ⁻² s ⁻¹ MPa ⁻¹) | K_{ox} (mmol m ⁻² s ⁻¹ MPa ⁻¹) |
|---------------------------------------|---|--|
| A ($n = 24$) | 0.09 | 0.31 |
| B ($n = 4$) | 0.43 | 0.05 |
| C ($n = 17$) | 4.09 | 2.37 |
| D ($n = 2$) | 6.11 | 5.18 |
| E ($n = 3$) | 11.61 | 8.67 |
| Functional type | | |
| C ₃ mesic terrestrials | 11.61 | 8.67 |
| C ₃ succulent terrestrials | 7.17 | 4.18 |
| CAM terrestrials | 0.14 | 0.09 |
| C ₃ tank-epiphytes | 2.54 | 1.74 |
| CAM tank-epiphytes | 0.60 | 0.43 |
| CAM atmospheric | 0.07 | 0.17 |

Table 4A.3.3. Mean values of xylem hydraulic conductance (K_x) and extra-xylary hydraulic conductance (K_{ox}) by venation pattern and functional type.

Because of the relationship between venation patterns and functional types, there were significant differences in both K_x ($F = 89.85$, $p < 0.001$) and K_{ox} ($F = 78.15$, $p < 0.001$) between functional types. The highest mean values of K_x and K_{ox} occurred in the C₃ mesic terrestrials, followed by C₃ succulent terrestrials, while the C₃ tank-epiphytes showed somewhat lower mean values. Among the three CAM functional types, the highest hydraulic conductances occurred in the CAM tank-epiphytes. Of all functional types, the lowest mean values of K_x and K_{ox} occurred in the CAM terrestrials and CAM atmospheric.

There was a strong, positive linear relationship between K_x and K_{ox} across all species ($r^2 = 0.93$, $p < 0.001$). The slope of the linear regression was 0.66, indicating that the value of K_{ox} is generally approximately one-third lower than that of K_x in the bromeliads. There were no significant differences in the ratio of K_x to K_{ox} between functional types ($F = 1.82$, $p = 0.129$) or venation patterns ($F = 1.69$, $p = 0.170$).

4A.3.2.4 Relationships between xylem diameter, vein order and density, and K_x

There were significant positive linear correlations across all 50 species between measured K_x and both the diameter of primary xylem conduits in 1° veins ($r^2 = 0.58$, $p < 0.001$) and in 2° veins ($r^2 = 0.57$, $p < 0.001$), suggesting that variation in xylem conduit diameter is an important driver of differential hydraulic capacity in the bromeliads.

Consideration of the theoretical contributions of individual vein orders (ΣK^*) to total theoretical K_x , based on conduit diameters, provided more detailed insights. Across venation patterns A-D there

was a positive linear relationship between log-transformed K_x and 1° vein ΣK^* ($r^2 = 0.44$, $p < 0.001$). The venation pattern E *Pitcairnia* species deviated from this relationship, showing somewhat lower K_x than would be expected from their 1° vein ΣK^* . The proportional contribution of the large midrib vein to $\Sigma \Sigma K^*_{\text{leaf}}$ in *Pitcairnia* species was between 7.65% and 10.85%, despite this vein being only one of approximately 100 in the vascular plane. The midrib therefore appears to be of disproportionate significance in the hydraulic architecture of *Pitcairnia* leaves. Excluding the midrib, the mean proportional contribution of 1° vein ΣK^* to theoretical K_x in *Pitcairnia* species was higher than in any other group (99.19%) due to the particularly strong differentiation of xylem conduit diameters across vein orders in these species.

Meanwhile venation pattern C species showed the lowest mean contribution of 1° vein ΣK^* to theoretical K_x (70.85%), but also the greatest variation, driven by a divergence in xylem composition between *Puya* species and the C₃ tank-epiphytes. Among the former, high-diameter vessels allowed 1° veins to contribute > 90% of theoretical K_x , whereas among the latter, greater equivalence between xylem conduit dimensions in 1° and 2° veins allowed 2° veins to contribute up to 45% of total theoretical K_x . Among venation pattern D and E species, the theoretical contribution of 3° veins to K_x was minimal (< 0.05%), perhaps suggestive of non-hydraulic roles.

Across all 50 species, IVD_1 was unexpectedly positively correlated with both K_x ($r^2 = 0.37$, $p < 0.001$) and K_{ox} ($r^2 = 0.37$, $p < 0.001$), whilst IVD_2 was not correlated with either K_x or K_{ox} . These results are inconsistent with a role for vein density in driving leaf hydraulic capacity at the level of the whole family. However, among venation pattern A species, there were significant negative relationships between K_x and both IVD_1 and IVD_2 ($r^2 = 0.59$, $p < 0.001$). Similarly, across the pooled venation pattern D and E species, there were negative linear correlations between K_x and each of IVD_1 , IVD_2 and IVD_3 ($r^2 = 0.84$, $p = 0.019$). Thus venation pattern appeared to modulate relationships between venation densities and K_x .

4A.3.2.5 Relationship between xylem conduit type and K_x

Species lacking leaf xylem vessels showed significantly lower K_x than species with vessels ($F = 18.93$, $p < 0.001$). This can probably be attributed primarily to the higher mean diameter of primary xylem conduits in species with leaf xylem vessels ($F = 13.46$, $p < 0.001$).

4A.3.2.6 Relationship between xylem hydraulic vulnerability and whole-leaf hydraulic vulnerability

Across all 50 species, the measured values of K_x were negatively correlated with values of P_{50L} reported in **Chapter 3** ($r^2 = 0.52$, $p < 0.001$), in apparent contravention of the classical safety-efficiency trade-off hypothesis. However, there was a significant positive linear correlation between P_{50X} and K_x , which is consistent with species with more efficient xylem transport being more vulnerable to cavitation.

P_{50X} was strongly positively correlated with log-transformed diameters of primary xylem conduits in 1° veins ($r^2 = 0.76$, $p < 0.001$), secondary conduits in 1° veins ($r^2 = 0.65$, $p < 0.001$), and primary conduits in 2° veins ($r^2 = 0.67$, $p < 0.001$). Since cavitation is most likely to occur in the highest diameter conduits, the correlations between P_{50X} and secondary conduits of 1° veins and primary conduits of 2° veins could be a coincidental result of anatomical scaling. P_{50X} was also negatively correlated with t/b^2 ($r^2 = 0.64$, $p < 0.001$), consistent with a role for xylem wall reinforcement against hoop stress in preventing embolism through conduit collapse.

Across all 50 species, there was a strong negative linear correlation between P_{50L} and P_{50X} ($r^2 = 0.42$, $p < 0.001$), with P_{50X} being on average 1.18 times more negative than P_{50L} . Major loss of overall leaf hydraulic conductance would therefore be expected to precede loss of xylem-specific hydraulic conductance during drought, consistent with an important role for dynamic changes in the conductance of the extra-xylary compartment. This would also reduce the physiological relevance of the xylem safety-efficiency trade-off. Furthermore, values of P_{50X} were uncorrelated with values of P_{50S} previously reported in **Chapter 3** ($r^2 = 0.03$, $p = 0.116$), providing further indication of the independent development and evolution of the sensitivity of different components of the transpiration stream to water potential.

B- Extra-xylary structure and function and leaf rehydration kinetics

4B.1 Introduction to strand B

Following the foregoing assessment of diversity in bromeliad venation architecture and its significance for leaf hydraulic function (strand A), the next strand of work focused on the relevance of the architecture of the extra-xylary compartment. The phrase 'hydraulic design' can be used to describe the multidimensional spatial configuration of hydraulic resistances and capacitances in a plant organ (Zwieniecki et al., 2007). In angiosperms, leaf hydraulic design is strongly influenced by variation in extra-xylary anatomical parameters and the presence or absence of discrete structures. Multiphasic rehydration kinetics approaches have been used to investigate the possibility that compartmentalised hydraulic design can result from particular anatomical arrangements (Zwieniecki

et al., 2007; Blackman and Brodribb, 2011). Notably, the presence of bundle sheath extensions (BSEs) has been implicated in decreased extra-xylary resistance by providing a low-resistance pathway for water transport from vascular bundles to the stomatiferous epidermis (Buckley et al., 2011; Zsögön et al., 2015). The great variety in internal leaf anatomy in the Bromeliaceae (Tomlinson, 1969; Males, 2016) could underpin critical divergences in hydraulic function. The presence of absorptive foliar trichomes, which could effectively bypass the vascular system (Benzing, 2000; Males, 2016), is an essentially unique factor that could strongly impact on the hydraulic design of bromeliad leaves.

4B.2 Methods for strand B

4B.2.1 *Taxon sampling and plant material*

For the survey of extra-xylary structural-functional traits, the same set of 50 species and sources of plant material were used as in the survey of venation architecture in strand A.

4B.2.2 *Anatomical measurements*

The light micrographs captured during the survey of venation architecture were imported in ImageJ to quantify the percentage of the transverse section occupied by different tissue types (epidermis, spongy mesophyll, palisade mesophyll, vein, bundle sheath extension (BSE), and hydrenchyma). A series of characteristic leaf anatomical designs with contrasting quantitative and qualitative extra-xylary anatomy were identified for comparison with hydraulic trait data.

4B.2.3 *Vein- and trichome-mediated rehydration kinetics*

Measurements of vein-mediated rehydration kinetics were performed on a subset of 19 species (as identified in Table 4B.2.1) according to the protocol of Zwieniecki et al. (2007). Briefly, leaves were allowed to dehydrate by drying on the bench for an appropriate, species-specific period of time, until Ψ_{leaf} reached approximately -0.8 MPa. The base of each leaf was then re-cut under the rehydration solution (dH₂O + 15 mM KCl) and attached into a tube that drew solution from a reservoir on a balance interfaced with a PC. Thus water-uptake by the leaf ($\Delta mass_{\text{reservoir}}$) could be measured in real time. The mass of the leaf was also periodically measured, which allowed the calculation of change in leaf water content ($\Delta mass_{\text{leaf}}$). The mass of water transpired during each time interval was derived by subtracting $\Delta mass_{\text{leaf}}$ from $\Delta mass_{\text{reservoir}}$, allowing the calculation of the proportion of water taken up that was transpired. Throughout the course of the rehydration measurements, the leaf was sealed in a cuvette under the same conditions as used for hydraulic

conductance measurements. Biphasic exponential kinetic equations were fitted to the data for the extraction of time-constants and pool sizes.

| Functional type | Subfamily | Species |
|--------------------------------------|-----------------|--|
| C ₃ mesic terrestrial | Pitcairnioideae | <i>Pitcairnia integrifolia</i> Ker Gawl. |
| C ₃ mesic terrestrial | Pitcairnioideae | <i>Pitcairnia xanthocalyx</i> Mart. |
| C ₃ succulent terrestrial | Bromelioideae | <i>Fascicularia bicolor</i> (Ruiz & Pav.) Mez |
| C ₃ succulent terrestrial | Bromelioideae | <i>Ochagavia elegans</i> Phil. |
| C ₃ succulent terrestrial | Puyoideae | <i>Puya alpestris</i> (Poepp.) Gay |
| C ₃ succulent terrestrial | Puyoideae | <i>Puya mirabilis</i> (Mez) L.B.Sm. |
| CAM terrestrial | Bromelioideae | <i>Ananas comosus</i> (L.) Merr. |
| CAM terrestrial | Bromelioideae | <i>Bromelia humilis</i> Jacq. |
| CAM terrestrial | Bromelioideae | <i>Cryptanthus bivittatus</i> (Hook.) Regel |
| CAM terrestrial | Pitcairnioideae | <i>Dyckia remotiflora</i> A.Dietr. |
| C ₃ tank-epiphyte | Tillandsioideae | <i>Guzmania lingulata</i> (L.) Mez |
| C ₃ tank-epiphyte* | Tillandsioideae | <i>Guzmania monostachia</i> (L.) Rusby ex Mez |
| C ₃ tank-epiphyte | Tillandsioideae | <i>Lutheria splendens</i> (Brongn.) Barfuss & W.Till |
| C ₃ tank-epiphyte | Bromelioideae | <i>Nidularium innocentii</i> Lem. |
| CAM tank-epiphyte | Bromelioideae | <i>Aechmea fendleri</i> André ex Mez |
| CAM tank-epiphyte | Bromelioideae | <i>Aechmea nudicaulis</i> (L.) Griseb. |
| CAM tank-epiphyte | Tillandsioideae | <i>Tillandsia utriculata</i> L. |
| CAM atmospheric | Tillandsioideae | <i>Tillandsia paucifolia</i> Baker |
| CAM atmospheric | Tillandsioideae | <i>Tillandsia stricta</i> Sol. ex Ker Gawl. |

Table 4B.2.1. Species used in rehydration kinetics measurements. *C₃-CAM intermediate species.

Trichome-mediated rehydration kinetics were measured by sealing the cut end of the dehydrated leaf with silicone grease and immersing it in the same rehydration solution (dH₂O + 15 mM KCl). At regular intervals, the leaf was removed from the solution, carefully surface-dried and weighed. These measurements were performed under the same temperature and light conditions as the vein-mediated rehydration measurements. Monophasic exponential kinetic equations were fitted to the data for the extraction of time-constants and pool sizes. For both vein-mediated and trichome-mediated rehydration, six replicates were performed per species.

4B.3 Results for strand B

4B.3.1 Relationships between extra-xylary leaf anatomical designs and hydraulic function

A series of characteristic leaf anatomical designs was identified on the basis of tissue geometry (Fig. 4B.3.1). These were strongly phylogenetically determined, and coincided closely with functional types. Leaf anatomical design A, with direct contact between vascular bundles and adaxial hydrenchyma, well-defined interveinal aerenchyma, and a reasonably distinct palisade layer,

occurred in *Pitcairnia* species only. Leaf anatomical design B, characterised by undifferentiated chlorenchyma, pronounced abaxial bundle sheath extensions (BSEs), was limited to *Puya*. Leaf anatomical design C occurred throughout the Bromelioideae subfamily, with the thickness of the palisade layer and size of the aerenchyma channels varying widely. Leaf anatomical design D was observed in the CAM terrestrials of the Pitcairnioideae subfamily (*Deuterocohnia* and *Dyckia* species), and is distinguished by undifferentiated chlorenchyma, extensive adaxial hydrenchyma, hydrenchyma-buttressed veins and narrow, poorly-defined aerenchyma. In leaf anatomical design E, which prevailed in the C₃ tank-epiphytes, the capacious aerenchyma was clearly continuous with substomatal cavities, vascular bundles were in contact with adaxial hydrenchyma, and there was relatively well-developed abaxial hydrenchyma. Finally, leaf anatomical design F was characterised by very densely packed chlorenchyma of varying degrees of differentiation, and the strong reduction or absence of aerenchyma. Leaf anatomical design F occurred only in the CAM atmospheric *Tillandsia* species.

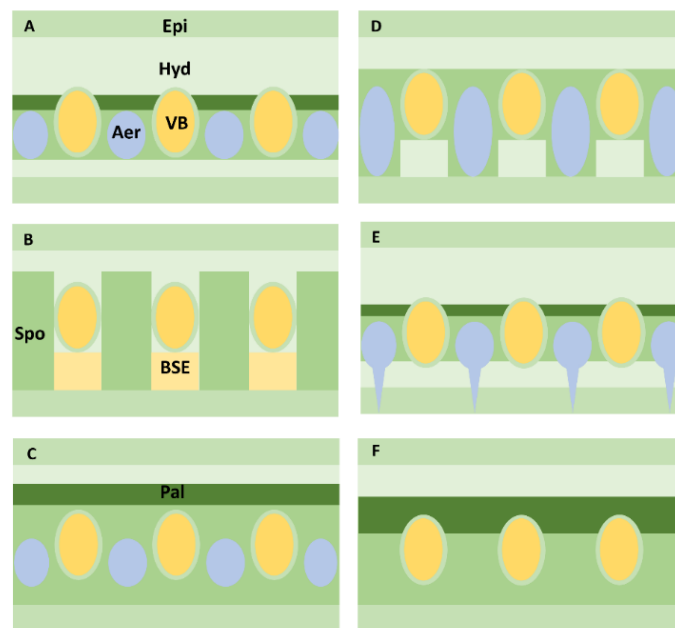


Figure 4B.3.1. Characteristic leaf anatomical designs (LAs; A-F) in the Bromeliaceae shown in transverse section. Tissue ratios not to scale.

Further consideration of the significance of these anatomical differences for hydraulic function was made by controlling for the venation patterns identified in strand A (see above). Among all species with venation pattern A ($n = 24$), there were representatives of leaf anatomical designs C, E and F. LA was a significant factor in explaining variation in K_{ox} among venation pattern A species ($F = 26.11$, $p <$

0.001), with the highest mean K_{ox} occurring in leaf anatomical design E species, and the lowest in leaf anatomical design F. Species-specific values of P_{50L} recovered from **Chapter 3** also differed significantly between leaf anatomical designs ($F = 30.86$, $p < 0.001$), being most negative in leaf anatomical design C species and least negative in leaf anatomical design F species. Similarly, among the species with venation pattern C ($n = 17$), there were representatives of leaf anatomical designs B and E. Leaf anatomical design again had a significant effect on K_{ox} in these species ($F = 10.48$, $p = 0.006$), with mean K_{ox} being approximately twice as high in leaf anatomical design B species. P_{50L} was also significantly more negative in leaf anatomical design B species ($F = 73.84$, $p < 0.001$). Differences in extra-xylary anatomy therefore appeared to modulate the relationship between venation pattern and hydraulic efficiency and vulnerability, with intercellular air spaces and BSEs perhaps being of particular significance.

4B.3.2 *Vascular and extra-xylary determinants of vein-mediated rehydration kinetics*

Biphasic exponential kinetics provided a good fit ($r^2 > 0.90$) to the rehydration profiles of all 19 species used for characterisation of rehydration kinetics. Time-constants and pool sizes for fast and slow phases of vein-mediated rehydration are provided in Table 4B.3.1. Although venation pattern was not a significant factor for the fast-phase time-constant ($F = 2.23$, $p = 0.118$), there was a significant negative correlation between the fast-phase time-constant and values of log-transformed ΣK^* for 1° veins reported above in strand A ($r^2 = 0.44$, $p = 0.001$). This is consistent with the hydraulic conductance of 1° veins being of particular importance in the process of rehydration. There were also significant negative correlations between the fast-phase time-constant and both measured K_x ($r^2 = 0.38$, $p = 0.003$) and the proportional contribution of 1° veins to theoretical K_x ($r^2 = 0.27$, $p = 0.013$), suggesting that species with high K_x driven by highly-conductive 1° veins are faster to rehydrate.

| Subfamily | Species | Functional type | Fast-phase time-constant (s) ± SE | Slow-phase time-constant (s) ± SE | Fast-phase pool size (g) ± SE | Slow-phase pool size (g) ± SE | Proportion of water transpired (%) ± SE |
|-----------------|--------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------|-------------------------------|---|
| Bromelioideae | <i>Aechmea fendleri</i> | CAM tank-epiphyte | 788 ± 31 | 8133 ± 394 | 0.204 ± 0.01 | 1.341 ± 0.13 | 14.55 ± 1.22 |
| Bromelioideae | <i>Aechmea nudicaulis</i> | CAM tank-epiphyte | 704 ± 18 | 7894 ± 502 | 0.304 ± 0.02 | 1.561 ± 0.12 | 12.12 ± 0.72 |
| Bromelioideae | <i>Ananas comosus</i> | CAM terrestrial | 664 ± 24 | 5977 ± 421 | 0.801 ± 0.05 | 2.087 ± 0.21 | 10.03 ± 0.91 |
| Bromelioideae | <i>Bromelia humilis</i> | CAM terrestrial | 404 ± 14 | 6266 ± 449 | 0.842 ± 0.06 | 2.304 ± 0.16 | 11.70 ± 1.13 |
| Bromelioideae | <i>Cryptanthus bivittatus</i> | CAM terrestrial | 488 ± 17 | 5231 ± 504 | 0.770 ± 0.04 | 1.319 ± 0.12 | 14.98 ± 1.26 |
| Bromelioideae | <i>Fascicularia bicolor</i> | C ₃ terrestrial | 689 ± 18 | 8943 ± 656 | 0.295 ± 0.03 | 2.893 ± 0.22 | 26.65 ± 1.89 |
| Bromelioideae | <i>Nidularium innocentii</i> | C ₃ tank-epiphyte | 791 ± 32 | 8223 ± 704 | 0.157 ± 0.02 | 0.964 ± 0.07 | 20.41 ± 2.01 |
| Bromelioideae | <i>Ochagavia elegans</i> | C ₃ terrestrial | 725 ± 30 | 9997 ± 821 | 0.369 ± 0.01 | 3.000 ± 0.27 | 22.28 ± 2.14 |
| Pitcairnioideae | <i>Dyckia remotiflora</i> | CAM terrestrial | 613 ± 33 | 5198 ± 603 | 1.936 ± 0.13 | 2.459 ± 0.25 | 10.51 ± 0.77 |
| Pitcairnioideae | <i>Pitcairnia integrifolia</i> | C ₃ terrestrial | 326 ± 26 | 5643 ± 313 | 0.202 ± 0.02 | 0.643 ± 0.51 | 57.88 ± 3.04 |
| Pitcairnioideae | <i>Pitcairnia xanthocalyx</i> | C ₃ terrestrial | 301 ± 14 | 5375 ± 426 | 0.252 ± 0.01 | 0.535 ± 0.30 | 54.31 ± 3.66 |
| Puyoideae | <i>Puya alpestris</i> | C ₃ terrestrial | 279 ± 18 | 4223 ± 391 | 0.947 ± 0.08 | 3.078 ± 0.32 | 34.15 ± 3.21 |
| Puyoideae | <i>Puya mirabilis</i> | C ₃ terrestrial | 314 ± 24 | 4967 ± 288 | 0.783 ± 0.08 | 2.245 ± 0.21 | 30.91 ± 3.03 |
| Tillandsioideae | <i>Guzmania lingulata</i> | C ₃ tank-epiphyte | 884 ± 44 | 8975 ± 570 | 0.142 ± 0.01 | 0.618 ± 0.04 | 24.89 ± 2.26 |
| Tillandsioideae | <i>Guzmania monostachia</i> | C ₃ -CAM tank-epiphyte | 852 ± 40 | 9100 ± 723 | 0.174 ± 0.02 | 0.503 ± 0.03 | 23.65 ± 1.83 |
| Tillandsioideae | <i>Lutheria splendens</i> | C ₃ tank-epiphyte | 911 ± 38 | 9221 ± 618 | 0.147 ± 0.01 | 0.877 ± 0.06 | 19.65 ± 1.04 |
| Tillandsioideae | <i>Tillandsia paucifolia</i> | CAM atmospheric | 1023 ± 62 | 3396 ± 288 | 0.567 ± 0.04 | 1.585 ± 0.11 | 3.52 ± 2.57 |
| Tillandsioideae | <i>Tillandsia stricta</i> | CAM atmospheric | 956 ± 56 | 4128 ± 376 | 0.299 ± 0.04 | 1.169 ± 0.11 | 5.01 ± 4.08 |
| Tillandsioideae | <i>Tillandsia utriculata</i> | CAM tank-epiphyte | 812 ± 62 | 5895 ± 457 | 0.634 ± 0.03 | 2.926 ± 0.30 | 6.22 ± 2.14 |

Table 4B.3.1. Mean time-constants and pool sizes for fast and slow phases of vein-mediated rehydration and proportion of water transpired during rehydration for 19 bromeliad species.

Species identified in strand A as displaying vessel elements in leaf xylem showed lower fast-phase time-constants than species with tracheids only ($F = 21.02$, $p < 0.001$), corroborating the notion that vessels are associated with reduced resistance in bromeliad leaf xylem. Vessels were however not significant in slow-phase rehydration rates ($F = 1.93$, $p = 0.183$). The slow-phase time-constant was meanwhile independent of measured K_{ox} ($r^2 = -0.05$, $p = 0.760$) as well as K_x ($r^2 = -0.06$, $p = 0.820$).

Species with BSEs showed lower fast-phase time-constants ($F = 21.44$, $p < 0.001$) despite not having smaller absolute fast-phase pool sizes ($F = 0.007$, $p = 0.932$). Among these were the *Puya* species, which also show dense cell packing and relatively low slow-phase time-constants.

Comparison of the relative volumes of different tissues with the relative volumes of the fast- and slow-phase pool sizes suggested that in species with BSEs, fast-phase rehydration was restricted to the vasculature, BSEs and epidermis (Fig. 4B.3.2). In species lacking BSEs, the relative volume of the fast-phase pool generally tallied with the combined relative volume of the veins and palisade mesophyll. However, in the CAM terrestrials *Cryptanthus bivittatus* and *Dyckia remotiflora* and the CAM atmospheric *Tillandsia paucifolia* and *T. stricta*, the relative volume of the fast-phase pool was equivalent to the combined volume of the veins, palisade mesophyll, and a significant proportion or the entirety of the spongy mesophyll. The chlorenchyma therefore does not appear to be as strongly hydraulically isolated in these species, possibly demonstrative of physiologically-relevant chlorenchyma hydraulic capacitance.

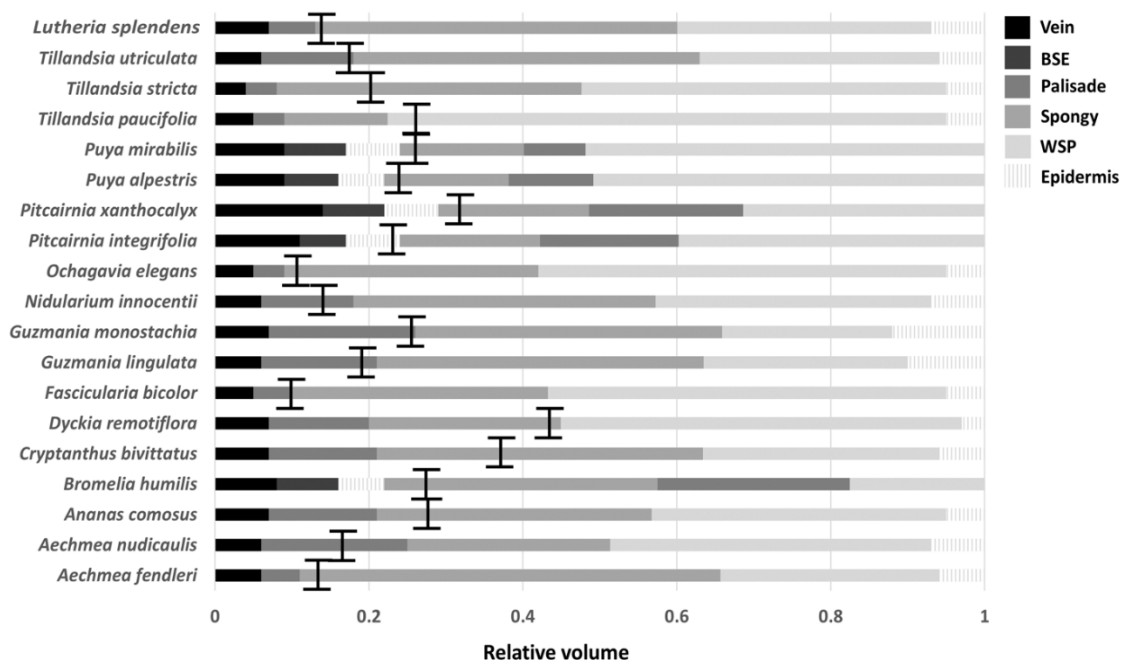


Figure 4B.3.2. Relative volume of fast- and slow-phase rehydration pool sizes (vertical bars) for vein-mediated rehydration overlaid on relative volumes of tissues types for leaves of 19 bromeliad species.

Overall, leaf anatomical design was a significant factor for fast-phase time-constant ($F = 14.07$, $p < 0.001$), slow-phase time-constant ($F = 6.19$, $p = 0.004$), absolute fast-phase pool size ($F = 11.31$, $p < 0.001$), and absolute slow-phase pool size ($F = 3.71$, $p = 0.026$), but not for relative fast- and slow-

phase pool sizes ($F = 2.04$, $p = 0.140$), the latter probably due to extensive interspecific variation in the thickness of the hydrenchyma tissue.

Species with low fast-phase time-constants showed the highest proportional use of water for transpiration during rehydration ($r^2 = 0.39$, $p = 0.003$). The transpirational ratio was significantly higher in species with BSEs ($F = 11.52$, $p = 0.003$) and species with vessels ($F = 7.77$, $p = 0.013$), and was positively correlated with K_x ($r^2 = 0.78$, $p < 0.001$) and K_{ox} ($r^2 = 0.64$, $p < 0.001$). This implies that species with higher hydraulic capacity make use of this to drive recharge after drought. There was also a negative correlation between the transpirational ratio and VED ($r^2 = 0.40$, $p = 0.002$), suggesting that species with vascular planes set closer to the stomatiferous epidermis spend more water during recharge.

4B.3.3 *Relationship between vein-mediated rehydration kinetics and trichome-mediated rehydration kinetics*

A monophasic exponential-curve provided a good fit to trichome-mediated rehydration data for the same 19 species as used in characterisation of vein-mediated rehydration kinetics ($r^2 > 0.90$). This highlights the importance of vascular tissues in generating the biphasic rehydration kinetics observed in vein-mediated rehydration experiments. Time-constants and pool sizes for trichome-mediated rehydration are provided in Table 4B.3.2. The fitted pool size for trichome-mediated rehydration was positively correlated with the time-constant ($r^2 = 0.59$, $p < 0.001$), and with the pool sizes of both the fast ($r^2 = 0.58$, $p < 0.001$) and slow ($r^2 = 0.87$, $p < 0.001$) phases of vein-mediated rehydration. Comparing functional types, the lowest time-constants for trichome-mediated rehydration occurred in the epiphytic types, particularly the CAM atmospherics (5365 s), while the highest time-constant occurred in the CAM terrestrials (782,147 s). Meanwhile the mean pool sizes were largest in the C_3 succulent terrestrials (3.36 g) and CAM terrestrials (3.18 g) and lowest in the C_3 mesic terrestrials (0.79 g).

| Subfamily | Species | Functional type | Time-constant (s) ± SE | Pool size (g) ± SE |
|-----------------|--------------------------------|------------------------------|------------------------|--------------------|
| Bromelioideae | <i>Aechmea fendleri</i> | CAM tank-epiphyte | 19,680 ± 286 | 0.965 ± 0.12 |
| Bromelioideae | <i>Aechmea nudicaulis</i> | CAM tank-epiphyte | 18,332 ± 199 | 1.844 ± 0.11 |
| Bromelioideae | <i>Ananas comosus</i> | CAM terrestrial | 986,891 ± 178 | 2.969 ± 0.09 |
| Bromelioideae | <i>Bromelia humilis</i> | CAM terrestrial | 813,949 ± 134 | 3.198 ± 0.10 |
| Bromelioideae | <i>Cryptanthus bivittatus</i> | CAM terrestrial | 204,124 ± 297 | 2.042 ± 0.10 |
| Bromelioideae | <i>Fascicularia bicolor</i> | C ₃ terrestrial | 587,025 ± 301 | 2.928 ± 0.11 |
| Bromelioideae | <i>Nidularium innocentii</i> | C ₃ tank-epiphyte | 13,334 ± 133 | 1.135 ± 0.12 |
| Bromelioideae | <i>Ochagavia elegans</i> | C ₃ terrestrial | 656,354 ± 176 | 3.278 ± 0.09 |
| Pitcairnioideae | <i>Dyckia remotiflora</i> | CAM terrestrial | 1,123,626 ± 408 | 4.522 ± 0.14 |
| Pitcairnioideae | <i>Pitcairnia integrifolia</i> | C ₃ terrestrial | 220,136 ± 311 | 0.850 ± 0.10 |
| Pitcairnioideae | <i>Pitcairnia xanthocalyx</i> | C ₃ terrestrial | 224,183 ± 284 | 0.724 ± 0.02 |
| Puyoideae | <i>Puya alpestris</i> | C ₃ terrestrial | 819,334 ± 244 | 4.170 ± 0.14 |
| Puyoideae | <i>Puya mirabilis</i> | C ₃ terrestrial | 730,830 ± 203 | 3.057 ± 0.15 |
| Tillandsioideae | <i>Guzmania lingulata</i> | C ₃ tank-epiphyte | 15,914 ± 186 | 0.707 ± 0.09 |
| Tillandsioideae | <i>Guzmania monostachia</i> | C ₃ tank-epiphyte | 14,775 ± 156 | 0.643 ± 0.03 |
| Tillandsioideae | <i>Lutheria splendens</i> | C ₃ tank-epiphyte | 18,748 ± 198 | 0.965 ± 0.02 |
| Tillandsioideae | <i>Tillandsia paucifolia</i> | CAM atmospheric | 4052 ± 110 | 2.123 ± 0.12 |
| Tillandsioideae | <i>Tillandsia stricta</i> | CAM atmospheric | 4342 ± 99 | 1.471 ± 0.13 |
| Tillandsioideae | <i>Tillandsia utriculata</i> | CAM tank-epiphyte | 7702 ± 91 | 3.471 ± 0.15 |

Table 4B.3.2. Mean time-constants and pool sizes for trichome-mediated rehydration for 19 bromeliad species.

Time-constants for trichome-mediated rehydration were much higher than for vein-mediated rehydration, indicative of the high resistance associated with the symplastic bottleneck in the former. When the time-constants for vein-mediated and trichome-mediated rehydration were log-transformed, they showed a negative correlation ($r^2 = 0.46$, $p < 0.001$), consistent with the hypothesis that high-efficiency vein-mediated and trichome-mediated water distribution do not co-occur in the bromeliads.

4.4 Discussion

The two strands of this investigation, focussed on (A) vein placement and venation architecture and (B) extra-xylary leaf architecture, have demonstrated the role of structural variety in supporting divergences in hydraulic function among bromeliad species. The implications of key observations are here discussed in turn, before being brought together in a discussion of the ecophysiological differentiation of bromeliad functional types.

4.4.1 Vein placement

In strand A of this chapter, a survey of vein placement in 376 species identified wide ranges in the absolute values of IVD and VED as well as the ratio of IVD: VED, reflecting the remarkable ecological diversity of the bromeliads (Benzing, 2000). The identification of significant differences in IVD: VED

between functional types adds to the growing corpus of evidence linking leaf structural properties, physiological functions and ecological divergences in the bromeliads. The most striking observation was the presence of numerous cases of both strong overinvestment and strong underinvestment in veins in different bromeliad lineages, which are now discussed in turn.

The association of vascular overinvestment with more arid habitat types (e.g. desert and xeric scrub) and of underinvestment with wetter habitats (e.g. tropical moist forest) is consistent with the operation of environmental filtering on the spatial arrangement of leaf veins. The C₃ succulent and CAM terrestrial bromeliads occupy a wide range of habitats and climate space. However, most occur in water-limited environments and share the capacity to take up and retain relatively large volumes of water in specialised foliar storage tissues. In these plants, much of the leaf thickness is taken up by the water storage tissue, but in the residual photosynthetic portion of the mesophyll, the vascular plane is generally set very deep relative to the abaxial epidermis. This results in a high extra-xylary apoplastic hydraulic path length, which is likely to make a major contribution to overall extra-xylary hydraulic resistance. Although symplastic hydraulic path length was not calculated here, extremely dense cell-packing in the succulent terrestrial bromeliads mean that it will not differ substantially from either the apoplastic path length or VED.

In hot environments and on loosely-structured soils, even following a moderate rainfall event, water availability can be fleeting. In such environments, natural selection may favour anatomical phenotypes conferring capacity for rapid refilling of succulent water storage tissue to meet transpirational, metabolic and turgor requirements during the ensuing drought period. The data presented here offer a preliminary insight into how low IVD in overinvesting species may be important in facilitating rapid recharge. This observation merits further investigation, which should focus on the interactions between IVD, xylem structure, leaf hydraulic capacitance, and hydrological niche, as well as the highly under-studied root biology of bromeliads (Males, 2016). The fact that succulent xerophytism has evolved convergently in multiple bromeliad lineages (the Xeric Clade of Pitcairnioideae, *Hechtia*, early-diverging Bromelioideae with *Puya*) provides natural evolutionary replication for testing the degree of similarity in the anatomical and physiological adaptations leading to independent origins of the same ecological syndrome.

Overinvestment was also common in CAM atmospheric *Tillandsia* species. These plants are dependent on pulses of wetting, often from occult precipitation, for the recharge of their succulent water storage tissue (Reyes-García *et al.*, 2008). While in theory dense vasculature could improve rehydration rates in these species as for the terrestrial succulents, the relative contribution of vascular water fluxes compared with absorptive trichome-mediated symplastic fluxes to the process

of hydraulic recharge in these species remains poorly understood. The imbalance between IVD and VED in many of these species may represent a 'side-effect' of developmental neoteny (Benzing, 2000). The atmospheric growth-form and many of its associated physiological properties occur in juvenile tank-epiphytes prior to their heteroblastic transition to tank formation and ontogenetic drift towards the physiological traits common to that growth-form (Meisner et al., 2013).

Vascular underinvestment was particularly pronounced in C₃ tank-epiphytes from the understoreys and lower canopies of tropical moist forests. These habitats are characterised by consistently high humidity and reduced incident light, which limits the potential for underinvestment to lead to hydraulic dysfunction (Zwieniecki and Boyce, 2014). One important scenario when the leaf micro-environment can change very suddenly and strongly is when a sun-fleck hits the leaf. Sun-flecks are far from being fatal to understorey tank-epiphytes; they probably represent a very important contribution to their carbon economy (Chazdon, 1988; Benzing, 2000). Nevertheless, sun-flecks do pose an immediate physiological challenge when they arrive, as they involve an increase not just in light intensity but also in temperature and leaf-air vapour pressure deficit (VPD). This could lead to excessive water loss and destabilisation of the distribution of water potential across the lamina in underinvesting species. To avoid hydraulic dysfunction, these plants rely on rapid and strong stomatal responses to environmental perturbation. C₃ tank-epiphytes indeed display more pronounced stomatal sensitivity to VPD and faster stomatal kinetics than C₃ terrestrial mesophytes (see **Chapter 5**).

For several reasons, underinvestment in veins, and the associated thinness of the leaves, is likely to be advantageous for lower-canopy and understorey epiphytic bromeliads. First, reduced vein density in the leaves could limit leaf hydraulic conductance, which, in combination with conservative stomatal behaviour, will help plants retain water in epiphytic microhabitats where this resource is often limiting (Males, 2016). Secondly, as has been shown here, underinvestment is closely associated with increased leaf-blade width relative to length, which could be important in maximising the biomechanically-supportable canopy surface area available for intercepting precipitation for refilling the plant's central or axillary tanks. Broader, thinner leaves may also be more efficient at exploiting diffuse light and sun-flecks in shaded forest environments. Finally, all else being equal, the areas-based construction costs of thinner leaves are lower, allowing greater canopy area to be achieved for the same resource investment.

Interestingly, some of the strongest expressions of underinvestment occurred in species of the genus *Catopsis*, including exposure-demanding species that typically inhabit the crowns of emergent trees and are subjected to both high light intensities and drying winds. The advantage of vein

underinvestment in this scenario could relate to reduced leaf hydraulic conductance, perhaps in combination with very strong stomatal sensitivity (see **Chapter 5**). Although this would come at the price of reduced productivity, it would allow these plants to occupy an extreme epiphytic niche that is virtually free of interspecific competition.

One important focus for future research will be to disentangle the interplay between vein placement, leaf hydraulics and the longitudinal air channels or aerenchyma that are frequently observed in bromeliad leaves and are especially well-developed among C_3 tank-epiphytes. While some of the other possible functions of these air channels were outlined in the **General Introduction**, they might act as low-resistance conduits for water vapour as an alternative to apoplastic or symplastic water transport (Rockwell et al., 2014; Buckley et al., 2015, 2017; Males, 2016), could be the innovation that has allowed vascular underinvestment to evolve in these plants. In this connection, the presence of the air channels could have several important effects. Two key examples are that it could 1) reduce the extra-xylary apoplastic and symplastic hydraulic path lengths between the vascular bundle and the site of evaporation; and 2) facilitate rapid hydraulic equilibration between relatively distant regions of the photosynthetic mesophyll. More empirical and theoretical work is needed to clarify the contribution of these enigmatic channels to bromeliad leaf physiology (Males, 2016).

Some additional comments on vein placement in bromeliad leaves, alongside further insights gained from a simple heuristic model linking vein placement parameters with physiological fitness components, are provided in **Appendix 4.2**.

4.4.2 Venation architecture and xylem anatomy

The existence of various hierarchical venation architectures in the Bromeliaceae has previously been noted (e.g. Tomlinson, 1969), but has never been systematically considered in a physiological context. While much attention has been given to the role of the iconic absorptive trichome in bromeliad leaf water relations, the vasculature has been relatively neglected. In this investigation, the venation architecture of 50 species (identical to those used in **Chapter 3** and a subset of those used for vein placement characterisation in this chapter) was analysed. There was some evidence of geometric scaling effects between interveinal distance (IVD) for veins of different orders, but variation in vein placement was independent of xylem conduit diameters. This developmental decoupling of vein density and structure could be an important mechanism for the generation of physiological diversity.

It is clear that interspecific differences in venation pattern are closely related to differences in hydraulic function. The conductive capacity of 1° veins appears to be of foremost importance as a driver of total xylem hydraulic conductance (K_x). In species where a complex hierarchical arrangement prevails, these 1° veins often make an overwhelmingly dominant contribution. High-conductance 1° veins were both associated with low fast-phase time-constants in rehydration kinetics, demonstrating the importance of this aspect of vascular anatomy in determining not only K_x , but also overall leaf hydraulic design. The presence of xylem vessels was associated with the same functional properties, highlighting the physiological significance of the remarkable evolutionary lability of xylem composition in the Bromeliaceae. Repeated losses of xylem vessels in leaves and other plant parts has probably played a fundamental role in the ecological diversification of these plants, and more research is needed to elucidate the developmental basis of this lability.

Given the apparent dominance of 1° veins in xylem hydraulic function, one must ask the question: what is the function of the 2° and 3° veins in these species? Many different roles can be assigned to leaf veins, ranging from mechanical support to sugar transport (Sack and Scoffoni, 2013). The highest-order veins, which are in close contact with the photosynthetic chlorenchyma, may be specialised for assimilate distribution (Sack and Scoffoni, 2013). Division of labour between vein orders appears to be a widespread hallmark of hierarchical venation networks (McKown et al., 2010; Sack and Scoffoni, 2013; Scoffoni et al., 2016a). There was good agreement between relative theoretical K_x based on xylem conduit radii and measured K_x , with the residual variance being perhaps attributable to differences in pits or end-walls of these cells, or the porosity of xylem conduit lateral walls and of the parenchymatous sheath surrounding vascular bundles (Tomlinson, 1969).

Observations reported in **Chapter 3** had suggested that the hydraulic properties of bromeliad leaves were not strongly connected with IVD. Consideration of the densities of veins of different size orders, as presented in this chapter, provides more nuanced elucidation of this issue. For both 1° and 2° veins, most variation in IVD was explained by the hierarchical arrangement of the venation, and the evidence therefore points towards conservatism in overall, order-nonspecific IVD. The high IVD values in C_3 succulent terrestrials are consistent with reports of a positive relationship between succulence and IVD in other plant groups (Ogburn and Edwards, 2013). Furthermore, venation pattern clearly modulates the importance of IVD for K_x , since relationships between IVD and K_x were specific to individual venation patterns.

It has recently been definitively established that wider leaf xylem conduits are more susceptible to cavitation (Scoffoni et al., 2016b). This seems to hold true within the Bromeliaceae, where there was

a positive correlation between K_x and xylem hydraulic vulnerability quantified as P_{50x} . The negative correlation between P_{50x} and the thickness-to-span ratio (t/b^2) is also consistent with evidence from other plant groups showing that investment in xylem wall reinforcement reduces the potential for loss of conductance due to buckling (Hacke et al., 2001). However, the relevance of these xylem hydraulic properties for overall leaf responses to water deficit are uncertain.

Variation in venation architecture is probably also important for other leaf functions and properties in the bromeliads, as in other plant groups. Veins are critical components of leaf structure, providing mechanical reinforcement to the lamina, and therefore provide a link between physiology and biomechanics (Kawai and Okada, 2016). Another important interaction is between leaf venation architecture and carbohydrate relations. At present, very little is known about phloem transport in the bromeliads, but selection for effective mobilisation of carbohydrate will likely have played a role in the evolution of venation architecture. Integrative structural-functional modelling approaches may be useful tools for casting light on the links between bromeliad leaf development and ecophysiology (DeJong et al., 2011).

4.4.3 *Extra-xylary structure and function*

In this chapter, strand B focussed on the role of extra-xylary structure in controlling leaf hydraulic function. A survey of extra-xylary structure in the same 50 species as used for analysis of venation architecture showed that there were clear differences among bromeliad species in terms of both continuous (e.g. leaf thickness) and discrete (e.g. presence or absence of bundle sheath extensions, BSEs) traits. This structured variation gave rise to a range of characteristic leaf anatomical types (LAs). Among groups of species that share the same venation pattern (as identified in strand A), differences in LA can explain contrasting hydraulic properties, including both extra-xylary hydraulic conductance (K_{ox}) and leaf hydraulic vulnerability quantified as the leaf water potential at 50% loss of maximal leaf hydraulic conductance (P_{50L}). These differences are attributable to contrasting resistances for apoplastic, symplastic, transcellular and vapour-phase flow, depending on the presence or absence of BSEs, the degree of palisade-spongy differentiation, cell-wall thicknesses, cell-packing, intercellular air space fraction and configuration, hydrenchyma thickness and distribution, and overall vein-epidermis path-lengths (Buckley et al., 2015).

4.4.4 *Recharge physiology*

Some succulent xerophytic bromeliads are morphologically convergent with other American semi-desert or dry grassland monocot succulents, such as *Agave*, *Dasyilirion*, *Furcraea*, and *Yucca* in the

Asparagaceae, and to a lesser extent with dicot succulents (e.g. Cactaceae). These bromeliads are in fact ecophysiologicaly representative of a particular hydraulic strategy common to many storage succulents (*sensu* Ihlenfeldt, 1985), which involves the gradual use of stored water to maintain low-level transpiration during extended drought periods and the recharge of depleted storage tissue with water during pulses of water availability associated with seasonal rainfall. One outstanding question in research into this functional group of plants is the extent to which the process of hydraulic recharge is dependent on transpiration (Males, 2017b). In this investigation, by quantifying the amount of transpiration that took place during vein-mediated rehydration kinetics measurements, it was possible to provide some insights into the role of transpiration in the recharge of water-storage tissues of succulent bromeliads. There was a clear negative correlation between vein-mediated rehydration time-constant and transpirational water loss, suggesting that fast recharge is generally achieved at the cost of a larger volume of transpired water. Interpretation of this observation is complicated by the fact that although the minimisation of transpirational water loss during recharge might seem obviously beneficial for a plant adapted to water-limited conditions, it may be that the gas exchange associated with that water loss represents a substantial positive contribution to the plant's net carbon balance. This could be particularly true of species with strong stomatal sensitivity to humidity, such as the CAM atmospherics (Reyes-García et al., 2008; see **Chapter 5**).

4.4.5 *Interactions between trichomes and leaf hydraulic design*

The presence of unique absorptive foliar trichomes in the Bromeliaceae makes the hydraulic properties and ecophysiology of these plants distinctive (Males, 2016). It has been hypothesised that in species displaying particularly efficient trichome-mediated water uptake, the vasculature may have become effectively redundant in terms of hydraulic function. In this investigation it has been shown that trichome-mediated rehydration is a monophasic process and considerably slower than vein-mediated rehydration, presumably because of the need for all water to pass through a narrow symplastic bottleneck during trichome-mediated uptake (Benzing, 1976; Benzing, 2000; Papini et al., 2010). However, the efficiencies of vein-mediated and trichome-mediated rehydration appeared to trade off, as originally hypothesised. The mechanistic explanation for this trade-off could relate to the considerable construction costs of high-capacity veins (often including in stems and roots as well as leaves) on the one hand, and dense arrays of elaborate, absorptive multicellular trichomes on the other. The mutual exclusivity of efficiency in either system is also reflective of the ecological differentiation of species into habitats where water is supplied in different forms. Films of liquid water from dewfall and condensing fog are critical to the water balance of trichome-dependent

atmospheric epiphytes, whereas groundwater is the principal source for most large terrestrial bromeliads. Variation in the time-constants for trichome-mediated rehydration can be attributed to differences in trichome absorptive capacity, which are known to be very large across the bromeliads (Benzing, 2000). Indeed, in some terrestrial species (e.g. *Pitcairnia* species), the trichomes are so poorly adapted for absorption that much of the water-uptake observed in these species was likely a consequence of gradual permeation of the cuticle or flooding of intercellular air spaces via open stomata.

4.4.6 Insights into the role of bundle sheath extensions

It has been hypothesised by others that BSEs serve a hydraulic role as low-resistance conduits for water transfer from vascular bundles to the epidermis, enhancing total K_{leaf} (Zwieniecki et al., 2007; Buckley et al., 2011; Szögön et al., 2015). This hypothesis was supported in the bromeliads, where species with BSEs show higher maximal K_{leaf} , as well as significantly lower sensitivity than homobaric species. This limited capacity for variation in K_{ox} in heterobaric species may be due to the relatively fixed, anatomically-defined conductance of BSEs compared with the dynamic hydraulic conductance of the mesophyll that may be associated with changes in cell turgor and aquaporin expression (see 4.4.2 above). The rehydration kinetics of bromeliad leaves with BSEs were also distinctive, showing redefined hydraulic compartmentalisation. When BSEs were present, the fast-phase compartment consisted of the veins, BSEs and epidermis, with a hydraulically isolated mesophyll (and hydrenchyma) as suggested by Zwieniecki et al. (2007). However, in the absence of BSEs, the fast-phase compartment generally comprised the veins and adjacent palisade mesophyll, with a slow-phase compartment extending to the spongy mesophyll, hydrenchyma and epidermis. BSEs therefore appear to have a major effect on the hydraulic design of bromeliad leaves. In this, they are probably supported by the frequent occurrence of large longitudinal air channels between the vascular bundles, which limit horizontal connectivity. Hydraulic isolation of the mesophyll in species with BSEs means that under transiently high evaporative demand, the hydraulic strain that occurs before stomatal closure falls on the apoplast and xylem, and the mesophyll does not have to be able to tolerate rapid reductions in water potential as it would if it were drawn on to buffer hydraulic supply under fluctuating environmental conditions. This explains why even in these species, which perform higher rates of gas exchange than other bromeliads, the solute potential of mesophyll cells is still usually remarkably high (see **Chapter 3**; Benzing, 2000; Martin et al., 2004). The implied close hydraulic connectivity between the xylem and epidermis also provides a mechanism by which efficient stomatal behaviour might be promoted in species with BSEs (Buckley et al., 2011), while the absence of BSEs would necessitate more sensitive and conservative stomatal behaviour (Zwieniecki

et al., 2007). This is broadly consistent with observations of the stomatal responses of bromeliads of different growth-forms (see **Chapter 5**).

4.4.7 *The distinctive ecology of functional types is underpinned by structure-function relationships*

The two strands of this investigation have shown how structural divergences can underlie interspecific differences in physiological function and ecological syndrome. Variation in vein placement, vascular and extra-xylary anatomy, and the efficiency of foliar trichomes in water uptake can all be considered important drivers of ecophysiological specialisation among functional types in the Bromeliaceae.

In C_3 mesic terrestrials, high vein-specific hydraulic conductances support high overall leaf hydraulic conductance and high rates of gas exchange (see also **Chapter 3**). In *Pitcairnia* species this high hydraulic capacity is associated with the presence of a midrib, but since comparable values of K_x were measured in other C_3 terrestrials lacking a midrib, it seems that this structure is not essential for the achievement of relatively high hydraulic capacity. Midribs may confer other, non-hydraulic benefits to *Pitcairnia* spp., and are probably particularly valuable for providing mechanical support to the long, narrow leaf blades. Nevertheless, midribs with high-diameter xylem conduits may be incompatible with more hydraulically stressful environments because of their intrinsic hydraulic vulnerability.

The C_3 succulent terrestrials characterised here included species from two phylogenetic lineages: the *Fascicularia-Ochagavia* clade (Bromelioideae) and the genus *Puya* (Puyoideae). Differences in venation pattern and extra-xylary anatomy defined differences in hydraulic properties between these two groups. In the *Puya* species, BSEs confer the capacity for higher hydraulic conductances than would otherwise be expected on the basis of their succulent anatomy. Indeed, hydraulic conductances were relatively high in both groups of C_3 succulent terrestrials, which may support higher rates of gas exchange than are seen in many other bromeliads (see **Chapter 3**). Water stored in extensive hydrenchyma tissue could be transiently coupled to the transpiration stream to reduce the likelihood of cavitation in the relatively wide xylem conduits of these species if extra-xylary or stomatal responses were insufficient to curtail increasing xylem tension during drought.

CAM terrestrials displayed low hydraulic conductances, explained by their venation patterns, xylem composition and extra-xylary anatomy. This is consistent with the conservative water use associated with CAM gas exchange, and the occurrence of many CAM terrestrial species in especially arid environments (see **Chapter 2**). As with the C_3 succulent terrestrials, hydraulic capacitance could

supplement the transpiration stream in these species, although more detailed work is required to explore this possibility.

Meanwhile the venation pattern C₃ tank-epiphytes probably benefit from the insertion of additional 2° veins between 1° veins in achieving greater leaf area with reduced hydraulic conductance in the water- and light-limited epiphytic niches they frequently inhabit. Maximisation of leaf area (preferably at low cost) increases the chances of intercepting sunflecks and improves the rosette's capacity to collect canopy throughfall in their impounding tanks. The relatively weak differentiation between xylem structure and theoretical conductance between 1° and 2°, sometimes driven by the absence of leaf xylem vessels, had important consequences for total K_x .

CAM tank-epiphytes all lacked xylem vessels and displayed venation pattern A in conjunction with relatively low hydraulic conductances. Although the independent origins of tank-epiphytism in the C₃ Tillandsioideae and CAM Bromelioideae are often referred to as a case of convergence (Benzing, 2000), the similarities occur primarily at the level of gross morphology rather than internal anatomy and physiology. This is partly related to the anatomical accommodation of CAM, which requires a distinctive anatomical background for optimal operational efficiency (Nelson and Sage, 2008; Zambrano et al., 2014; Heyduk et al., 2016; Males, in review). One similarity with C₃ tank-epiphytes was the degree of vascular reduction. Although differential xylem hydraulic vulnerability may not be of great ecophysiological significance among these bromeliads, vascular reduction in tank-epiphytes could be the result of enhanced trichome uptake efficiency and symplastic water distribution, as well as selection for reduced construction costs in resource-limited canopy environments (see **Chapter 3**). Furthermore, in both C₃ and CAM tank-epiphytes, K_x and K_{ox} were higher than in CAM terrestrials and atmospherics. The presence of external capacitance in the tanks of the tank-epiphytes allows them to maintain higher transpiration rates and probably to achieve higher growth rates (see **Chapter 3**).

The neotenic CAM atmospherics are very distinctive, with their morphological reduction and frequent occupation of highly exposed canopy microhabitats where they depend on brief seasonal pulses of moisture availability (Reyes-García et al., 2008). The extreme vascular reduction and succulent, densely-packed extra-xylary compartment in leaves of these species minimises hydraulic conductances, consistent with a highly conservative water-use strategy (Males, 2016).

4.5 Conclusions

Vein placement in bromeliad leaves varies widely between species and differs significantly between functional types. There is a trend towards overinvestment in more water-limited environments,

while underinvestment is more frequent in high-moisture environments. Underinvestment in veins is particularly common among C₃ tank-epiphytes with broad, thin leaf-blades and inhabiting moist, low-light microhabitats in tropical forests. Overinvestment in veins is common to multiple lineages of succulent terrestrials, and confers the dual benefit of increased extra-xylary hydraulic resistance and increased efficiency in the refilling of water storage tissue during brief pulses of water availability.

Venation architecture is a key determinant of bromeliad leaf hydraulic capacity, and vein-order-specific conductances, which depend largely on xylem conduit diameters, are of particular importance. Xylem hydraulic vulnerability, which can be explained by xylem conduit diameter and wall reinforcement, is probably of relatively limited physiological significance because of the high sensitivity of the extra-xylary compartment to leaf water potential. Variation in extra-xylary anatomy also affects hydraulic function. BSEs radically alter the hydraulic design of bromeliad leaves, isolating the mesophyll and hydrenchyma and facilitating enhanced hydraulic capacity. Trichome-mediated rehydration efficiency trades off with vein-mediated rehydration efficiency, leading to a spectrum of possible water distribution strategies that can be associated with the different functional types.

Overall, multiple divergences in anatomical traits, including vascular anatomy, venation architecture, extra-xylary leaf anatomy, and trichome structure independently and synergistically impact on hydraulic function to determine contrasting ecophysiological tolerances. Such structurally-determined divergences in physiological function have therefore probably been critical components of the evolution of ecologically distinctive functional types in the Bromeliaceae.

5. Variation and ecological significance of stomatal sensitivity to vapour pressure deficit in the Bromeliaceae

5.1. Introduction

Chapter 4 highlighted how divergences in vascular and extra-xylary leaf anatomy impact on physiological function and ecological differentiation in the bromeliads. Since it was identified in **Chapter 3** that stomatal sensitivity to leaf water status is strong and varies independently of other leaf traits, it was deemed probable that differences in stomatal structure and function could also make a pivotal contribution to the ecophysiological differentiation of the functional types. The aim of this chapter is therefore to address the following question posed in the **General Introduction**:

How does stomatal conductance and its sensitivity to vapour pressure deficit differ between species, and to what extent are these traits related to stomatal morphology?

Leaf-air vapour pressure deficit (VPD) is a key microenvironmental variable that directly impacts on leaf physiology by acting as the driving force for transpiration, and adaptation to contrasting VPD regimes is therefore expected to be an important aspect of bromeliad ecological diversity.

5.1.1 Stomatal conductance and sensitivity to VPD

The recognition of the importance of the sensitivity of the stomatal apparatus to micro-environmental factors in determining overall plant productivity, water-use efficiency, and drought resistance has spurred increasing interest in this subject in contemporary plant physiology (Bourne et al., 2015; Franks and Britton-Harper, 2016; McAdam and Brodribb, 2016). In certain functional groups of bromeliads which occur in water-limited microhabitats characterised by frequent fluctuations in light and VPD levels, rapid or strong stomatal responses might be expected to be particularly advantageous. Conversely, such a high degree of stomatal conservatism may not be required in soil-rooted terrestrials with access to a more constant supply of moisture. While no systematic survey has previously been performed to investigate the relevance of this phenomenon to ecological differentiation between bromeliad functional types, there are a few isolated reports of strong stomatal sensitivity to humidity in the bromeliads in the literature (Lange and Medina, 1979; Adams and Martin, 1986a; Lüttge et al., 1986a). Intriguingly, these studies suggested that the stomata of at least some bromeliad species could sense and respond to humidity directly, generating a so-called 'feedforward' response (Lange et al., 1971; Farquhar, 1978). Hypothetical feedforward mechanisms contrast with feedback mechanisms, which are dependent on a change in leaf water status to elicit a reduction in stomatal aperture (Bunce, 1997; Buckley, 2005). The relative

importance of feedforward and feedback effects remains controversial, and could differ between plant groups.

Alongside steady-state responses of stomatal conductance to water vapour (g_{sw}) to VPD and other stimuli, stomatal kinetics are increasingly recognised as an important determinant of overall intrinsic water-use efficiency (WUE; A/g_{sw}) and resilience to water limitation (Lawson and Blatt, 2014; McAusland et al., 2016; Vialet-Chabrand et al., 2016). When a plant at steady-state g_{sw} is exposed to a step-wise increase in VPD, g_{sw} typically displays a transient increase known as a ‘wrong-way response’ (WWR). The WWR remains an enigmatic phenomenon, with ongoing research suggesting a role for differential localised responses of plasma membrane aquaporin (PIP) activity (Nonami et al., 1991), but is generally attributed to the mechanical advantage of the epidermis (Mott and Franks, 2001; Buckley et al., 2003, 2011; Buckley, 2005). The physiological significance of the WWR is quantifiable in terms of the volume of excess water loss that it engenders (Lawson and Blatt, 2014). This is followed by a gradual decline in g_{sw} until a new, lower steady-state is attained. This adaptive ‘right-way response’ (RWR) minimises water loss under increased evaporative demand, thereby reducing the chances of a physiologically damaging drop in leaf water potential (Saliendra et al., 1995). However, because stomatal aperture affects not only water loss but also net CO₂ assimilation (A), the RWR to increased VPD restricts the supply of CO₂ for photosynthetic assimilation. Depending on the relative importance of stomatal, mesophyll and biochemical limitations on photosynthesis, A may therefore decrease in concert with reductions of stomatal aperture caused by elevated VPD. The degree of stomatal sensitivity to VPD can thus have important consequences for both plant carbon and water economies.

Stomatal sensitivity is known to differ widely across other plant groups (e.g. Morison and Gifford, 1983; Oren et al., 1999), and recent methodological and theoretical advances have been made in the field of stomatal kinetics (McAusland et al., 2016; Vialet-Chabrand et al., 2016). The ecophysiological diversity of the bromeliads make them an excellent study system, and the different stomatal complex morphologies found in the family could also provide insights into the structural contributions to stomatal sensitivity and kinetics (Tomlinson, 1969; Males, 2016). To explore the role of stomatal sensitivity in bromeliad ecophysiological diversity, the magnitude of stomatal responses to VPD were measured in 93 species representing each of the major functional types and displaying contrasting stomatal complex morphology. The kinetics of stomatal responses to small step-changes in VPD were also characterised in a subset of eight taxonomically and ecophysiological diverse C₃ species.

5.2 Methods

5.2.1 Taxon sampling and plant material

Ninety-three species were selected from across the Bromeliaceae for anatomical and physiological characterisation (see **Appendix 5.1** for full list). Taxon sampling was designed to include representatives of key radiations and critical lineages, as well as all of the principal functional types. The species included seven C₃ mesic terrestrials, seven C₃ succulent terrestrials, 21 CAM terrestrials, 22 C₃ tank-epiphytes, 23 CAM tank-epiphytes, and six CAM atmospheric. A full list of species is included in **Appendix 5.1**. Plant material was obtained from the living collections at Cambridge University Botanic Garden (CUBG) and the Royal Botanic Garden Kew (RBGK). Measurements on species from the collection at CUBG were made on intact plants, while measurements on species from RBGK were made on detached leaves. Preliminary comparisons of data produced using intact plants and detached leaves showed no statistically significant systematic effect of leaf detachment on the results of stomatal measurements (data not shown).

Eight C₃ bromeliad species from different ecological backgrounds were selected for characterisation of stomatal aperture and kinetics. Two C₃ mesic terrestrial *Pitcairnia* species (Pitcairnioideae), with small, simple anomocytic stomata and high stomatal densities were chosen: *P. integrifolia* Ker Gawl., a soft-leaved rupicolous species with a disjunct distribution in moist forests of Trinidad, northern Venezuela and southeastern Brazil, and *P. xanthocalyx* Mart., which is similar in morphology and habit, but native to tropical dry forest in Mexico. Although both of these species grow on substrates with poor water-retention capacity and are therefore frequently subjected to low levels of water availability, data presented in **Chapter 3** showed that they were nevertheless adapted to episodically high levels of water use. Two species from the Tillandsioideae subfamily were chosen: *Guzmania monostachia* (L.) Rusby ex Mez, a C₃-CAM intermediate tank-epiphyte with a wide distribution in evergreen and seasonally-deciduous forest across the Caribbean, Central America and north-eastern South America; and *Lutheria glutinosa* (Lindl.) Barfuss & W.Till, a C₃ tank-epiphyte with a poorly-developed tank that is restricted to high-rainfall montane forest in Trinidad and Venezuela. From the core Bromelioideae, *Nidularium innocentii* Lem. was selected. This thin-leaved C₃ tank-forming species is a facultative epiphyte native to the Atlantic Forest of Brazil. In these three species, large hypodermal cells protrude into the substomatal cavity. These cells can effectively occlude the pore, potentially providing a considerable resistance to gas exchange (Tomlinson, 1969). One terrestrial species from the Bromelioideae was also included: *Ochagavia elegans* Phil., a C₃ succulent terrestrial native to coastal scrub on Juan Fernandez Island, Chile. The final two species were C₃ succulent terrestrials from the genus *Puya* (Puyoideae): *Puya berteroniana* Mez, a large xerophytic species from Mediterranean scrub in central Chile; and *P. mirabilis* (Mez) L.B.Sm., a morphologically-reduced

species native to more arid scrub in Bolivia and north-western Argentina. In *O. elegans* and both *Puya* species, the large guard cells are subtended by two lateral subsidiary cells that appear to restrict effective pore aperture (Tomlinson, 1969).

5.2.2 Stomatal pore length and density

For the full set of 93 species, impressions of the abaxial epidermis of 30 replicate leaves sampled from at least three individuals of each species were taken using nail varnish. Stomatal pore length and stomatal density were measured by light microscopy, the latter across regions of at least 10 x 10 mm, depending on leaf size.

5.2.3 Stomatal aperture

To verify the coupling of guard cell movements with changes in g_{sw} measured by gas exchange, 20 leaves sampled from at least three individuals for each of the eight species in the subset used for stomatal kinetics measurements were detached and incubated under a universally-saturating photosynthetic photon flux density (PPFD) of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at a leaf-air vapour pressure deficit (VPD) of 0.02 kPa and at 0.25 kPa intervals of VPD between 0.25 kPa and 2.0 kPa. After at least 45 min of acclimation at each VPD level, epidermal impressions of the abaxial surface of the leaves were taken and observed under a light microscope. Stomatal aperture was quantified as the maximum diameter of the pore between pairs of guard cells.

5.2.4 Gas exchange measurements

Fully-expanded leaves in the second rosette layer were selected for measurements of gas exchange using a LI-6400-XT infra-red gas analyser (LI-COR, Lincoln, NE, USA). The 2 x 3 cm 6400-02B chamber was used for all species except the smaller CAM atmospheric, for which measurements were performed on whole plants using the 6400-24 bryophyte chamber fitted with a 6400-18A RGB light source. Leaves or plants were initially allowed to acclimate to chamber conditions with a VPD of 0.02 kPa at 25°C and a light intensity of either 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for C_3 species or zero for CAM species. External CO_2 was maintained at 400 ppm. All measurements for C_3 species were performed during the middle of the day, whereas for CAM species they were performed during the middle of the dark period (Phase I assimilation). Constant leaf temperature ($\pm 0.01^\circ\text{C}$) was maintained throughout all measurements. Preliminary assays showed that leaf water content did not change significantly during VPD response curves or VPD step-change analysis.

5.2.5 VPD response curves

Five replicate VPD response curves were performed for each species using the LI-6400-XT to measure gas exchange, using leaves from at least three individuals. VPD was varied by altering absolute water content of the air while holding leaf temperature constant (Buckley, 2005). Each leaf was allowed to acclimate at a VPD of 0.02 kPa until g_{sw} reached steady state (approximately one hour). Once steady-state g_{sw} was reached, this maximal value ($g_{sw,max}$) was recorded along with the mean assimilation rate (A_{max}), transpiration rate (E_{init}) and intrinsic water-use efficiency (WUE_{init}) at the same steady state. VPD was then increased by dehumidification of the air stream into the Li-6400XT to 0.25 kPa. When a new steady-state g_{sw} was attained, all gas exchange parameters were logged and VPD was increased to 0.5 kPa. This process was repeated at VPD intervals of 0.25 kPa up to 2.0 kPa. Normalised curves of g_{sw} , A , E and WUE were then plotted as functions of VPD. Stomatal sensitivity to VPD (S_s) was quantified as the slope of the linear relationship between the natural logarithm of g_{sw} and VPD.

5.2.6 VPD step-change analysis

Eight replicate VPD step-change responses were recorded for each of the eight species used for analysis of stomatal kinetics, sampling leaves from at least three individuals per species. Each leaf was allowed to acclimate at a VPD of 0.5 kPa until g_{sw} reached steady state (approximately 1 h). Gas exchange variables were continuously recorded at 120 s intervals from this point onwards. After 10 minutes of logging at steady-state g_{sw} , the air-inlet stream was immediately switched to a dehumidified stream to increase VPD to 0.8 kPa. The response of g_{sw} , E , and A to this step-change in VPD was monitored and logging continued until the WWR and subsequent RWR were completed with the arrival at a new steady state.

5.2.7 Phylogenetic patterning

Cladograms were produced for the sets of species belonging to the three major subfamilies (Bromelioideae, Pitcairnioideae, and Tillandsioideae) based on recent phylogenetic analyses (Givnish et al., 2014; Silvestro et al., 2014). Since species-level relationships are still not definitively resolved, all species within each genus were represented by a polytomy and all phylogenetic distances were set to 1. The 'contMap' function from the package 'phytools' (Revell, 2012) in R (R Development Core Team, 2008) was then used to produce heat-map trees to display semi-quantitative variation in $g_{sw,max}$ and S_s in the Bromelioideae, Pitcairnioideae, and Tillandsioideae.

5.3 Results

5.3.1 Variation in stomatal conductance and sensitivity to VPD across functional types

Maximal stomatal conductance to water vapour ($g_{sw,max}$) varied across all species by a factor of 10, and differed between functional types (Fig. 5.3.1a). The highest values of $g_{sw,max}$ ($\leq 0.39 \text{ mol m}^{-2} \text{ s}^{-1}$) occurred in the C_3 succulent terrestrials, followed by C_3 mesic terrestrials and C_3 tank-epiphytes. The C_3 functional types also showed the greatest within-group variation. In both CAM terrestrials and CAM tank-epiphytes, $g_{sw,max}$ was between 0.05 and 0.10 $\text{mol m}^{-2} \text{ s}^{-1}$, whereas CAM atmospheric showed especially low values ($< 0.05 \text{ mol m}^{-2} \text{ s}^{-1}$). Complete data for all gas exchange and other measurements are provided in **Appendix 5.1**.

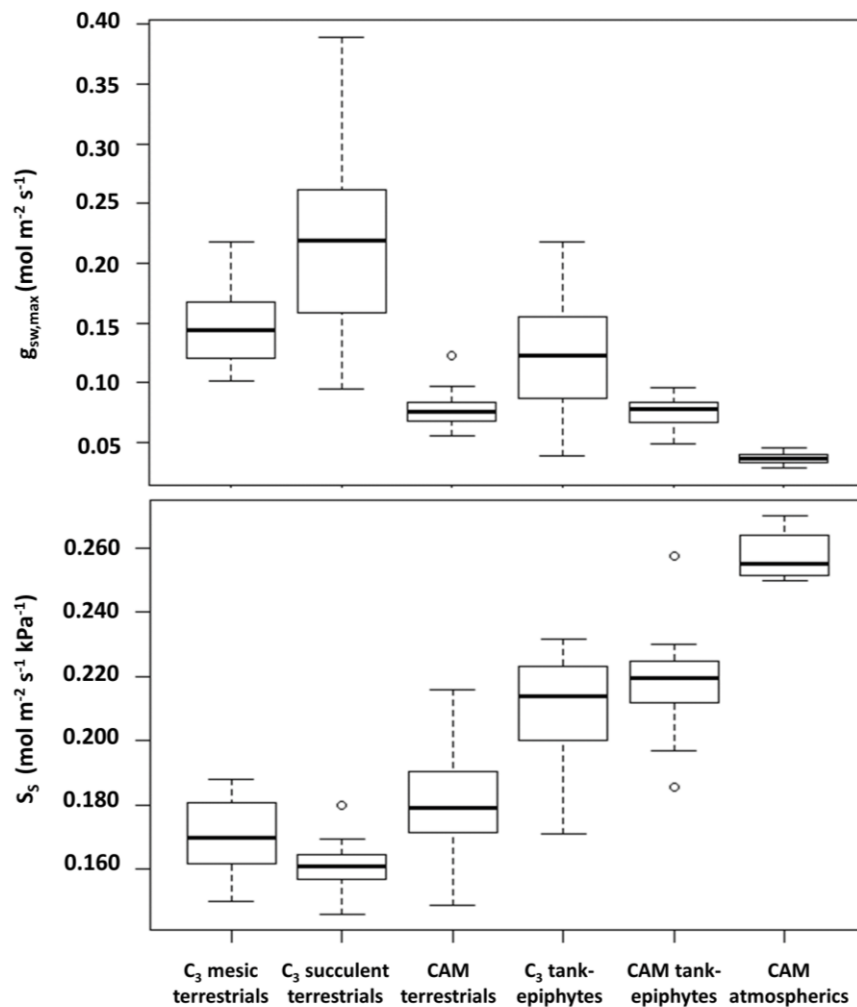


Figure 5.3.1. a) Variation in maximal stomatal conductance to water vapour ($g_{sw,max}$) among bromeliad functional types. b) Variation in instantaneous stomatal sensitivity to VPD (S_s) among bromeliad functional types.

Functional types differed in the response of g_{sw} to VPD. For each species, stomatal sensitivity to VPD (S_s) was quantified, and the resulting values are plotted by functional type in Fig. 5.3.1b. The full response curves of g_{sw} (normalised by $g_{sw,max}$) to VPD are shown by functional type in Fig. 5.2. The lowest values of S_s occurred in the C_3 terrestrials, with the median value being marginally lower in the succulent species ($0.160 \text{ mol m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$). CAM terrestrials were highly variable but the median value ($0.180 \text{ mol m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$) was slightly higher than for either of the C_3 terrestrial functional types. Both C_3 and CAM tank-epiphytes showed somewhat higher median values (ca. $0.220 \text{ mol m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$), with more interspecific variation occurring among the C_3 species. The highest values of S_s were observed in the CAM atmospheric (s) ($0.260 \text{ mol m}^{-2} \text{ s}^{-1} \text{ kPa}^{-1}$). The reduction in g_{sw} in response to VPD between 0.02 and 2.00 kPa was sufficient to elicit a feedforward decrease in E in half of the C_3 mesic terrestrials and CAM terrestrials, but none of the C_3 succulent terrestrials. All tank-epiphyte species showed feedforward behaviour of E , with some displaying zero transpiration by 2.00 kPa because of complete stomatal closure. Meanwhile all CAM atmospheric (s) showed zero transpiration by 1.25 kPa, making the feedforward response strongest in this group.

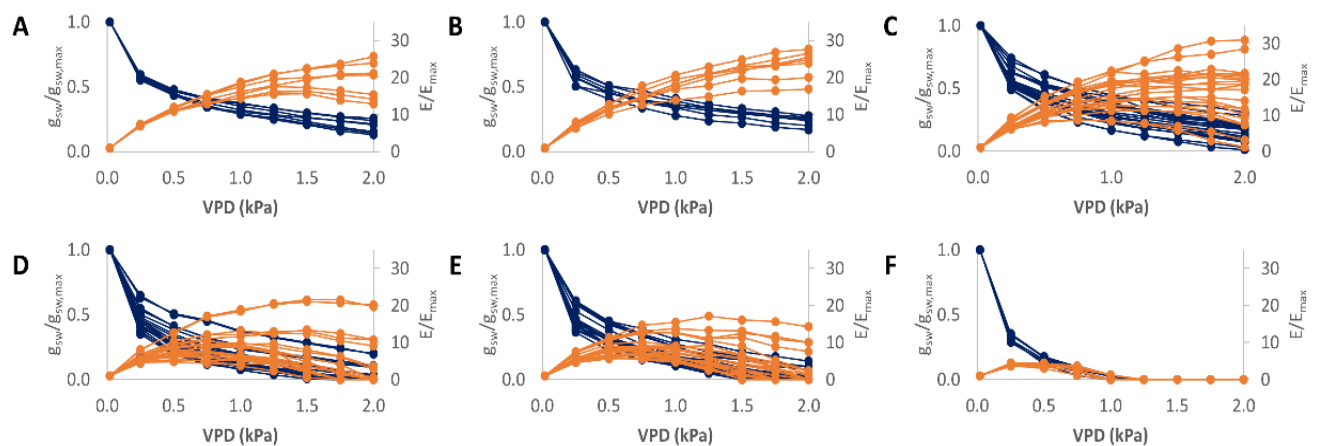


Figure 5.3.2. Blue lines: responses to leaf-air vapour pressure deficit (VPD) of stomatal conductance to water vapour (g_{sw}) normalised by maximal stomatal conductance to water vapour ($g_{sw,max}$). Orange lines: responses to VPD of transpiration rate (E) normalised by initial transpiration rate at 0.02 kPa (E_{init}). Data are for representative species of six functional types of bromeliads: a) C_3 mesic terrestrials ($n = 7$); b) C_3 succulent terrestrials ($n = 7$); c) CAM terrestrials ($n = 21$); d) C_3 tank-epiphytes ($n = 22$); e) CAM tank-epiphytes ($n = 23$); f) CAM atmospheric (s) ($n = 6$).

Across all species, there was a weak negative relationship ($r^2 = 0.33$, $p < 0.001$) between log-transformed values of $g_{sw,max}$ and S_s (Fig. 5.3.3).

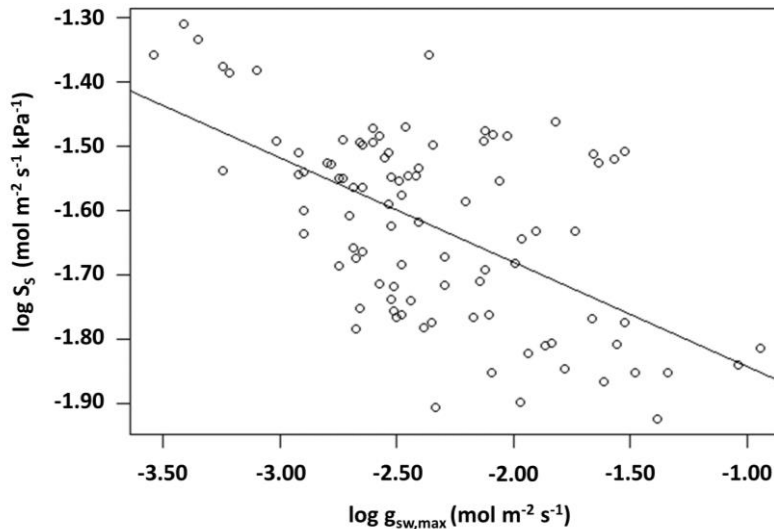


Figure 5.3.3 Relationship between log-transformed maximal stomatal conductance to water vapour ($g_{sw,max}$) and instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s) for 93 bromeliad species. Line shows linear regression.

Changes in stomatal aperture (α) in response to VPD were directly measured in a subset of C_3 species. Maximum stomatal aperture measured as pore width (α_{max}) was highest in the succulent terrestrials (5.39 μm in *Puya berteroniana*) and lowest in the tank-epiphytes (2.56 μm in *Lutheria glutinosa*). There was no significant correlation between pore length and α_{max} for these species ($r^2 = 0.29$, $p = 0.17$). Across the eight species, there was a strong positive correlation between α_{max} and $g_{sw,max}$ ($r^2 = 0.87$, $p < 0.01$). Equivalent sensitivity values calculated using stomatal aperture rather than conductance data (S_α) were positively correlated with S_s ($r^2 = 0.74$, $p < 0.01$).

5.3.2 Effect of stomatal sensitivity to VPD on assimilation and water-use efficiency

The increase in VPD also brought about a reduction in A for all species, indicative of the universal importance of stomatal limitations to photosynthesis in the bromeliads (Fig. 5.3.4). Differences in the response of A to VPD between functional types were broadly in line with those observed for g_{sw} , although variation in the instantaneous sensitivity of A to VPD (S_A) appeared to be driven largely by photosynthetic pathway, with CAM functional types showing higher values than C_3 types (Fig. 5.3.5). Characteristic responses of A and g_{sw} to VPD defined interspecific differences in the relationship between VPD and WUE (Fig. 4.13). In species of both C_3 terrestrial functional types, WUE showed a modest increase between 0.02 and 2.00 kPa VPD. In some CAM terrestrials, this improvement in WUE was considerably stronger. Among both C_3 and CAM tank-epiphytes, WUE either peaked around 1.50 kPa prior to stomatal closure, or continued to increase to 2.00 kPa if stomata remained

open. In CAM atmospheric, WUE peaked between 0.75 and 1.00 kPa before complete stomatal closure occurred.

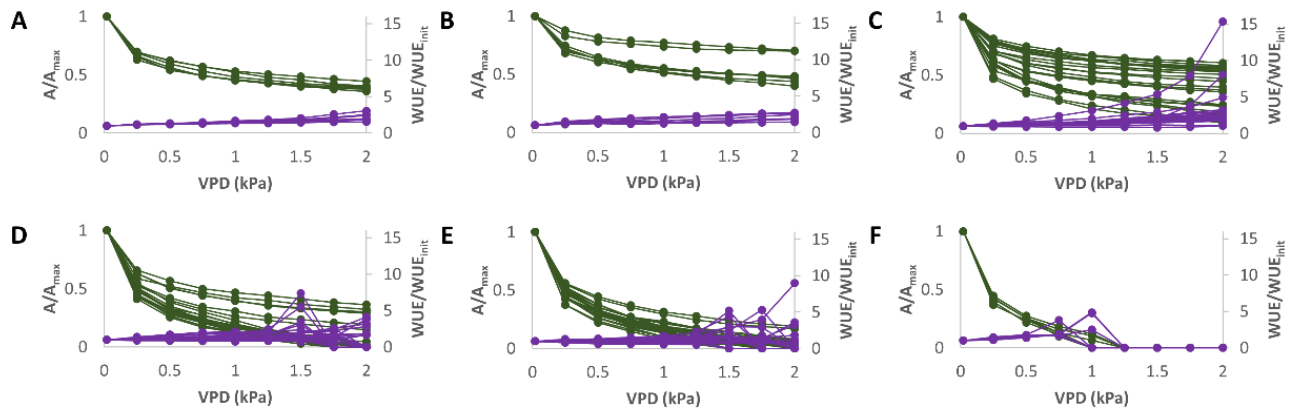


Figure 5.3.4. Green lines: responses to leaf-air vapour pressure deficit (VPD) of assimilation rate (A) normalised by maximum photosynthetic capacity (A_{max}). Purple lines: responses to VPD of intrinsic water-use efficiency (WUE) normalised by initial WUE at 0.02 kPa (WUE_{init}). Data are for representative species of six functional types of bromeliads: a) C_3 mesic terrestrials ($n = 7$); b) C_3 succulent terrestrials ($n = 7$); c) CAM terrestrials ($n = 21$); d) C_3 tank-epiphytes ($n = 22$); e) CAM tank-epiphytes ($n = 23$); f) CAM atmospheric ($n = 6$).

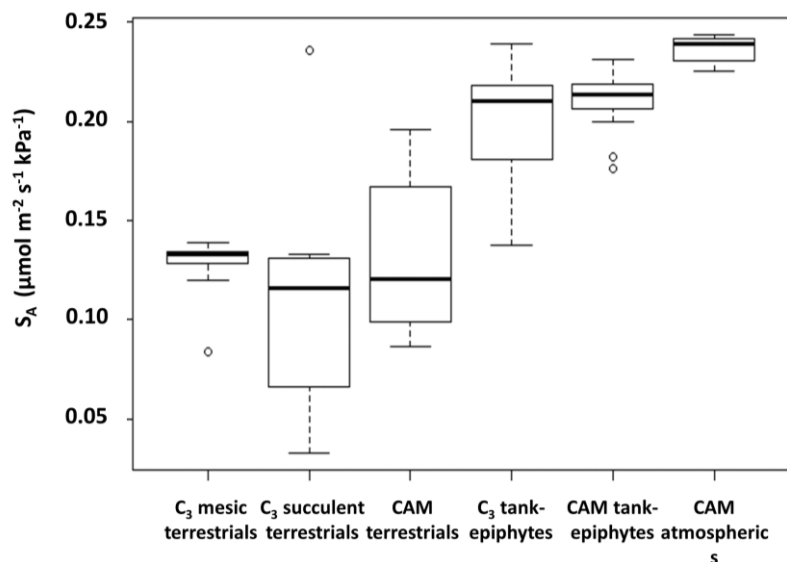


Figure 5.3.5. Variation in the instantaneous sensitivity of assimilation rate to VPD (S_A) among bromeliad functional types ($n = 93$).

There was a weak positive correlation between A_{\max} and $g_{\text{sw,max}}$ ($r^2 = 0.36$, $p < 0.001$; Fig. 5.3.6a), and a much stronger positive correlation between S_A and S_S ($r^2 = 0.70$, $p < 0.001$; Fig. 5.3.6b). These relationships are consistent with an important role for stomatal conductance in limiting photosynthetic performance in bromeliads.

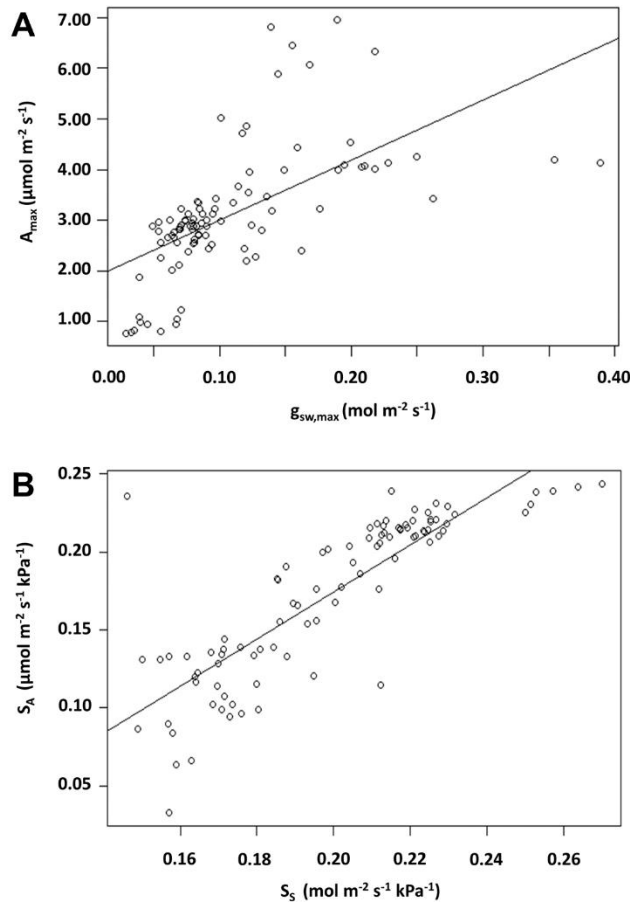


Figure 5.3.6. a) Relationship between maximal stomatal conductance to water vapour ($g_{\text{sw,max}}$) and maximum photosynthetic capacity (A_{\max}) for 93 bromeliad species. b) Relationship between instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_S) and instantaneous sensitivity of assimilation rate to leaf-air vapour pressure deficit (S_A) for 93 bromeliad species. Lines show linear regression.

5.3.3 Phylogenetic trait patterning

In the Bromelioideae subfamily, the highest values of $g_{\text{sw,max}}$ were concentrated in the C_3 terrestrial clade (*Greigia-Fascicularia-Ochagavia*; Fig. 5.3.7a), which also showed the lowest values of S_S (Fig. 5.3.7b). Particularly low values of $g_{\text{sw,max}}$ occurred among the CAM tank-epiphytes in the Core Bromelioideae, and this clade also displayed considerably higher S_S than the rest of the subfamily.

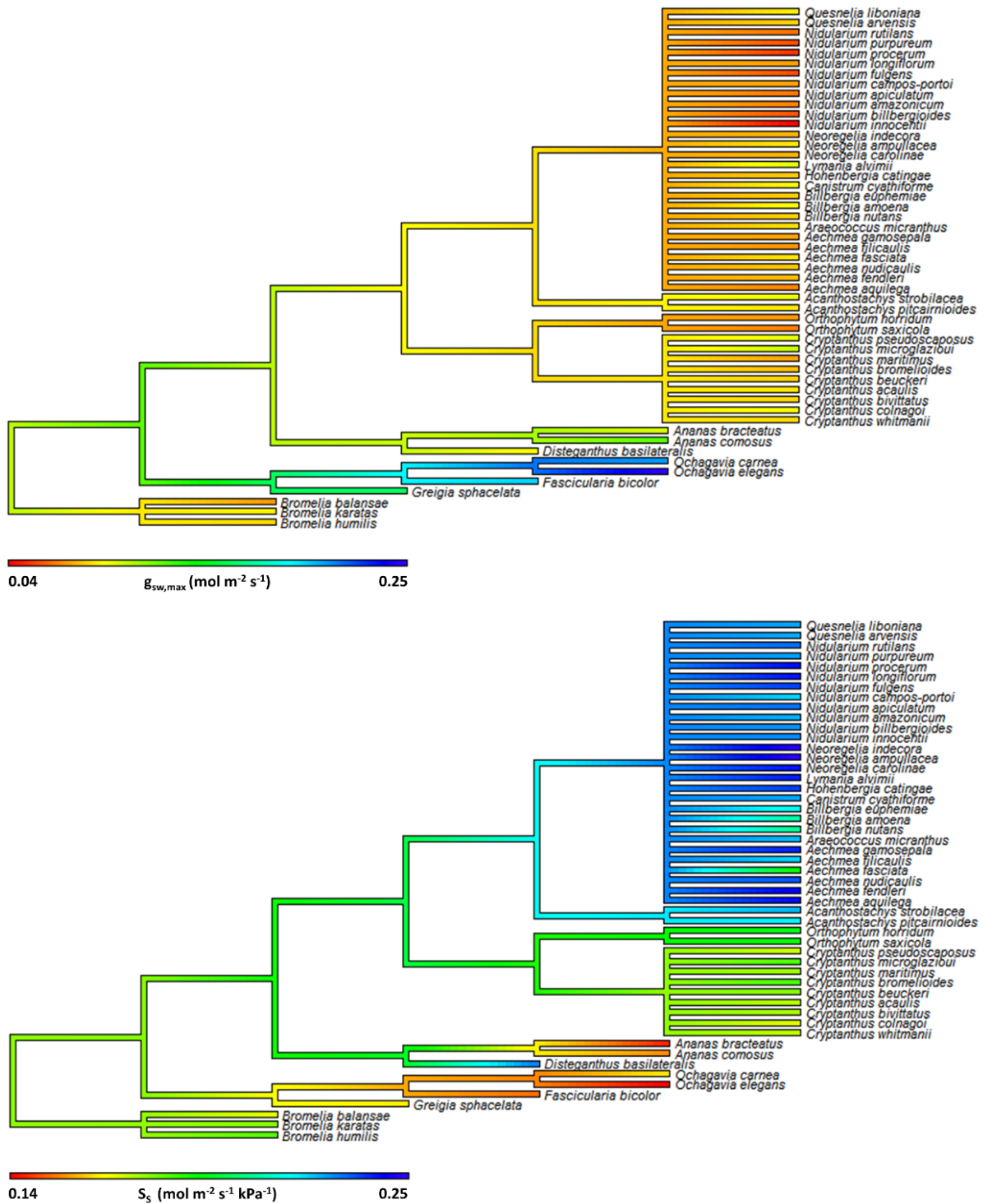


Figure 5.3.7. Phylogenetic patterning of stomatal traits in the Bromelioideae: a) maximal stomatal conductance to water vapour ($g_{sw,max}$); b) instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s). Phylogenetic topology based on Givnish et al. (2014) and Silvestro et al. (2014); all genetic distances set equal.

In the Pitcairnioideae, there was a clear evolutionary trend in both $g_{sw,max}$ and S_s (Fig. 5.3.8a,b). *Pitcairnia* species showed the highest values of $g_{sw,max}$ and the lowest values of S_s , while the Xeric

Clade species (*Deuterocohnia-Dyckia*) displayed the lowest values of $g_{sw,max}$ and highest values of S_s . The semi-xeromorphic C_3 mesic terrestrial *Fosterella* species showed intermediate values for both traits.

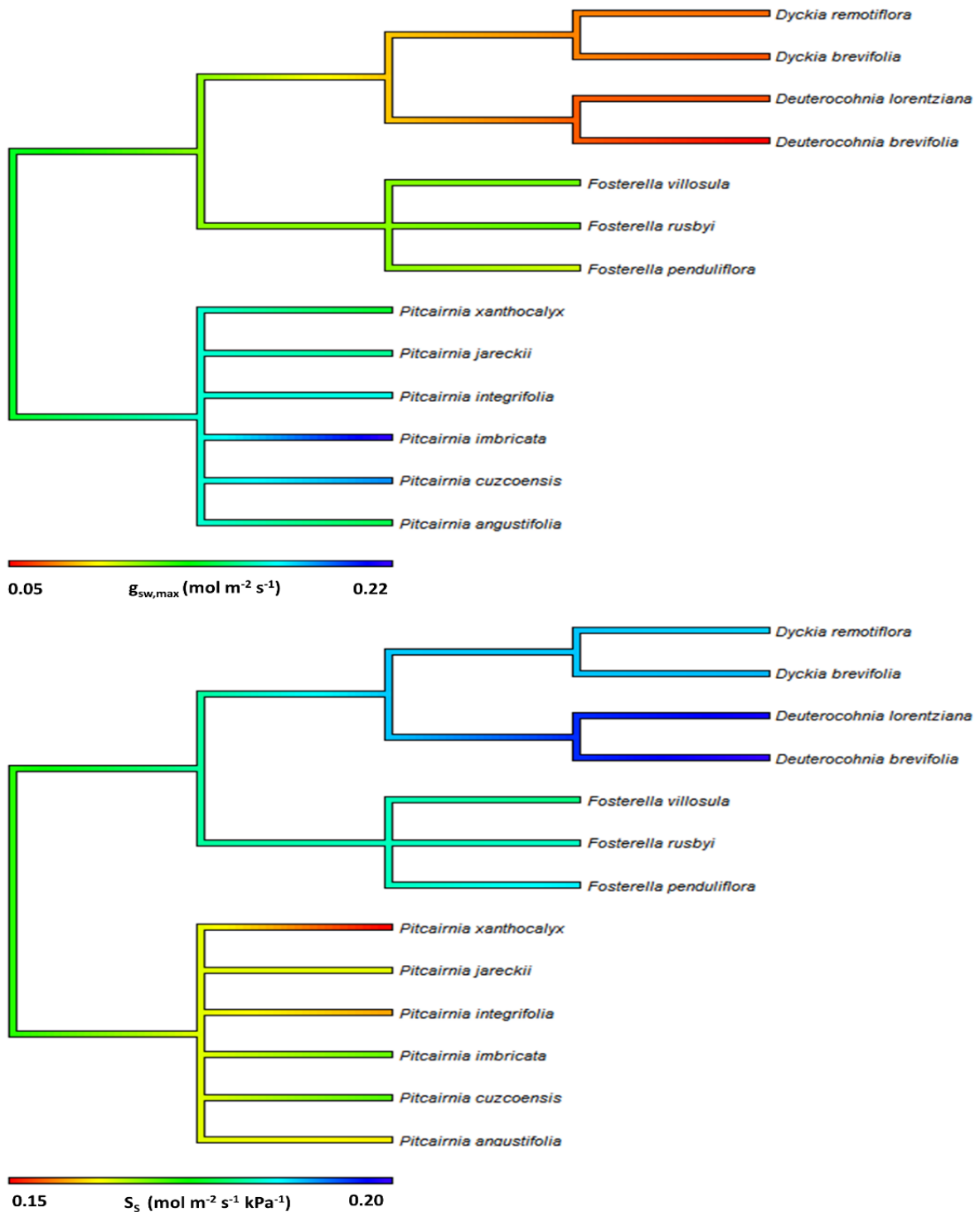


Figure 5.3.8. Phylogenetic patterning of stomatal traits in the Pitcairnioideae: a) maximal stomatal conductance to water vapour ($g_{sw,max}$); b) instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s). Phylogenetic topology based on Givnish et al. (2014); all genetic distances set equal.

Similarly clear patterns were discernible in the Tillandsioideae (Fig. 5.3.9a,b). The atmospheric *Tillandsia* species were most distinctive, displaying the lowest values of $g_{sw,max}$ and the highest values of S_s . Among the rest of the subfamily, *Lutheria* and *Vriesea* species showed the highest values of $g_{sw,max}$, whereas the lowest values of S_s occurred in *Guzmania* species.

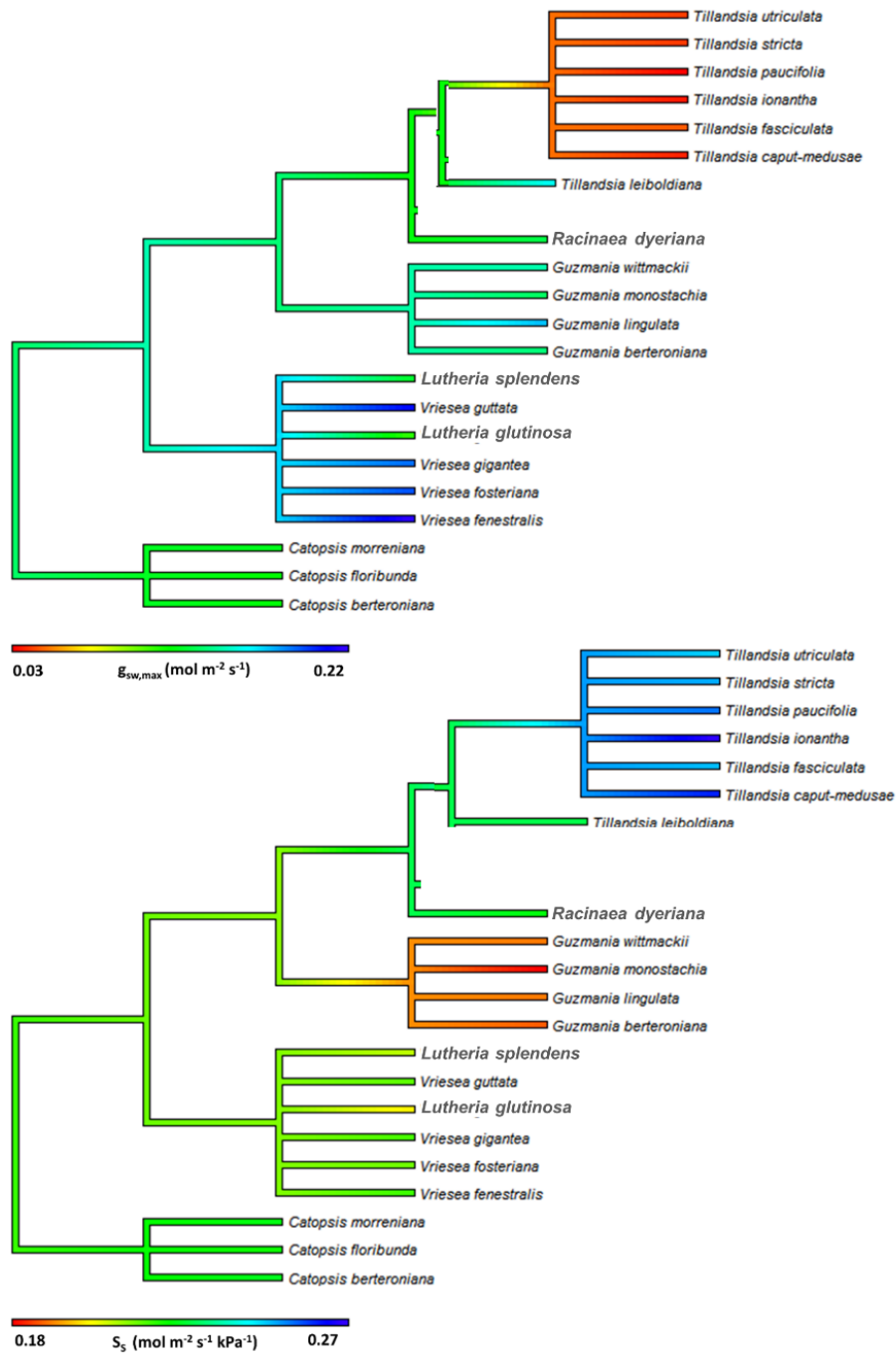


Figure 5.3.9. Phylogenetic patterning of stomatal traits in the Tillandsioideae: a) maximal stomatal conductance to water vapour ($g_{sw,max}$); b) instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s). Phylogenetic topology based on Givnish et al. (2014); all genetic distances set equal.

5.3.4 Stomatal density and morphology

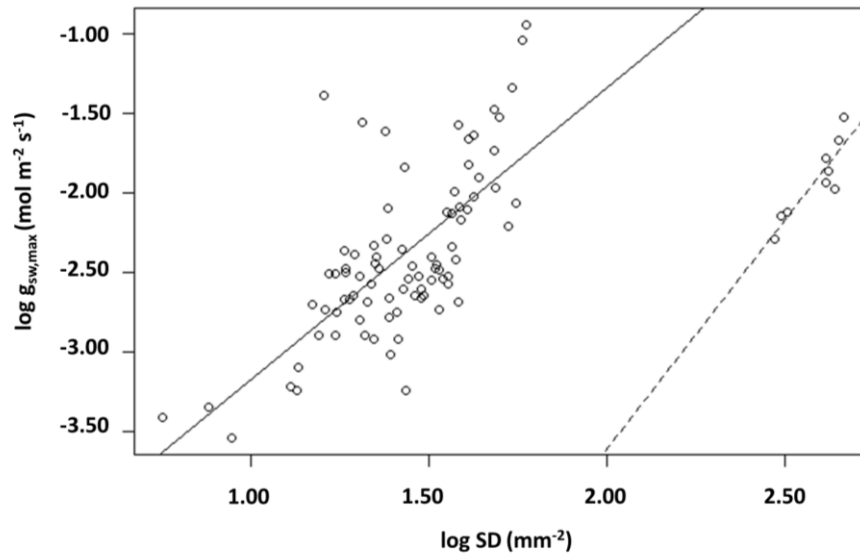


Figure 5.3.10. Relationship between log-transformed stomatal density (SD) and maximal stomatal conductance to water vapour ($g_{sw,max}$) in *Fosterella* and *Pitcairnia* species (dashed line shows linear regression) and all remaining species (solid line shows linear regression).

Stomatal density was an order of magnitude higher in the genera *Fosterella* and *Pitcairnia* than in any other genus. However, there were similar positive linear relationships between log-transformed stomatal density and $g_{sw,max}$ both among *Fosterella* and *Pitcairnia* species ($r^2 = 0.76$, $p = 0.001$) and among all remaining species ($r^2 = 0.48$, $p < 0.001$), as shown in Fig. 5.3.10.

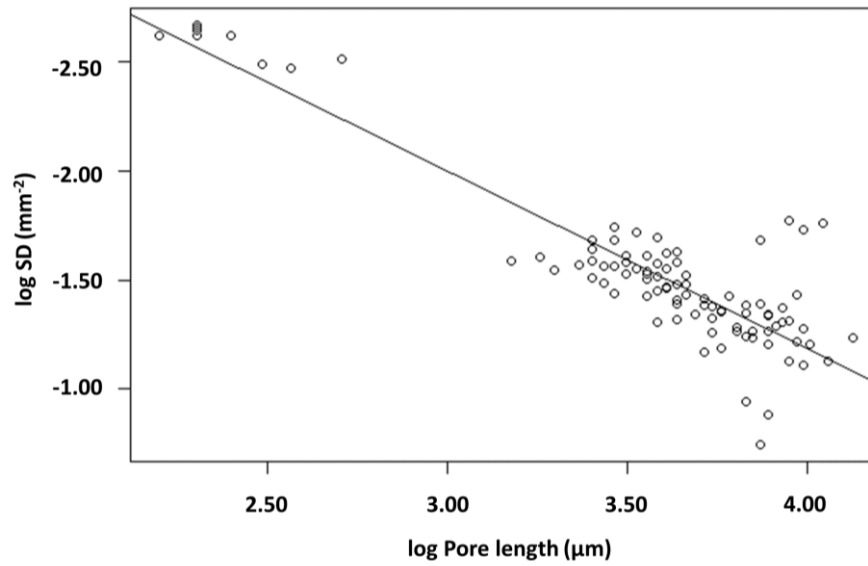


Figure 5.3.11. Relationship between log-transformed stomatal pore length and stomatal density (SD). Line shows linear regression.

There was a negative correlation between log-transformed stomatal pore length and stomatal density ($r^2 = 0.81$, $p < 0.001$; Fig. 5.3.11). However, there was no correlation between pore length and $g_{sw,max}$.

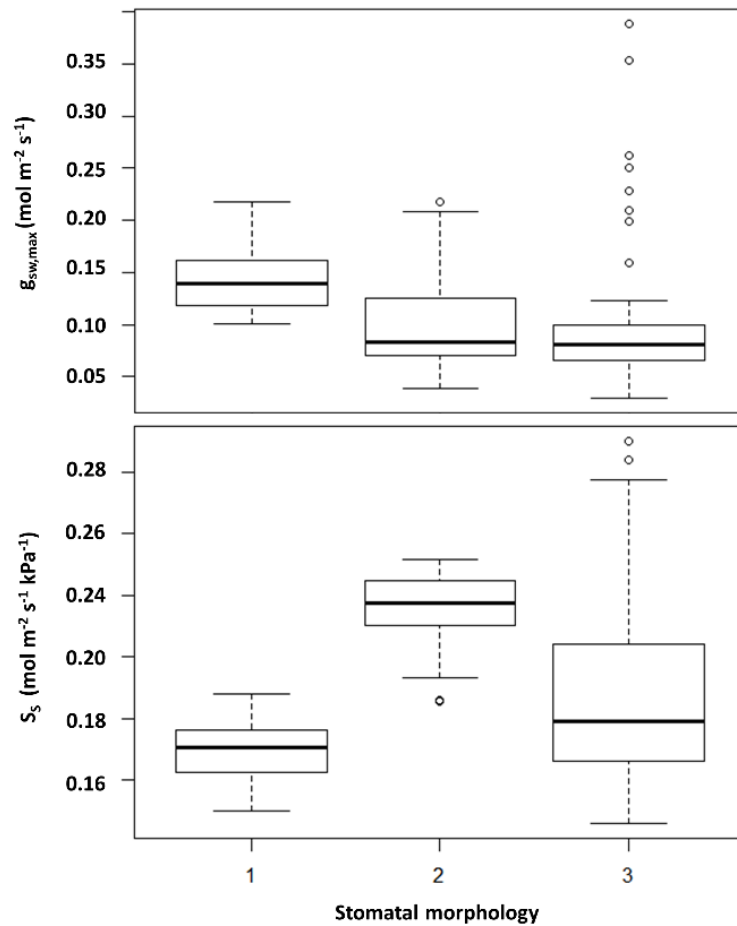


Figure 5.3.12. Variation of stomatal traits among species of different stomatal morphologies: a) maximal stomatal conductance to water vapour ($g_{sw,max}$); b) instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s). Key to stomatal morphologies: 1) unmodified; 2) modified hypodermal cells; 3) modified epidermal subsidiary cells.

Modified stomatal morphology was associated with lower $g_{sw,max}$ and higher S_s (Fig. 5.3.12). Species with either modified hypodermal cells or subsidiary cells did not differ significantly in $g_{sw,max}$, but the median value of S_s was higher for species with modified hypodermal cells than those with modified subsidiary cells. There was considerably more variation among species of the latter group, suggesting that other factors may be important determinants of stomatal sensitivity in these plants.

4.3.3.5 Coordination with drought tolerance

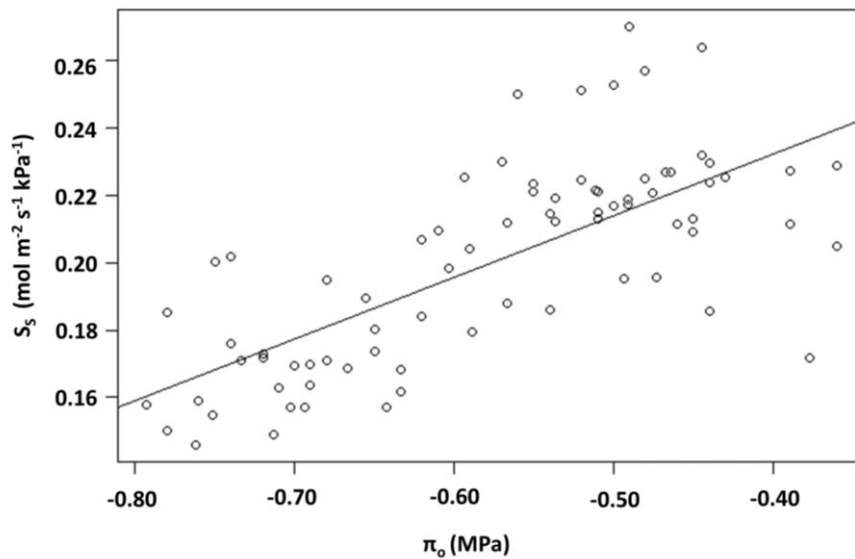


Figure 5.3.13. Relationship between osmotic potential at full turgor (π_0) and instantaneous stomatal sensitivity to leaf-air vapour pressure deficit (S_s) among 77 bromeliad species. Line shows linear regression.

For a subset of 77 species, values of π_0 from **Chapter 3** were available for comparison with S_s . The two traits showed a strong positive correlation ($r^2 = 0.50$, $p < 0.001$; Fig. 5.3.13), indicating that species which are less capable of osmolyte accumulation and are therefore more sensitive to drought also tend to have stomata with higher sensitivity to VPD.

5.3.5 VPD step-change analysis

As expected, a step-increase in VPD from 1.3 kPa to 1.8 kPa elicited a transient WWR followed by a decline in g_{sw} to a new, lower steady state in all species (Fig. 5.3.14). There was an equivalent decline in A in all cases, such that intrinsic WUE was lower in the new steady state for all species except *Puya mirabilis*. This contrasts with the steady-state data presented above, perhaps because adjustments in photosynthetic activity had not been completed across the time period for which non-steady-state gas exchange was recorded. The kinetics of the stomatal response to step-changes varied between species. Interspecific differences in response profiles could be expressed in terms of a set of key parameters, displayed in Table 5.3.1. The temporal duration of the WWR, t_{WWR} , was quantified, and found to be lowest in the three C_3 tank-epiphyte species (*G. monostachia*, *L. glutinosa*, and *N. innocentii*). The highest values of t_{WWR} were recorded in the C_3 succulent terrestrials. The area under the g_{sw} curve for the duration of the WWR (AUC_{WWR}) was used as an

indicator of the amount of additional water lost during the initial WWR above the amount of water that would be transpired if the initial steady-state g_{sw} did not change. AUC_{WWR} was highest in the C_3 succulent terrestrials and lowest in the C_3 tank-epiphytes, indicating that this group lost the least water during the WWR. The close positive correlation between t_{WWR} and AUC_{WWR} across species ($r^2 = 0.80$, $p < 0.01$) highlights the importance of stomatal kinetics in determining the efficiency of responses to environmental cues. There was a strong negative correlation between t_{WWR} and S_s ($r^2 = 0.59$, $p = 0.03$), suggesting that species with faster stomatal kinetics in response to VPD also show the highest sensitivity to VPD in steady-state g_{sw} .

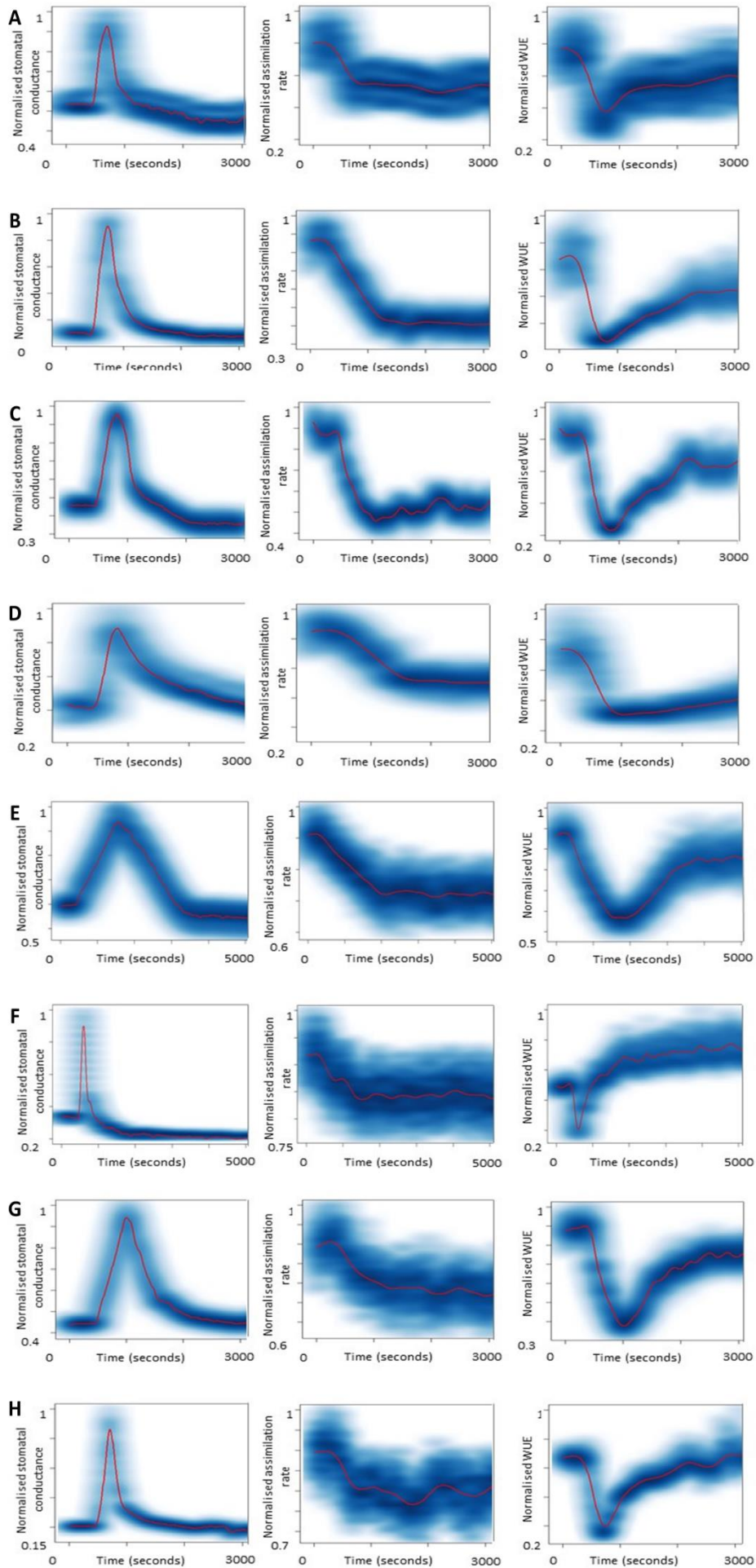


Figure 5.3.14 (overleaf). Response of stomatal conductance to water vapour (g_{sw}), assimilation rate (A), and intrinsic water-use efficiency (WUE) to an instantaneous increase of leaf-air vapour pressure deficit (VPD) from 1.3 kPa to 1.5 kPa at constant temperature. a) *Nidularium innocentii*; b) *Ochagavia elegans*; c) *Guzmania monostachia*; d) *Lutheria glutinosa*; e) *Puya berteroniana*; f) *Puya mirabilis*; g) *Pitcairnia integrifolia*; h) *Pitcairnia xanthocalyx*. Blue point clouds show data from eight biological replicates; red lines are smoothed splines.

Parameters related to the RWR were also quantified for each species (Table 5.3.1). The duration of the RWR, t_{RWR} , varied between 1010 s in the C₃ mesic terrestrial *P. xanthocalyx* and 2560 s in the C₃ succulent terrestrial *P. mirabilis*. Unlike with t_{WWR} , t_{RWR} was not correlated with S_s ($r^2 = 0.11$, $p = 0.43$). Whereas the C₃ mesic terrestrial species showed both low t_{RWR} and low S_s , the C₃ succulent terrestrials showed high t_{RWR} but low S_s . By contrast, C₃ tank-epiphytes combined high S_s with low t_{RWR} , which could be considered the most efficient trait combination for water conservation.

| Functional type | Species | t_{WWR} (s) ± SE | AUC _{WWR} (mol m ⁻²) ± SE | t_{RWR} (s) ± SE |
|--------------------------------------|--------------------------------|-----------------------|---|-----------------------|
| C ₃ tank-epiphyte | <i>Nidularium innocentii</i> | 670 ± 22 | 3 ± 0.2 | 1425 ± 54 |
| C ₃ succulent terrestrial | <i>Ochagavia elegans</i> | 1920 ± 114 | 61 ± 4.5 | 1950 ± 79 |
| C ₃ tank-epiphyte | <i>Guzmania monostachia</i> | 900 ± 58 | 28 ± 0.9 | 1120 ± 62 |
| C ₃ tank-epiphyte | <i>Lutheria glutinosa</i> | 910 ± 50 | 12 ± 1.4 | 1520 ± 105 |
| C ₃ succulent terrestrial | <i>Puya berteroniana</i> | 2560 ± 188 | 219 ± 7.3 | 2360 ± 194 |
| C ₃ succulent terrestrial | <i>Puya mirabilis</i> | 1950 ± 103 | 138 ± 2.9 | 2560 ± 177 |
| C ₃ mesic terrestrial | <i>Pitcairnia integrifolia</i> | 1210 ± 97 | 100 ± 1.8 | 1310 ± 92 |
| C ₃ mesic terrestrial | <i>Pitcairnia xanthocalyx</i> | 1490 ± 156 | 72 ± 1.4 | 1010 ± 81 |

Table 5.3.1. Mean species values of parameters describing wrong- and right-way responses of g_{sw} to an increase of VPD from 0.3 kPa to 0.8 kPa. t_{WWR} = duration of wrong-way response; AUC_{WWR} = area under stomatal conductance curve during wrong-way response; t_{RWR} = time until completion of right-way response.

The step-change in VPD caused a reduction in A in all species, with a new steady-state value of A always being reached within 3000 s. The physiological significance of the WWR is reflected in the strong transient decrease in intrinsic WUE observed in all species, where excessive water loss occurs alongside reducing carbon assimilation. The rate of recovery of WUE associated with stomatal closure differed between species and was controlled by the rate of change in g_{sw} rather than A .

5.4 Discussion

5.4.1 Stomatal sensitivity to VPD and kinetics

The characterisation presented here of steady-state stomatal sensitivity to leaf-air vapour pressure deficit (VPD) in 93 bromeliad species and of stomatal kinetics in response to changes in VPD in eight diverse C_3 bromeliad species demonstrates the potential for divergences in stomatal structure and function to modulate water-use efficiencies and bioclimatic relations. Comparison of the stomatal properties of the bromeliads studied here with those of other plant groups (e.g. Oren et al., 1999) suggests that stomatal conductance is low and stomatal sensitivity to VPD is high in all bromeliads. This is consistent with their occupation of the 'slow-growing' end of the plant economics spectrum (see **Chapter 3**). The duration of the responses were broadly comparable with those measured for stomatal opening in response to light in a range of herbaceous and woody species by McAusland et al. (2016).

The operation of the feedforward effect of S_s on transpirational water loss in C_3 and CAM epiphytic functional types, even across the relatively modest range of VPD values used in this investigation, clearly shows that S_s is able to restrict rates of water loss in these species. Visual inspection of the E -VPD curves for other species suggests that at higher values of VPD, further reductions in g_{sw} will be sufficient to cause E to begin to fall. The feedforward effect, which occurs in some but not all plant groups and has stoked some controversy (Lange et al., 1971; Farquhar, 1978; Meinzer et al., 1984; Grantz, 1990; Assmann and Gershenson, 1991; Mott and Parkhurst, 1991; Monteith, 1995; Saliendra et al., 1995; Franks et al., 1997; Buckley, 2005; Peak and Mott, 2011), is therefore probably ubiquitous in the Bromeliaceae, and could be critical to their ecological variety (see 5.4.2 below). The underlying mechanisms, which could involve direct sensing of humidity in the stomatal pore (Mott and Parkhurst, 1991; Peak and Mott, 2011) or of transpiration rate (Franks et al., 1997), will require further investigation.

Interspecific variation in S_s had clear consequences for net carbon fixation and WUE. The tight correlation between S_s and S_A is strong evidence for a major role for stomatal conductance in limiting photosynthetic capacity in the bromeliads, consistent with the results of photosynthetic limitation analysis in **Chapter 3**. S_A showed a particularly clear distinction between terrestrial and epiphytic functional types, with the latter showing higher values. For epiphytes, the effect of high S_s on A is compounded by environmentally-constrained low nitrogen content limiting biochemical photosynthetic capacity (see **Chapter 3**). WUE either increased or remained relatively constant in most species until stomatal closure occurred.

Stomatal sensitivity to VPD appears to represent an integral component of bromeliad drought resistance syndromes. Using data from **Chapter 3**, it has been shown that there is a strong positive relationship between the bulk osmotic potential at full turgor (π_o) and S_s . Thus species which are less tolerant of drought at the cellular level (i.e. less negative π_o) tend to have stomata that are more sensitive to VPD. This supports the notion of a continuum of drought resistance strategies running from tolerance of low water potentials (e.g. by investment in osmolyte accumulation) to avoidance of water loss (e.g. by conservative stomatal behaviour). This coordination contrasts with the apparent independence of stomatal sensitivity to leaf water status (quantified as P_{50s} ; see **Chapter 3**). This is perhaps because stomatal responses to VPD are so efficient at curtailing excessive water loss that perturbations of leaf water potential are minimal, and P_{50s} has therefore probably been subjected to rather weaker selection that might lead to coordination with other drought resistance traits.

While the inferences drawn here are based on measurements made on mature plants, the heteroblastic development of rosette form in many tank bromeliads means that juvenile plants are morphologically and physiologically distinctive. Adams and Martin (1986) showed that juvenile and mature plants of *Tillandsia depeana* Steud. differed in their VPD responses, suggesting that stomatal sensitivity can change within individuals between developmental stages, although this is likely to result from ontogenetic drift (Meisner et al., 2013). Further work should be performed to definitively ascertain the potential for developmental effects and short- or long-term environmental acclimation to determine stomatal responses to humidity in different functional types. It seems probable that the light sensitivity of stomata of tank-epiphytes is also high, particularly among those that are adapted to forest environments where light availability is concentrated in sun-flecks (Tinoco-Ojanguren and Percy, 1992; Company et al., 2016). However, some other vascular epiphytes show slow stomatal opening in response to sunflecks (Zotz & Mikona, 2003; Zhang et al., 2009), minimising water expenditure but also limiting their carbon gain during sunfleck exposure. The question of whether the same paradigm holds true for the bromeliads is another area ripe for investigation.

5.4.2 Stomatal aspects of functional type differentiation

Despite the very high stomatal densities measured in C_3 terrestrial *Pitcairnia* spp., these species did not display significantly higher maximal stomatal conductance than some C_3 tank-epiphytes. This is consistent with the fact that even these comparatively mesomorphic bromeliads show rather low absolute values of hydraulic conductance that are strongly sensitive to plant water potential (**Chapter 3**). However, steady-state stomatal conductance is less sensitive to VPD in the mesic

terrestrials than in tank-epiphytes. Even in the most mesomorphic *Pitcairnia* species, a certain amount of foliar hydrenchyma is always present, and this internal reservoir may help buffer against changes in transpiration rate induced by variation in VPD. Stomatal sensitivity is nevertheless sufficient for modest increases in VPD to engender a rise in WUE. In terms of stomatal kinetics, C₃ mesic terrestrial species appeared to be less conservative than tank-epiphytes, losing more water during WWRs, although they did display rapid RWRs in response to step-changes in VPD.

It is interesting to note that the C₃ succulent terrestrial bromeliads used in this investigation displayed a strong reduction in actual stomatal conductance to water vapour (g_{sw}) relative to the maximum value ($g_{sw,max}$) across the comparatively modest VPD levels to which they were subjected. In nature, these plants probably frequently experience somewhat higher VPD for much of the day (e.g. in coastal Chile; Giliberto and Estay, 1978), which would restrict g_{sw} even further. One possible consequence would be a midday depression of net CO₂ fixation, as has been observed in other bromeliads (Griffiths et al., 1986; Lüttge et al., 1986b; Maxwell et al., 1994, 1995; Griffiths and Maxwell, 1999; Maxwell, 2002; Pierce et al., 2002). Net CO₂ uptake could even be primarily limited to fog events (i.e. low-VPD conditions), which could be common occurrences for species such as *O. elegans* on Juan Fernández Island (Valdebenito et al., 1992). Zizka et al. (2009) have remarked on the restriction of some succulent *Puya* species to areas of coastal Chile that are frequently subject to fog events. Restriction of stomatal aperture in response to high VPD may have promoted selection for high thermal tolerance in these succulents, since the potential for evaporative cooling will be diminished (Chaves et al., 2015). It is also possible that long-term acclimation to low soil water potential could modify the stomatal responses of plants in nature relative to the well-watered individuals used in this study.

In the Pitcairnioideae, the transition from non-succulent leaf anatomy and C₃ photosynthesis (*Pitcairnia-Fosterella*) to succulence and CAM (Xeric Clade) was associated with a substantial decline in $g_{sw,max}$ and an increase in S_s. Since soil water availability is considerably lower in the arid environments to which the Xeric Clade species are native, this more conservative stomatal behaviour represents an important adaptation. Among the terrestrial Bromelioideae, the only species sampled that showed a relatively high value of S_s was *Disteganthus basilateralis* Lem., which is an example of the intriguing (pseudo-)petiolate, spatulate or obovate foliar morphology that recurs in isolated species throughout the Bromeliaceae (see **Chapter 2** for further discussion). This, coupled with the fact that *D. basilateralis* is native to perhumid lowland forest in French Guiana (Luther, 1990), could account for the presence in this species of especially strong constriction of stomatal conductance at high VPD. The origin of CAM in the Pitcairnioideae, and those which occurred in other bromeliad lineages (e.g. at the base of the Hechtioideae), were probably all

associated with an increase in S_s . This could simply be the result of correlative adaptation, with no immediate mechanistic interaction between CAM and stomatal sensitivity. Alternatively, CAM biochemistry and stomatal rhythms may be intrinsically linked with enhanced sensitivity. Many aspects of CAM stomatal biology remain understudied, although some recent advances have been made in terms of circadian control and CO_2 responses (reviewed in Males and Griffiths, 2017a). Ongoing research is seeking possible mechanisms based on the differences in malate metabolism between C_3 and CAM species, since malate is a key regulator of stomatal movements (Araújo et al., 2011).

The C_3 tank-epiphytes examined in this investigation showed both high sensitivity of steady-state stomatal conductance to VPD, sufficient to generate a feedforward effect of VPD on transpiration, and rapid stomatal kinetics in response to changes in VPD. This combination presumably maximises water conservation in dynamic canopy microenvironments, helping plants to maintain water balance. If leaf water potential begins to decline during episodes of low water availability or high evaporative demand, the stomata also close sooner than those of terrestrial species, which may be of particular importance because of their lower internal hydraulic capacitance (see **Chapter 3**). C_3 tank-epiphyte bromeliads therefore exhibit a multifaceted stomatal water-conservation syndrome. Even this may not be sufficient to allow *N. innocentii* to occupy all but the most humid forest microhabitats, since it shows a strong decline in WUE as VPD rises. In a survey of the spatial structure of Atlantic Forest bromeliad communities, Fischer and Araujo (1995) found that this species was most frequent in moist, shady riparian forest. Similarly, the steep decline in WUE observed after an initial increase with rising VPD for both *G. monostachia* and *L. glutinosa* could represent a physiological threshold on bioclimatic tolerance. The relatively high sensitivity of CO_2 assimilation to VPD in the tank-epiphytes, particularly *N. innocentii*, curtails the potential improvement in WUE that could be provided by their stomatal sensitivity. It could be partly explained by a low foliar N content restricting photosynthetic capacity. Several studies have found evidence of N- and P-limitation in epiphytic bromeliads lacking access to soil nutrients (Laube and Zotz, 2003; Zotz and Richter, 2006; Zotz and Asshoff, 2010; Wanek and Zotz, 2011; Lasso and Ackerman, 2013). One could also speculate on the possibility of a non-stomatal signal between VPD and photosynthesis, but the mechanism for any such effect is unknown. It was perhaps surprising that, relative to C_3 mesic terrestrials, the C_3 tank-epiphytes showed a strong restriction of the duration and intensity of the WWR, but not the RWR. This could imply that different sets of structural and biochemical factors are involved in determining the speed of the WWR and the RWR. Similar to C_3 tank-epiphytes, CAM tank-epiphytes showed low $g_{\text{sw,max}}$ and high S_s . Lüttge et al. (1986a) have previously noted close tracking of ambient humidity by stomatal conductance in the

CAM tank-epiphyte *Aechmea aquilega* (Salisb.) Griseb. in Trinidad. This highly conservative stomatal behaviour is likely to be of particular adaptive value to epiphytes exposed to frequent water limitation, which is true of many CAM tank-epiphyte species native to seasonally-dry tropical forests and upper-canopy species exposed to high levels of insolation and wind.

The origin of CAM and the atmospheric habit in *Tillandsia* was associated with a strong decrease in $g_{sw,max}$ and dramatic increase in S_s , consistent with an early dataset for the species *T. recurvata* (L.) L. published by Lange and Medina (1979). Exceptionally high S_s could be of critical importance to confining gas exchange to periods of low VPD. There is abundant evidence for the coupling of water uptake and photosynthetic activity in these species during such windows of opportunity, on both diurnal and seasonal timescales (De Santo et al., 1976; Andrade, 2003; Reyes-García et al., 2008a,b, 2012). This opportunistic ecophysiological strategy has enabled the atmospheric bromeliads to colonise bioclimatic and geographic space that is virtually inaccessible to other plants, including a range of desert and semi-desert environments (Bernal et al., 2005; Pinto et al., 2006; Hesse, 2012).

5.4.3 *The possible structural basis of contrasting stomatal functionality*

The variety of stomatal morphologies in the Bromeliaceae have long been recognised and over five decades ago Tomlinson (1969) postulated ideas regarding their significance since for gas exchange, although no concrete advances have since been made in this area. This investigation provided an opportunity to seek evidence for the hypothesis that either modified guard cells or hypodermal or epidermal subsidiary cells could be associated with distinctive stomatal functionality. The significant correlation that was found between changes in stomatal aperture and changes in stomatal conductance in response to VPD suggest that guard cell movements make the overriding contribution to changes in conductance. However, some variation could not be explained by changes in aperture, raising the possibility that VPD responses of conductance could be partly controlled by changes in resistance not associated with guard cells. In the succulent terrestrials, stomata are equipped with a pair of large subsidiary cells. By analogy with their counterparts in the grasses, these subsidiary cells may enhance the efficiency of guard cell movements through osmotic coupling and mechanical antagonism (Franks and Farquhar, 2007). During the opening of grass stomata, the rapid increase in turgor in guard cells is associated with a loss of turgor in the adjacent subsidiary cells, with the reverse process occurring during stomatal closure. Interestingly, although C_3 succulent terrestrial bromeliad species did show strong stomatal sensitivity to VPD, their dynamic responses to changes in VPD were relatively slow and involved greater water loss associated with the WWR than was observed in either C_3 mesic terrestrials or C_3 tank-epiphytes. This could be indicative of

biochemical or biophysical limitations on the rate of change of stomatal aperture such that the strongest declines in absolute conductance in response to VPD also take the greatest length of time.

It has previously been mooted that the hypodermal cells underlying the guard cells in most C_3 and CAM tank-epiphyte species could play a more direct mechanical role in controlling stomatal conductance (Tomlinson, 1969; Males, 2016). One possible mechanism would be that if the water potential of the hypodermal cells is in equilibrium with water vapour in the stomatal pore, they could act as an additional hydropassive guard-cell-like resistor. Under high humidity, the hypodermal cells could gain turgor pressure and move away from the lumen of the pore. When humidity falls, the hypodermal cells could lose turgor pressure and fall back into the pore, reducing stomatal conductance. Alternatively, the hypodermal cells could be mechanically engaged with the guard cells such that increasing guard cell turgor causes the hypodermal cells to swing back from the lumen of the pore, and decreasing guard cell turgor draws them in to enhance resistance to gas exchange.

In the Bromeliaceae, modified stomata always occur in association with greater guard cell size and pore length and reduced stomatal density relative to the simple, unmodified morphology seen in mesic terrestrial *Pitcairnia* species. This complicates interpretation of measurements of stomatal responses, since stomatal size and density have both been implicated in variation in leaf-level conductance (Drake et al., 2013; Fanourakis et al., 2014; Franks et al., 2015; Monda et al., 2016). Stomatal density was a strong predictor of $g_{sw,max}$, and the adaptive reductions in $g_{sw,max}$ associated with evolutionary transitions to more specialised epiphytic functional types were probably driven in large part by the evolution of lower stomatal density. The tight negative relationship between stomatal density and pore length suggests that there is also a developmentally-determined negative correlation between stomatal size and density in the bromeliads, as has been observed across the flowering plants (Beaulieu et al., 2008; Doheny-Adams et al., 2012; Drake et al., 2013). The apparent independence of $g_{sw,max}$ from pore length probably means that variation in pore area is more dependent on maximum pore width than length. The potential involvement of stomatal pore size in interspecific differences in $g_{sw,max}$ therefore requires more detailed investigation.

Interestingly, the observation of higher sensitivity in bromeliad lineages with greater stomatal size and reduced stomatal density bucks the trend among the angiosperms, where studies have generally identified correlations in the opposite direction (Hetherington and Woodward, 2003; Franks and Beerling, 2009; Drake et al., 2013) and Raven (2014) has described a possible energetic and metabolic basis. Large, low-density stomata that are highly responsive to VPD (but not light) are found in the ferns (Brodribb and Holbrook, 2004b; McAdam and Brodribb, 2012, 2013), but this is likely due to the hydropassive nature of stomatal regulation in ferns (Brodribb and McAdam, 2011).

Similarly, in the bromeliads there was a negative relationship between maximal stomatal conductance and stomatal sensitivity rather than the classical positive relationship (Kaufmann, 1982; McNaughton and Jarvis, 1991; Yong et al., 1997; Oren et al., 1999). These unusual trends could be driven by divergent stomatal morphologies with contrasting functional syndromes. The absence of subsidiary cells in groups with small, high-density stomata (e.g. *Pitcairnia*) could limit their responsiveness, while the presence of subsidiary cells could compensate for the energetic costs of larger guard cells in other groups (e.g. *Vriesea*; Franks and Farquhar, 2007; Chen et al., 2017). It may be that across the Bromeliaceae as a whole there is a trade-off between investment in stomatal capacity (density x pore size) and stomatal sensitivity. Moreover, while the bromeliad species sampled here are extremely ecologically diverse and inhabit very different areas of macro- and micro-climatic space, most other reports in the literature have sampled taxa from communities of plants coexisting in the same climate space. Further investigation with wider taxon sampling could explore whether more classical relationships can be recovered among bromeliad taxa united by shared stomatal morphology.

5.5 Conclusions

There are pronounced differences in stomatal conductance, sensitivity to VPD, and kinetics between bromeliad functional types. These differences are of direct relevance to ecological divergences between functional types, with evolutionary transitions to more specialised epiphytic habits in multiple bromeliad lineages being associated with reductions in maximal stomatal conductance, increases in steady-state stomatal sensitivity to VPD, and improvements in stomatal kinetics. Through stomatal and perhaps also non-stomatal effects, VPD has strongly species-specific effects on intrinsic WUE. The distinctive properties of stomata of different functional types may have a basis in stomatal complex morphology and stomatal size and density.

6. Trait-mediated environmental niche differentiation in Trinidadian *Aechmea* bromeliads

6.1 Introduction

In **Chapter 4** and **Chapter 5** it was shown that differences in leaf structural traits underpinned divergences in physiological function between bromeliad species of contrasting functional types. As laid out in the **General Introduction**, the next stage of this research project was to determine the extent to which such structure-function differences might explain niche segregation between bromeliad species in a specific ecological system. This chapter presents the results of an investigation into how leaf anatomical and physiological factors drive divergences in the environmental niches and geographical distributions of *Aechmea* species on the southern Caribbean island of Trinidad.

6.1.1 Background

Across geographical landscapes and ecosystems, including highly diverse tropical forests, the coexistence of closely-related species may be maintained by ecological divergence and the partitioning of niche space (Wright, 2002; Silvertown, 2004). Differences in both biotic and abiotic ecology may be involved. In particular, the environmental relations of congeneric plant species may differ markedly as a result of contrasting physiological characteristics (e.g. Barker et al., 1997; Baraloto et al., 2007). In this chapter, a case study of ecological differentiation underpinned by structural-functional trait complexes is presented, drawing on extensive physiological and anatomical characterisation to explain differences in the geographical and altitudinal distributions of CAM tank-epiphyte bromeliads of the genus *Aechmea* native to the island of Trinidad.

6.1.2 Trinidad: environmental context

The continental island of Trinidad lies approximately 10° N of the equator, off the Venezuelan coast of the South American mainland (Fig. 6.1.1). To the south and west, the island is surrounded by the Columbus Channel and Gulf of Paria, which are fed by the Orinoco Delta and the estuary of the Río de San Juan. The Caribbean Sea and Atlantic Ocean meet at the northeast point of Trinidad. Along with its oceanic sister island of Tobago (Frost and Snoke, 1989), Trinidad's floristic affinities are primarily Guianese rather than Antillean (Beard, 1946a). This is a reflection of its close proximity to the mainland, which lies at a distance of less than 20 km from both the Chaguaramas peninsula in the northwest of Trinidad and Icacos Point in the southwest. During the last glacial maximum,

Trinidad was connected to the mainland by a land bridge, facilitating recent free biotic exchange (Trelease, 1918; Beard, 1946a; Lawlor, 1986).



Figure 6.1.1 Geographical location of Trinidad.

Topographically, Trinidad can be divided into five distinct bands running roughly parallel to each other in an east-west orientation (Pittendrigh, 1948; Fig. 6.1.2). In the south, the prominent feature is the sandstone Southern Range (Pindell et al., 2009), which rises to the eminences of the Trinity Hills (303 m at Morne Derrick) between Guayaguayare and Moruga. To the north of the Trinity Hills extends the Southern Basin, drained by the South Oropouche and Ortoire Rivers. This is followed by the Central Range, which runs in a south-west to north-east direction. The highest peak in this Miocene limestone range is Tamana (307 m; Erlich et al., 1993). North of the Central Range is the Sangre Grande Basin, drained primarily by the Caroni River in the west and the Oropouche River in the east. Finally, the northern end of Trinidad is dominated by the Northern Range, which is of metamorphic origin (Algar and Pindell, 1993) and culminates at El Cerro del Aripo (940 m), the highest point in Trinidad.

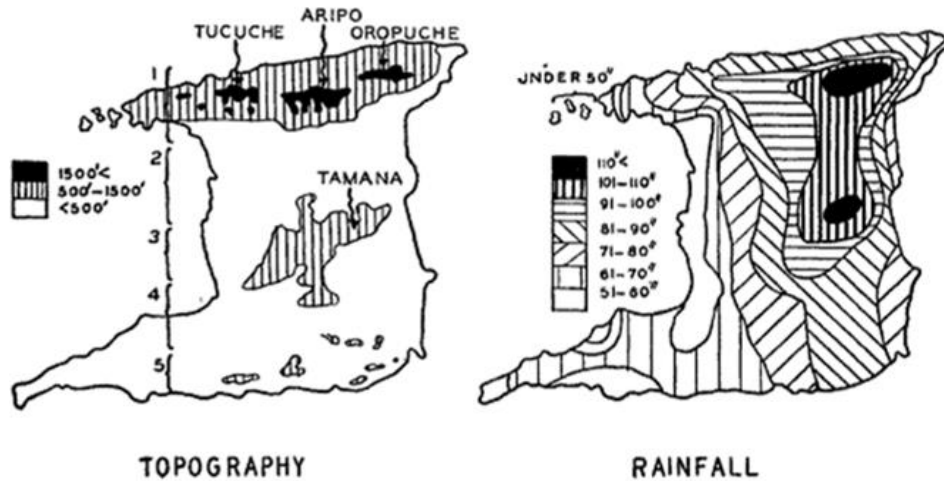


Figure 6.1.2. Distribution of topography (ft above sea-level) and mean annual precipitation (in) in Trinidad, reproduced from Pittendrigh (1948).

The Northern Range is the region of greatest interest in this study, and therefore warrants further description. Geologically, the Northern Range is a continuation of the mountains of the Paria Peninsula of Venezuela, with which it is collinear and separated by only a few kilometres of ocean (the Bocas del Dragón). The Main Ridge of the Northern Range extends from Macqueripe on the northern coast of the Chaguaramas peninsula to the Heights of Platanal in the east (Fig. 6.1.3a). Alongside El Cerro del Aripo, the Main Ridge includes the peaks of El Tucuche (936 m) and Morne Bleu (834 m). A series of north-south-oriented ridges drop down from the Main Ridge, defining a chain of valleys (Fig. 6.1.3b). The names used in this chapter to identify these valleys are, from east to west: Tucker Valley; Diego Martin Valley; Maraval; Santa Cruz Valley; Maracas Valley; Caura Valley; Lopinot Valley; Arima Valley; Heights of Guanapo; Heights of Aripo; Hollis Reservoir Watershed; and the Cumaca Valley. Beyond Cumaca, there is less pronounced relief and the land lies largely below 300 m until the Atlantic coast is reached between Matura and Rampanalgas. A secondary ridge (culminating at Mars, 580 m) begins north of Brasso Seco and runs eastwards to the Atlantic coast near Rampanalgas. Historically, this ridge provided the administrative boundary between the counties of St Andrew to the south and St David to the north, and is therefore referred to here as the St David's Ridge. North of the St David's Ridge is a chain of lower hills (including Shark and Manantial) that are termed here the Northeast Coastal Hills.

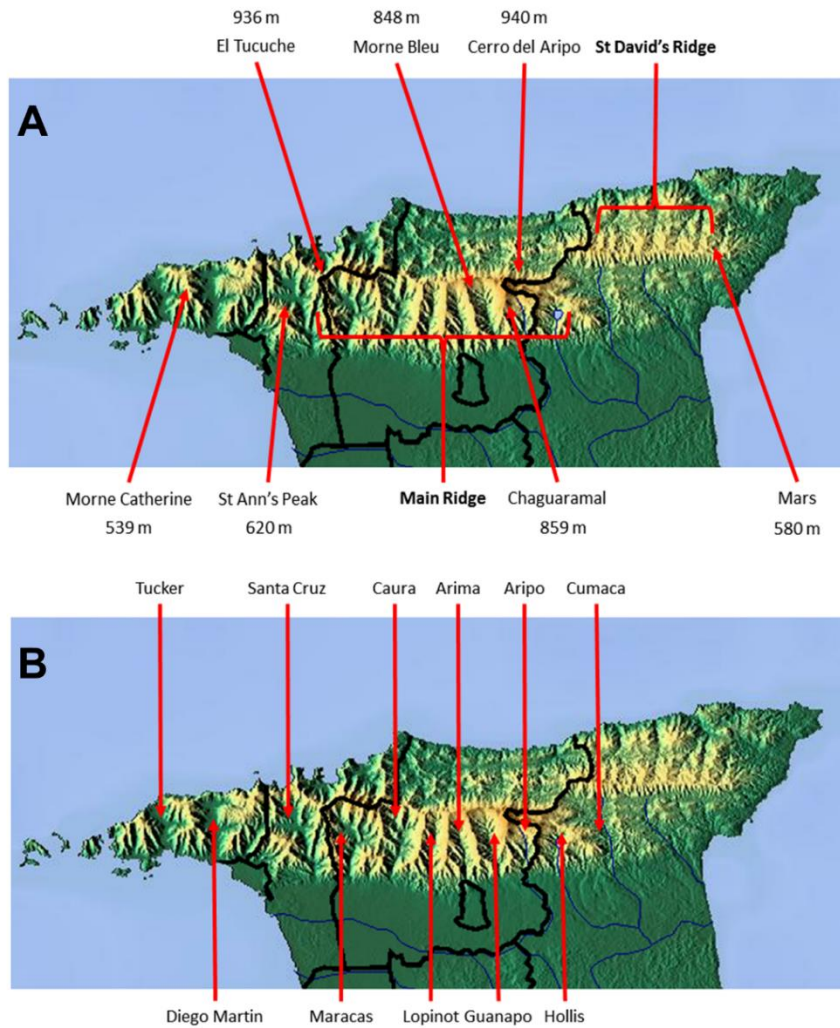


Figure 6.1.3. a) Prominent topographic features of the Northern Range. b) Valleys to the south of the Main Ridge.

The climate of Trinidad, as described by Beard (1946a), is driven by the annual migration of the intertropical convergence zone (ITCZ). The dry season typically lasts from January to May, with precipitation usually reaching its lowest level in March (16.9 mm mean for Port-of-Spain). Easterly winds of 20-30 kph are common in the dry season. The wet season runs from June to December, and there is a peak of precipitation in August (244.0 mm mean for Port-of-Spain). Two-thirds of the annual precipitation falls during the wet season, which is often punctuated by a few weeks of drier weather in September/October (the *petit carême*). Temperatures are relatively invariant throughout the year (28.0 - 33.1°C monthly mean highs for Port-of-Spain). Trinidad lies on the extreme southern periphery of the hurricane belt and rarely suffers from hurricane events.

The interplay between the ITCZ, Northeast Trade winds, Trinidad's topography, and the oceanography of the surrounding seas drives the complex distribution of precipitation across the

island (Beard, 1946a; Fig. 6.1.2). The highest levels of precipitation (up to 3800 mm yr^{-1}) are concentrated in the north-eastern corner of the island, where the Northern Range meets the Atlantic coast. High rainfall is also observed in the Central Range around Tamana and Mount Harris. To the west and south, precipitation levels fall to a low of approximately 1200 mm yr^{-1} . Relative to the Lesser Antilles, rainfall at any given elevation in Trinidad is low, and this is reflected in the vegetation (Beard, 1949).

6.1.3 Trinidad: vegetation

At present, the principal vegetation of the Northern Range is a mixture of primary and secondary forest (Helmer et al., 2012). Many of the less precipitous slopes were cleared and planted with crops such as citrus, cocoa and timber trees (e.g. mahogany and teak) from the 18th century until the mid-20th century (Leiter and Harding, 2004). Regular interplanting with shade trees (particularly the immortelles, *Erythrina poeppigiana* (Walp) O.F.Cook and *E. fusca* Lour.) was common practice (Shepard, 1928; Beer, 1987). Changes in the global economic value of these commodities, particularly cocoa, and the switch to an oil-based economy in Trinidad and Tobago, led to a gradual abandonment of managed plantations and reversion to secondary forest (Pollard, 1985). A recent study based on multiseason satellite imagery estimated that 73% of Trinidad is currently forested (Helmer et al., 2012), although this figure includes invasive bamboo thicket.

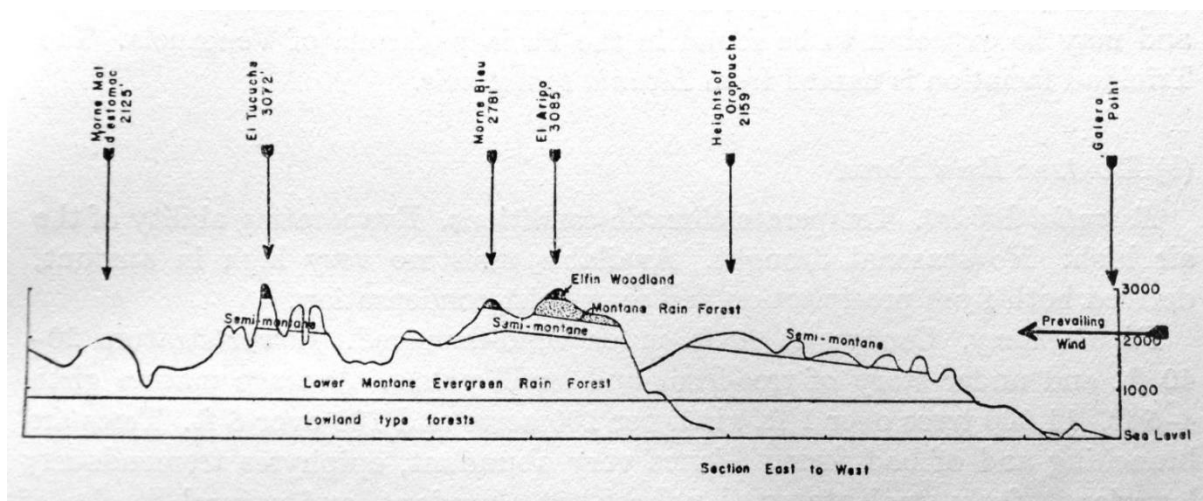


Figure 6.1.4. Vegetation zonation in the Northern Range of Trinidad: transect from Morne Mal d'Estomac (W) to Galera Point (E) reproduced from Beard (1946a). Heights in ft.

The most comprehensive classification system for the forest types and tree communities of Trinidad was established by John Stewart Beard (1946a). In the Northern Range, a subset of the forest types and communities defined by Beard predominate (illustrated in a representative transect in Fig. 6.1.4), spanning elfin woodland (EW), montane forest (MF), lower montane forest (LMF), evergreen seasonal forest (ESF), seasonal montane forest (SMF), and deciduous seasonal forest (DSF). Further details of the physiognomy and floristics of these formations are given in **Appendix 6.1**.

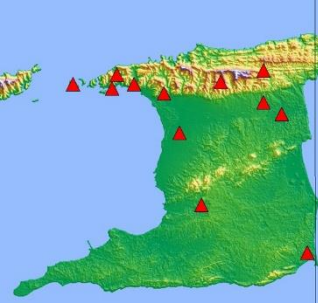




Intermixed with native forest vegetation are large pockets of invasive bamboo thicket (*Bambusa vulgaris* Schrad.), which often exploits areas newly disturbed by fire, wind-damage, or logging. There are also some forestry plantations of Caribbean pine (*Pinus caribaea* Morelet) in the vicinity of Matura, Mount St Benedict and Macqueripe. The matrix of protected areas in Trinidad includes the Matura National Park Environmentally Sensitive Area (designated in 2001) at the eastern end of the Northern Range. This encompasses the Matura and St David Forest Reserves, which have been estimated to include 80% 'undisturbed' forest (EMA, 2007). Across the remainder of the Northern Range there are frequent clearings for subsistence agriculture and market gardening, either on privately-owned land or as a continuation of the long history of squatting in Trinidad (Driver, 2002).

6.1.4 Bromeliaceae in Trinidad

The bromeliad flora of Trinidad comprises 59 species, of which five (including the pineapple, *Ananas comosus* (L.) Merr.) are considered exotic (Baksh-Comeau et al., 2016). The distributions of the Trinidadian bromeliads at various spatial scales have been considered by several authors, most notably Colin Pittendrigh. During his research in the 1940s into the malarial mosquitoes of Trinidad, many of which breed in bromeliad phytotelmata, Pittendrigh developed an interest in how functional traits interacted with the light and humidity environment to determine the vertical and horizontal distributions of bromeliads within forest canopies (Pittendrigh, 1948). This strand of research was continued by Griffiths and Smith (1983), who made use of advances in the understanding of photosynthetic physiology to examine the role of CAM in bromeliad species ecology. Further investigations by these authors and colleagues provided critical early insights into some physiological characteristics of ecologically divergent bromeliads (Smith et al., 1985; Griffiths et al., 1986; Lüttge et al., 1986a,b; Smith et al., 1986a,b).

A bromeliad genus of particular interest in Trinidad is *Aechmea*. There are ten *Aechmea* species on the island, one of which, *Ae. magdalenae* (André) André ex Baker, is an introduced exotic and does not occur far from human habitation. The remaining nine species display a wide range of ecologies and distributions. Brief accounts of the existing knowledge of each species are given in Table 6.1.1,

including maps of approximate occurrence points for each species obtained from vouchers deposited at the National Herbarium of Trinidad and Tobago (full details in **Appendix 6.2**).

| Species | Subgenus | Functional type | Global distribution | Tobago? | Herbarium records |
|--|----------------------|-----------------------|---|--------------------------------|---|
| <i>Aechmea aquilega</i> (Salisb.) Griseb. | <i>Aechmea</i> | Nesting tank-epiphyte | Northern South America; Costa Rica; Jamaica | Yes |  |
| <i>Aechmea aripensis</i> (N.E.Br.) Pittendr. | <i>Aechmea</i> | Tank-epiphyte | Venezuela (Paria Peninsula) | No |  |
| <i>Aechmea bromeliifolia</i> (Rudge) Baker | <i>Macrochordion</i> | Tank-epiphyte | Northern South America; Central America | No |  |
| <i>Aechmea dichlamydea</i> var. <i>trinitensis</i> L.B.Sm. | <i>Platyaechmea</i> | Tank-epiphyte | Venezuela (Paria Peninsula; var. <i>parianensis</i>) | Yes (var. <i>dichlamydea</i>) |  |
| <i>Aechmea downsiana</i> Pittendr. | <i>Aechmea</i> | Tank-epiphyte | - | No |  |



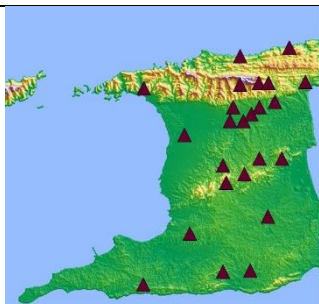

| | | | | | |
|---|-----------------|-----------------------|--|-----|---|
| <i>Aechmea fendleri</i> André ex Mez | <i>Aechmea</i> | Nesting tank-epiphyte | Venezuela (Paria Peninsula) | No |  |
| <i>Aechmea lingulata</i> (L.) Baker | <i>Aechmea</i> | Nesting tank-epiphyte | Northern South America; Costa Rica and Panama; Lesser Antilles, Puerto Rico, Bahamas | Yes |  |
| <i>Aechmea mertensii</i> (G.Mey.) Schult. & Schult.f. | <i>Aechmea</i> | Tank-epiphyte | Northern South America | No |  |
| <i>Aechmea nudicaulis</i> (L.) Griseb. | <i>Pothuava</i> | Tank-epiphyte | Northern South America; Central America; Mexico; Cuba; Hispaniola; Puerto Rico | Yes |  |

Table 6.1.1 Characteristics and herbarium records of native *Aechmea* species in Trinidad.



Figure 6.1.5 Key bromeliad species in this investigation. a-b) *Aechmea aquilega*; c-d) *Aechmea fendleri*; e-f) *Aechmea nudicaulis*; g-h) *Aechmea dichlamydea* (var. *dichlamydea* shown).

For the purposes of undertaking a comparative investigation into ecophysiological divergence and distributional segregation, three of these *Aechmea* species were selected on the basis of preliminary

observations of apparent altitudinal zonation: *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis*. *Ae. aquilega* in Trinidad is a very common epiphyte or lithophyte, noted for occurring around mud volcanoes (Young et al., 2004). It also occurs on Tobago and in Brazil, Jamaica, Costa Rica, French Guiana, Guyana, Suriname, and Venezuela. *Ae. fendleri* is another nesting tank-epiphyte. Outside Trinidad it occurs only in Venezuela, including on the adjacent Paria Peninsula, Cerro Copey on Isla Margarita (Enrech et al., 1998), and in montane forests in the states of Aragua, Distrito Federal, Miranda, Monagas, Nueva Esparta, Sucre and Yaracuy (Holst, 1994). Like *Ae. aquilega*, *Ae. fendleri* occasionally occurs as a terrestrial (Britton, 1921). In the early 20th century it was frequently referred to by the synonym *Ae. porteoides* Britton. *Ae. nudicaulis*, meanwhile, is a strongly clonal tubular tank-epiphyte (Loh et al., 2015). This species occurs on both Trinidad and Tobago, and is widely distributed throughout the Caribbean, Central America, and northern South America.

During the course of fieldwork, it became apparent that the distribution of *Ae. dichlamydea* var. *trinitensis* in northeast Trinidad showed interesting patterns when compared with the other species. Although it was not possible to export living material of this species for detailed anatomical and physiological characterisation, it was included in some elements of the spatial analyses presented here. This variety is endemic to Trinidad, but other varieties of *Ae. dichlamydea* occur on the Venezuelan Paria Peninsula (var. *pariaensis*) and on Tobago (var. *dichlamydea*). All three varieties are predominantly epiphytic, rarely terrestrial, and vegetative rosettes appear similar to those of *Ae. fendleri*. *Ae. dichlamydea* (var. *dichlamydea*) is pictured with *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis* in Fig. 6.1.5.

6.1.5 Aims

The aims of this investigation were to determine the extent of environmental habitat occupancy in selected *Aechmea* species in Trinidad, and how this can be explained by divergence in ecophysiological trait-based niches. This involved addressing the following central questions:

1. What are the differences in the distributions of *Aechmea aquilega*, *Ae. dichlamydea* var. *trinitensis*, *Ae. fendleri*, and *Ae. nudicaulis* across the Northern Range of Trinidad?
2. How do these differences relate to environmental variables and vegetation composition and structure across this area?
3. How do the species distributions on Trinidad relate to their global distributions?
4. For *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis*, which ecophysiological traits might account for differences in geographical and altitudinal distributions?

6.2 Methods

6.2.1 Assessment of realised and potential environmental niches

The realised environmental niches (i.e. habitat occupancy) of *Aechmea aquilega*, *Ae. fendleri* and *Ae. nudicaulis* were established by carrying out field surveys to assess the distribution of each species across northern Trinidad, followed by estimation of potential niches using correlative species distribution modelling (SDM).

6.2.1.1 Field surveys

To establish the distributions and altitudinal zonation of the focal *Aechmea* species across the Northern Range of Trinidad, transects were followed across various parts of the Range. Some transects followed roads, traces or forest trails, whereas in other cases it was necessary to cut a trail through the forest. Generally, transect routes were chosen that would involve a gain or loss of altitude and thereby maximise the possibility of identifying the transition between the altitudinal zones occupied by *Ae. aquilega* and *Ae. fendleri* where such a transition occurred. Some additional transects were followed in the plain to the south of the Northern Range, and around Mount Tamana in the Central Range, where *Ae. fendleri* had been collected in the 1920s (see **Appendix 6.2**). Plants were identified by visual inspection from the forest floor. Inflorescences and infructescences were used as taxonomically diagnostic features whenever present. Vegetative rosettes were only recorded when sufficient evidence, including habit, rosette architecture, and leaf morphology, was available to make a positive identification. The coordinates and elevation of each individual or clone were then logged using a Garmin Etrex 10 GPS device (Garmin, Schaffhausen, Switzerland). Distribution mapping was carried out in Trinidad in January-February 2014, April-May 2015, March 2016, May 2016, September 2016, and April 2017. The first three trips and the final trip coincided with the island's dry season and the flowering and fruiting season of the *Aechmea* species, improving the ease of progress in the field and the accuracy of plant identification.

In April 2017, a three-day visit to Tobago was also undertaken to carry out rapid ground-truthing of projected species distributions on that island based on models trained on presence data from Trinidad. Recording was performed along several altitudinal transects across the Main Ridge, as well as around the coastal fringe of the island.

6.2.1.2 Correlative species distribution modelling

Correlative SDM was performed to estimate the environmental niches of *Ae. aquilega*, *Ae. dichlamydea*, *Ae. fendleri* and *Ae. nudicaulis*. Prior to SDM, it is imperative to minimise spatial bias in

presence data used for model training (Segurado et al., 2006). In order to reduce spatial bias associated with the transect-based method of data collection, subsampling of the full distributional datasets was performed. This is considered the most robust method for removing spatial autocorrelation from biased presence datasets (Kramer-Schadt et al., 2013; Fourcade et al., 2014). Residual spatial autocorrelation was tested for using average Nearest Neighbour Index (NNI) testing with the 'nni' function in the 'spatialEco' package in R (Evans, 2016). The resolution of subsampling was adjusted on a species-by-species basis until satisfactory NNI results were obtained ($p > 0.05$; see **Appendix 6.3** for details).

For each species, the pruned presence data (columns of latitude/longitude coordinates) were then loaded into the software MaxEnt (version 3.3.3k; <http://www.cs.princeton.edu/~schapire/maxent/>; Phillips et al., 2004, 2006) for species distribution modelling (SDM). MaxEnt utilises the maximum entropy approach for SDM with presence-only data. The software algorithms minimise the relative entropy between the probability density estimated from presence data and probability density estimated from 'background' points in the landscape in covariate space (Elith et al., 2011). Separate modelling runs were performed for each species. The environmental layers incorporated in the models are listed in Table 6.2.1, and raster images showing the distribution of values across Trinidad are available in **Appendix 6.4**. The bioclimatic layers (Bio1, Bio2, Bio12, Bio15, Bio17) were retrieved from the Worldclim database (Hijmans et al., 2005). These data layers were estimated by interpolation of multiple climatic datasets as described in Hijmans et al. (2005). Bio1 corresponds to an estimate of mean annual temperature (MAT, °C), which could affect plant carbon balance via the thermal sensitivity of biochemical processes. Bio2 is the diurnal temperature range (DTR, °C), which is likely to be of particular relevance to CAM bromeliads with contrasting thermal optima for daytime and night-time phases of carbon fixation. Bio12 is mean annual precipitation (MAP, mm), which is a basic measure of the overall availability of water. Bio15 is the precipitation seasonality (P_{seas} , %), as used in previous chapters. Precipitation seasonality could be an important determinant of the relative intensity of drought stress during the dry season, to which species may be differentially resistant. Bio17 is the mean quantity of precipitation that falls during the driest quarter of the year (P_{dry} , mm). In Trinidad, the driest conditions generally occur over a period of several months (December-May), and it was therefore decided that the quarterly quantity would capture differences in the intensity of seasonal drought across the geographical extent of the island, which could be an important factor in shaping the distributions of species of contrasting drought resistance.

More nuanced metrics of water availability were obtained by retrieving aridity index (AI, mm mm^{-1}) actual and potential evapotranspiration (AET and PET, mm month^{-1}) rasters from the CGIAR-CSI

portal (Zomer et al., 2008; Trabucco and Zomer, 2010). AI (MAP/ annual PET, mm mm⁻¹) represents a measure of the relative sizes of inputs and outputs in the water balance at a given location. The ratio of AET to PET (AET/PET, mm mm⁻¹) expresses the extent to which evapotranspiration is reduced by water availability limitations or other constraints on transpiration. Elevation, slope and aspect rasters were generated from the ArcGIS World Elevation Series (Esri, Redlands, CA, USA). All bioclimatic and elevational layers were encoded in ASCII raster grids with a resolution of 30 arc-seconds and set to the WGS 84 datum.

In addition to the bioclimatic and elevational layers, a 2 m-resolution vegetation layer ('vegtype') was created using mosaic data from the Helmer et al. (2012) satellite imagery-based vegetation map of Trinidad via the USDA website (<http://data.fs.usda.gov/geodata/rastergateway/caribbean/index.php>). This raster was cropped and aligned with the bioclimatic and elevational layers, which were then resampled to match the resolution of the vegetation layer. Each pixel in the vegetation raster was encoded with one of 49 possible scores, referred to here as 'vegtypes'. The full list of vegtypes is shown in **Appendix 6.5**, using updated taxonomic nomenclature checked on The Plant List (<http://www.theplantlist.org/>). Because the Helmer et al. (2012) classification is based on Beard (1946a), it incorporates various floristic inconsistencies evident in that scheme (including misidentification and partial identification; Baksh-Comeau, pers. comm.). Preliminary analysis of new field data has shown that the floristic projections of Helmer et al. are accurate only in MF vegetation. Even if they fall short of accurate identification of the major floristic elements, the Helmer et al. maps highlight differences in vegetation composition and structure across Trinidad and Tobago.

Model performance was assessed using the area under the curve (AUC) of the Receiver Operating Characteristic (ROC) as the primary criterion (referred to as test-AUC). The ROC function relates the proportion of true positives ('sensitivity') to the proportion of false negatives (1-'specificity') over the range of probability thresholds possible (Pearce and Ferrier, 2000). The test-AUC value essentially gives the probability that the model will classify a presence point with greater accuracy than random prediction (Phillips et al., 2006). A test-AUC value of 0.50 indicates that the model does not differ from random prediction. The maximum possible value (equivalent to the most informative model) differs depending on model construction, but is always 1.00 or less (Phillips et al., 2006). Presence-only SDM using MaxEnt is considered most appropriate for estimating the potential (rather than actual) distributions of species (Pearce and Boyce, 2006). Approximate estimation of the fundamental environmental niche of the *Aechmea* species was the aim in this investigation, making MaxEnt a suitable choice of SDM approach.

Automatic selection of model features was enabled, and the regularisation parameter was varied to find optimal fit (Radosavljevic and Anderson, 2014). Random seeding was enabled, and the maximum number of background points was specified as 1000. For each model run, 100 cross-validation replicates were carried out to verify test-AUC robustness, and jackknife test plots displaying variable importance were produced. Model output was saved in html files. Omission and Predicted Area and ROC curves were inspected to check for independence between test and training data and to retrieve the model test- AUC. Three types of results of jackknife testing of the explanatory power of predictor variables are provided by MaxEnt: i) the gain of the full model with all variables; ii) the gain of the model with the focal variable only; iii) the gain of the model with all variables except the focal variable. These results were compared to identify the overall ranked contribution of each variable to model performance for each species. Probability distribution grid (PDG) maps were also generated for interpretation.

Models were initially developed for each of the four species (*Ae. aquilega*, *Ae. dichlamydea*, *Ae. fendleri*, and *Ae. nudicaulis*) across the rectangular geographical extent of the Northern Range, bounded by -61.795 ° W, -60.900 ° W, 10.640 ° N, 10.850 ° N.

6.2.1.3 Projecting current distributions on Tobago and the Paria Peninsula

Species distribution models generated for the Northern Range were used to project probability of occurrence under present climatic conditions onto three other geographical regions: the whole of the island of Trinidad (-61.94°W, -60.90°W, 10.00°N, 10.85°N), the island of Tobago (-60.87°W, -60.47°W, 11.10°N, 11.36°N), and the Paria Peninsula in Venezuela (-62.64°W, -61.84°W, 10.50°N, 10.78°N). Images of the environmental layer rasters for Tobago and the Paria Peninsula equivalent to those used for the Northern Range are displayed in **Appendix 6.4**.

6.2.2 Anatomical and physiological characterisation

6.2.2.1 Plant material

Approximately 50 individuals of *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis* were imported from Trinidad to the UK and allowed to acclimate to conditions in a tropical glasshouse at Cambridge University Botanic Garden (CUBG) for one year. It was not possible to collect *Ae. dichlamydea* var. *trinitensis* plants to include in comparative anatomical and physiological characterisation.

Glasshouse daytime temperatures were maintained at approximately 25-30°C, and night-time temperatures did not fall below 15°C. Supplemental illumination (300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf-level) was provided during the UK winter, and a relative humidity of over 80% was maintained at all times.

Prior to physiological characterisation, batches of plants were transferred to a Fitotron Proface growth chamber (Weiss Technik UK, Loughborough, UK) in the Department of Plant Sciences, University of Cambridge, under a reverse day-night cycle. Except during temperature response experiments, temperature during the light period was maintained at 28°C and relative humidity at 80%. A photosynthetic photon flux density (PPFD) of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was maintained throughout the light period. In the dark period, temperature was reduced to 18°C and relative humidity increased to 90%.

6.2.2.2 Anatomy and morphology

Transverse sections of at least 20 leaves sampled from at least six plants of each species were hand-cut and mounted for examination by light microscopy. Trichome traits were measured by carefully removing trichomes from the leaf surface using adhesive tape and mounting this on a slide for inspection by light microscopy. New anatomical data are presented in Table 6.2.1, while parameters that had been quantified in **Chapter 3** (interveinal distance (IVD), vein-epidermis distance (VED), internal air space fraction (IAS), leaf mass per unit area (LMA), stomatal density (SD)) were also analysed here.

| Parameter | Description |
|---|--|
| Trichome diameter (d_{trichome} , μm) | Diameter of the shield of a multicellular trichome |
| Trichome density (TD, trichomes mm^{-2}) | Density of absorptive trichomes on abaxial epidermis |

Table 6.2.1 Anatomical parameters measured for each species ($n \geq 20$ leaves from \geq six plants per species).

For five representative individuals of each species, tank volume was determined by filling with water and emptying into a measuring cylinder. The exposed surface area of the tank of each individual was quantified by filling the tank, taking a photograph and measuring the area of the water surface in ImageJ (NIH, Bethesda, MD, USA). The ratio of tank volume to surface area was then calculated for comparison between species.

6.2.2.3 Physiological measurements

6.2.2.3.1 Nocturnal acid accumulation and gas exchange profiles

Discs of 1 cm diameter were cut from the central portion of leaves of each species at dawn and dusk and immediately frozen at -80°C. Tissue was then crushed and boiled in distilled water to release stored acids. Titrations were performed using KOH and phenolphthalein as an indicator. Nocturnal

acid accumulation was calculated as the difference between dawn and dusk measurements of acidity, using the assumed stoichiometric ratio of malate: 2H^+ (Lüttge et al., 1975). The percentage of CO_2 recycling (i.e. CAM cycling activity) was quantified as $((\Delta\text{H}^+/2) - \Delta\text{CO}_2)/(\Delta\text{H}^+/2) \times 100$, where ΔCO_2 is integrated total nocturnal CO_2 uptake measured by gas exchange (Griffiths, 1988). Representative day-night gas exchange profiles were recorded for all species using the LI-COR 6400 XT (LI-COR, Lincoln, NE, USA) fitted with the standard 2 x 3 LED leaf cuvette and external CO_2 concentration of 400 ppm. Between 18h00 and 06h00, the cuvette lights were switched off and a leaf temperature of 18°C and relative humidity of 90% were specified, providing a leaf-air vapour pressure deficit (VPD) of 0.21 kPa. Between 06h00 and 18h00, the lights were set to provide $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, and a leaf temperature of 28°C and relative humidity of 80% were specified to provide a VPD of 0.76 kPa.

For the drought course experiment (see 6.2.2.4 below), mesophyll conductance to CO_2 (g_m) was quantified during the weakly-expressed phase II of CAM by combined gas exchange and chlorophyll fluorescence measurements using the method and calibration of Bellasio et al. (2016). Gas exchange and fluorescence were measured using the Li-Cor 6400-40 leaf chamber fluorometer using a multiphase flash (Loriaux et al., 2013), with standard chamber conditions set to a leaf temperature of 26°C, relative humidity of 80%, PPFD of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and external CO_2 concentration of 400 ppm. For each species, four replicate A-Ci curves and A-PPFD curves were measured under ambient (~21%) O_2 and 2% O_2 for each species, using leaves drawn from different individuals. Measurements were repeated at each time point in the drought course.

6.2.2.3.2 Leaf hydraulic conductance and vulnerability

Values of total leaf hydraulic conductance (K_{leaf}), leaf hydraulic vulnerability (P_{50L}), maximal stomatal conductance ($g_{s\text{max}}$), stomatal sensitivity (S_s) to leaf-air vapour pressure deficit (VPD), stomatal hydraulic vulnerability (P_{50S}), pressure-volume curve parameters (Ψ_{tlp} , ϵ , C_{FT}), and saturated water content (SWC) were retrieved from the dataset presented in **Chapter 3** for further analysis here.

6.2.2.3.3 Thermal sensitivity of photochemistry and nocturnal acid accumulation

Leaf discs (20 mm diameter) were sampled during Phase III of CAM and incubated for 10 min at a series of temperatures ranging from 15-60°C, and the quantum yield of photosystem II (Φ_{PSII}) was measured using a MINI-PAM-II (Heinz Walz GMBH, Effeltrich, Germany). Ten leaf discs cut from at least four individuals per species were used for each temperature level.

The sensitivity of nocturnal acid accumulation to acute (rather than chronic) night-time temperature variation was assessed by incubating detached leaves at a series of night-time temperatures ranging from 8-28°C and measuring dusk-dawn change in titratable acidity as outlined in 6.2.2.3.1 above. Five leaves sampled from at least three individuals per species were used at each temperature level. The effect of variation in night-time temperature was expressed as the percentage deviation in ΔH^+ relative to the value observed at 18°C, which was representative of growth conditions at CUBG and used in all other measurements. Daytime temperature was maintained at 28°C.

6.2.2.3.4 Vein- and trichome-mediated rehydration kinetics

The efficiency of vein- and trichome-mediated water uptake was measured using the methodology described in **Chapter 4**. Measurements were made using five replicate leaves drawn from at least three individuals per species.

6.2.2.4 Drought course

In order to determine how plants of each species respond to long-term drought, a drought course study was carried out. Ten well-watered plants of each species were allowed to acclimate to controlled conditions in a growth chamber for one month under the conditions described in 6.2.2.1. At the end of the acclimation period, baseline measurements of gas exchange, titratable acidity, chlorophyll fluorescence (using a Walz Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany) and leaf water potential and hydraulic conductance were performed, and leaf cross-sections were examined by light microscopy to quantify the relative contribution of the hydrenchyma to total leaf thickness. Water was then withheld completely, initiating the drought period. At regular intervals, measurements of leaf gas exchange, dusk-dawn changes in titratable acidity, chlorophyll fluorescence, leaf water potential and hydraulic conductance, and anatomical traits were repeated in order to track the changes in structure and function that took place as drought progressed. Leaf and whole-rosette mortality was also recorded. The drought course continued for a period of 60 d.

6.3 Results

6.3.1 Assessment of environmental niches

6.3.1.1 Distributional data

Maps showing all presence points across the Northern Range for each species (*Ae. aquilega*, *Ae. dichlamydea*, *Ae. fendleri* and *Ae. nudicaulis*) are shown in Fig. 6.3.1a-d. Excluding additional records

for southern Trinidad and Tobago, a total of 893 geo-referenced presence points were recorded for *Ae. aquilega*, 47 for *Ae. dichlamydea*, 380 for *Ae. fendleri*, and 944 for *Ae. nudicaulis*. More detailed descriptions of field observations are included in **Appendix 6.6**.

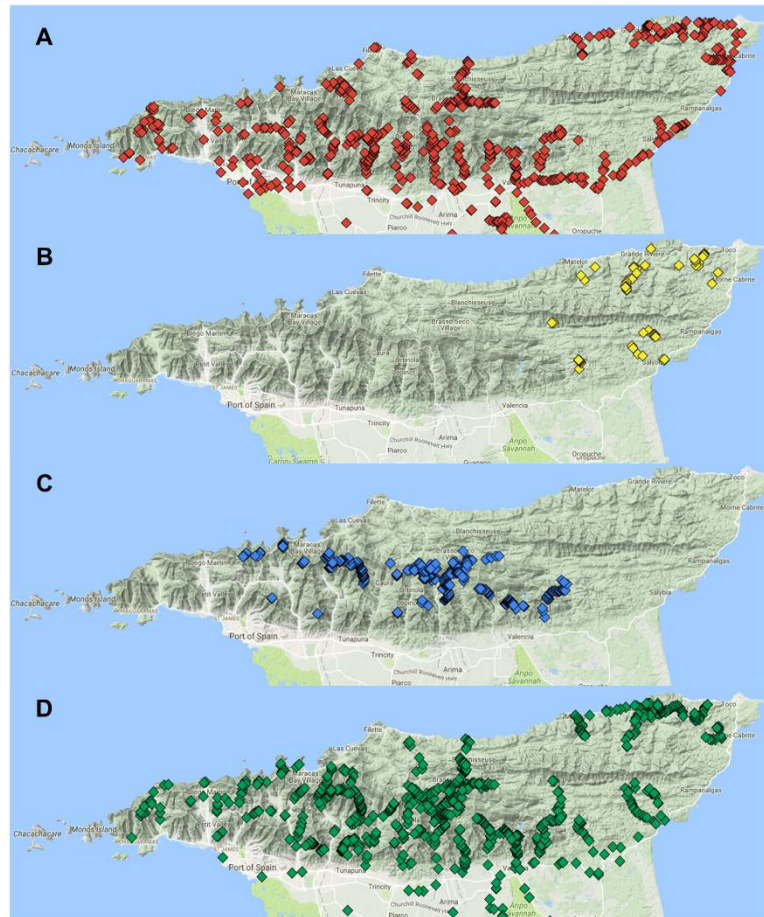


Figure 6.3.1. Presence points recorded during Northern Range field surveys for a) *Ae. aquilega*, b) *Ae. dichlamydea*, c) *Ae. fendleri* and d) *Ae. nudicaulis*.

6.3.1.2 Altitudinal and latitudinal components of segregation of *Aechmea aquilega* and *Ae. fendleri*

Based on previous observations made largely in the Arima Valley (Griffiths et al., 1986), it had been expected that *Ae. aquilega* and *Ae. fendleri* would display altitudinal segregation across the Northern Range. The data collected for this investigation partly upheld this hypothesis, but also reflected a more complex reality. Towards the western end of the Northern Range, there was clear altitudinal zonation, with *Ae. aquilega* being replaced by *Ae. fendleri* above a distinct threshold altitude. At the dry, extreme western end of the Northern Range, *Ae. aquilega* occurred to the highest ground (e.g. the summit of Morne Catherine, 539 m) whereas *Ae. fendleri* was absent. Moving eastwards from

here towards the Main Ridge, the upper limit for *Ae. aquilega* began to shift downwards, and *Ae. fendleri* appeared at the highest elevations. The transition from *Ae. aquilega* to *Ae. fendleri* occurred at decreasing elevation towards the east. Overlap of *Ae. aquilega* and *Ae. fendleri* was observed at Hollis and Cumaca (< 200 m), and at Brasso Seco (140 m). At the wettest end of the longitudinal gradient, around the Matura Forest, *Ae. aquilega* was much reduced in abundance. *Ae. fendleri* was also absent, perhaps due to mean temperature being too high or their insufficient day-night changes in temperature at low elevation. Overall, these results are consistent with moisture and/or temperature playing a role in the segregation of these two species.

6.3.2 Species distribution modelling

6.3.2.1 Testing model fit

The SDMs produced in MaxEnt varied in goodness of fit. For *Ae. fendleri* and *Ae. dichlamydea*, which have particularly clearly constrained distributions on Trinidad, it was possible to fit models with high test-AUC scores. Increasing the regularisation parameter to 3.0 or 4.0 improved model performance considerably for these species. However, for *Ae. aquilega* and *Ae. nudicaulis*, it was more difficult to generate a model with high test-AUC, because the distributions of these species are much wider and less strongly bioclimatically constrained than those of *Ae. dichlamydea* and *Ae. fendleri*. This was especially true of *Ae. nudicaulis*, which is almost ubiquitous on Trinidad. Regardless of model structure, performance was always poor for this species, generally being no better than random prediction. The results of SDM are displayed in Table 6.3.1.

| Species | | Regularisation parameter | Mean test-AUC | Standard deviation test-AUC |
|----------------------------|-----------------|--------------------------|---------------|-----------------------------|
| <i>Aechmea aquilega</i> | With vegtype | 18.0 | 0.657 | 0.232 |
| | Without vegtype | 8.0 | 0.656 | 0.223 |
| <i>Aechmea dichlamydea</i> | With vegtype | 4.0 | 0.879 | 0.091 |
| | Without vegtype | 3.0 | 0.875 | 0.100 |
| <i>Aechmea fendleri</i> | With vegtype | 4.0 | 0.888 | 0.130 |
| | Without vegtype | 3.0 | 0.866 | 0.156 |
| <i>Aechmea nudicaulis</i> | With vegtype | 5.0 | 0.479 | 0.177 |
| | Without vegtype | 3.0 | 0.480 | 0.218 |

Table 6.3.1. Results of MaxEnt species distribution models for the Northern Range of Trinidad, showing regularisation parameters, mean test-AUC scores, and standard deviations.

6.3.2.2 Responses of P_{occ} to predictor variables

Aechmea aquilega

For *Ae. aquilega*, probability of occurrence (P_{occ}) was not affected by vegtype. P_{occ} and MAT showed a linear positive relationship between 21.2°C ($P_{occ} = 0.17$) and 26.8°C ($P_{occ} = 0.63$). There was also a linear positive relationship between P_{occ} and temperature diurnality, with P_{occ} rising from 0.40 at 7.9°C to 0.60 at 9.5°C. P_{occ} was negatively related with MAP, falling in a linear fashion from 0.61 at 1500 mm to 0.39 at 2900 mm. The relationship between P_{occ} and precipitation seasonality was positive and linear, with P_{occ} increasing from 0.42 at 39% to 0.64 at 57%. P_{occ} was negatively correlated with dry season precipitation, falling from 0.60 at 120 mm to 0.41 at 320 mm. P_{occ} decreased sigmoidally with increasing altitude, from 0.60 at sea-level to 0.05 at 900 m and above. In response to AET/PET, P_{occ} declined linearly from 0.62 at AET/PET = 0.75 to 0.42 at AET/PET = 0.97. P_{occ} was 0.62 at the lowest AI values, and declined to 0.36 at 20000. P_{occ} declined parabolically in response to increasing slope, falling from 0.57 at 0° to 0.30 at 29°. P_{occ} increased linearly with aspect, rising from 0.43 when slope was below 0° to 0.57 when it exceeded 250°. Jackknife testing of variable importance (Table 6.3.2) suggested that precipitation seasonality was the strongest predictor of occurrence, followed by altitude, AET/PET and vegtype.

Aechmea dichlamydea

For *Ae. dichlamydea*, P_{occ} was highest in vegtype 13 (*Mora* ESF; $P_{occ} = 0.66$), followed by vegtype 21 (Young Secondary Forest, the dominant vegtype in the northeast corner of Trinidad; $P_{occ} = 0.62$). P_{occ} was relatively high and stable between MAT values of 21.0-25.5°C, falling steeply above this. P_{occ} was highest at low temperature diurnality values (0.82 at 7.8°C), falling sharply to 0.17 by 8.8°C. P_{occ} remained at approximately 0.30 when MAP was below 2000 mm, but then rose steeply to 0.63 at 2900 mm. For precipitation seasonality, P_{occ} was only high at the lowest values, declining steeply from 0.76 at 38% to 0.12 at 42%. P_{occ} was low (0.18) when dry season precipitation was below 240 mm. Above this threshold, P_{occ} increased rapidly, reaching 0.65 by 320 mm. P_{occ} was insensitive to altitude, remaining near-constant around 0.50. P_{occ} remained low at 0.20 at AET/PET values below 0.88, then rose sharply to 0.70 by 0.97. There was a sigmoid increase in P_{occ} in response to AI, from 0.23 at 9000 to 0.63 at 20000. P_{occ} was highest at low slope values, remaining at 0.54 up to 12° and then dropping to 0.36 by 29°. As with altitude, aspect had no effect on P_{occ} . Jackknife testing showed that the strongest predictor variables for *Ae. dichlamydea* when the vegtype layer was excluded were DTR, P_{seas} , P_{dry} and AET/PET. Inclusion of the vegtype layer did not affect this.

Aechmea fendleri

For *Ae. fendleri*, P_{occ} was highest in vegtype 19 (MF; $P_{occ} = 0.725$), followed by vegtypes 16 (LMF; $P_{occ} = 0.63$) and 21 (Young Secondary Forest; $P_{occ} = 0.40$). P_{occ} showed a sigmoid decline with rising MAT, suggesting that lower mean temperatures were most favourable to *Ae. fendleri* occurrence. DTR meanwhile showed an optimum at 8.55 °C, declining steeply both above and below. The response of P_{occ} to MAP was positive and linear up to a break point at 2400 mm, above which the relationship was again linear but less steep. P_{seas} showed an optimum at 41, declining very sharply below this value and less steeply but linearly above it. There was also an optimum value of P_{dry} , at approximately 295 mm. P_{occ} rose gradually and linearly below this point, and above it dropped off very steeply. P_{occ} increased sigmoidally with altitude, from 0.05 at sea-level to 0.90 at 900 m. As a function of AET/PET, P_{occ} rose from 0.08 at AET/PET = 0.75 to a peak value of 0.58 at AET/PET = 0.95. Above this, there was a slight decline in P_{occ} to 0.50 by 0.97. In response to increasing AI, P_{occ} rose approximately linearly from 0.10 at 9000 to a plateau of approximately 0.59 at 18500 and above. The relationship between P_{occ} and slope was positive and sigmoid, with P_{occ} reaching 0.90 by 30°. Aspect, which was the worst-performing predictor variable, showed a weak and essentially linear positive relationship with P_{occ} . The results of jackknife testing of variable importance were consistent regardless of whether the vegetation layer was included or not. Altitude was the highest-scoring variable, followed by MAT, slope and DTR. In summary, the distribution of *Ae. fendleri* appeared to be most strongly controlled by temperature-related factors.

Aechmea nudicaulis

Reflecting the near-ubiquitous distribution of *Ae. nudicaulis*, for this species P_{occ} was unresponsive to most environmental variables. The only apparent exceptions were a decline in P_{occ} from 0.51 to 0.32 above AET/PET values of 0.94 and AI values of 18000. Similarly, above MAP values of 2700 mm, P_{occ} fell from 0.51 to 0.35, and when P_{dry} exceeded 310 mm P_{occ} decreased from 0.51 to 0.34. Given the extremely limited discriminatory power of any model for *Ae. nudicaulis*, these results should be treated with caution. However, they are consistent with qualitative observations made in the field, where the abundance of *Ae. nudicaulis* tended to decline somewhat in zones of especially high rainfall.

| Species | | 1 st highest-scoring predictor | 2 nd highest-scoring predictor | 3 rd highest-scoring predictor | 4 th highest-scoring predictor |
|----------------------------|-----------------|---|---|---|---|
| <i>Aechmea aquilega</i> | With vegtype | P _{seas} | Alt | AET/PET | Vegtype |
| | Without vegtype | AET/PET | AI | MAT | Alt |
| <i>Aechmea dichlamydea</i> | With vegtype | DTR | P _{seas} | P _{dry} | AET/PET |
| | Without vegtype | DTR | P _{seas} | P _{dry} | AET/PET |
| <i>Aechmea fendleri</i> | With vegtype | Alt | MAT | Slope | DTR |
| | Without vegtype | Alt | MAT | Slope | DTR |
| <i>Aechmea nudicaulis</i> | With vegtype | Vegtype | - | - | - |
| | Without vegtype | MAP | P _{seas} | AET/PET | AI |

Table 6.3.2. Results of jackknife testing of variable importance for Maxent species distribution models using all environmental variables, with and without vegtype layer. P_{seas} = precipitation seasonality; Alt = altitude; AET/PET = ratio of actual to potential evapotranspiration; AI = aridity index; MAT = mean annual temperature; DTR = diurnal temperature range; P_{dry} = precipitation in driest quarter; Slope = angle of terrain slope.

6.3.2.3 Probability distribution grid maps

Aechmea aquilega

The projected distribution of *Ae. aquilega* in Trinidad under present-day climate conditions in the Northern Range is shown in Fig. 6.3.2a, which matches field observations well. The distribution of P_{occ} was projected to be fairly uniform outside of the Northern Range (Fig. 6.3.2b), with a value of 0.50-0.60 assigned to most pixels. Although this is partly a reflection of limited model predictive capacity, the relative uniformity of the probability distribution is in keeping with the fact that *Ae. aquilega* is known to occur widely across Trinidad outside the Northern Range. Inclusion of vegtype in the projection for Trinidad had little effect on the PDG (Fig. 6.3.2c-d). The projected present-day probability distribution for Tobago (Fig. 6.3.2e) shows a notable reduction in P_{occ} (0.20-0.30) along the spine of the Main Ridge. This was confirmed by ground-truthing on Tobago, where *Ae. aquilega* was observed primarily at low elevations, particularly in the wetter northeast of the island (Fig. 6.3.2f). On the Paria Peninsula (Fig. 6.3.2g), the probability distribution for *Ae. aquilega* indicates relatively high P_{occ} (0.50-0.60) only on the peninsula's low-elevation southern plain and around the coastal fringe of the remainder of the peninsula.

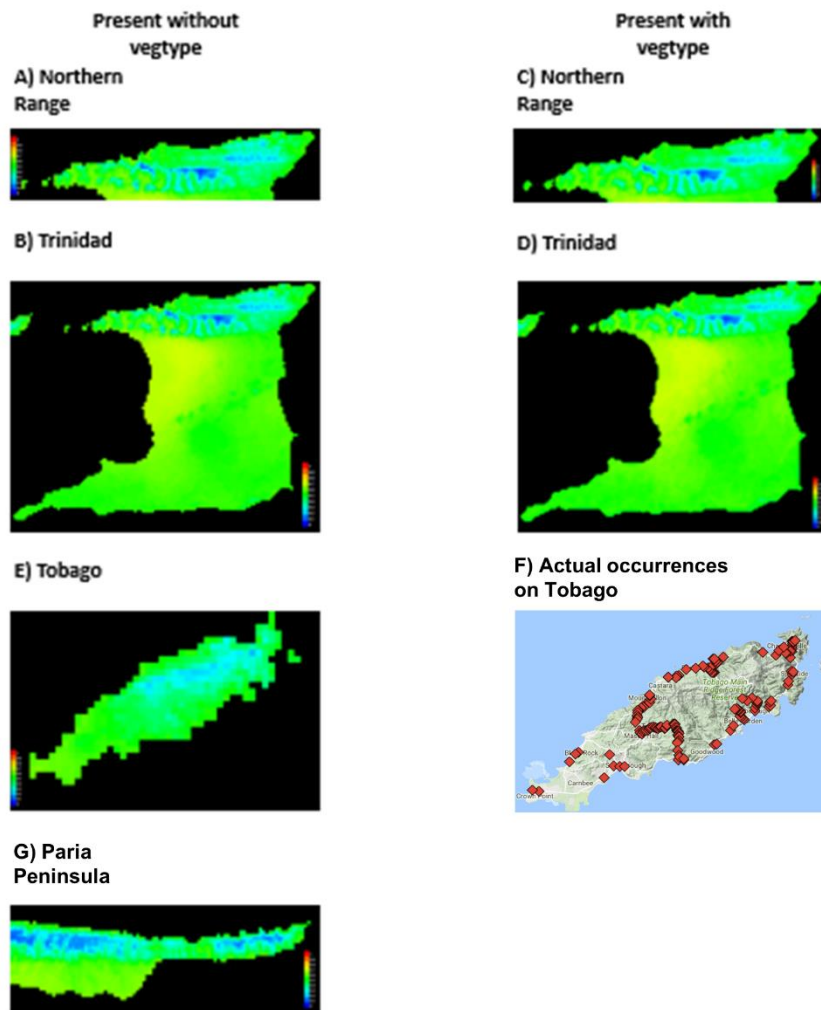


Figure 6.3.2. Maxent probability distribution grid maps and comparative Tobagonian distributional data for *Ae. aquilega* under present-day climate for: a) the Northern Range (model without vegtype); b) Trinidad (model without vegtype); c) the Northern Range (model with vegtype); d) Trinidad (model with vegtype); e) Tobago (model without vegtype); f) actual occurrences recorded on Tobago; g) the Paria Peninsula (model without vegtype).

Aechmea dichlamydea

Across the Northern Range, the model successfully reflected that there was a high P_{occ} for *Ae. dichlamydea* only in the northeast corner regardless of whether the vegtype layer was included or excluded (Fig. 6.3.3a,c). Projections for the whole of the island suggested that P_{occ} for *Ae. dichlamydea* should be low across much of central and southern Trinidad (Fig. 6.3.3b,d). However, two regions of elevated P_{occ} could be identified. The first (Zone D1), which was evident only when vegtype was included in the model (Fig. 6.3.3d, cf. Fig. 6.3.3b), was centred on an area of ESF to the south of the Central Range. The second (Zone D2), which showed especially high values of P_{occ} (\geq

0.80) when vegtype was included but was also discernible when vegtype was excluded, was located in the southwestern Cedros peninsula. Inspection of the environmental layer maps reveals that Zone D1 is similar to northeast Trinidad in displaying particularly low seasonality of rainfall. The same is true of Zone D2, which shows a further similarity with the northeast in exhibiting a low diurnal temperature range. However, the vegetation of Zone D2 continues to experience extensive disturbance associated with the petrochemical industry, and is unlikely to harbour stable populations of long-lived epiphytes.

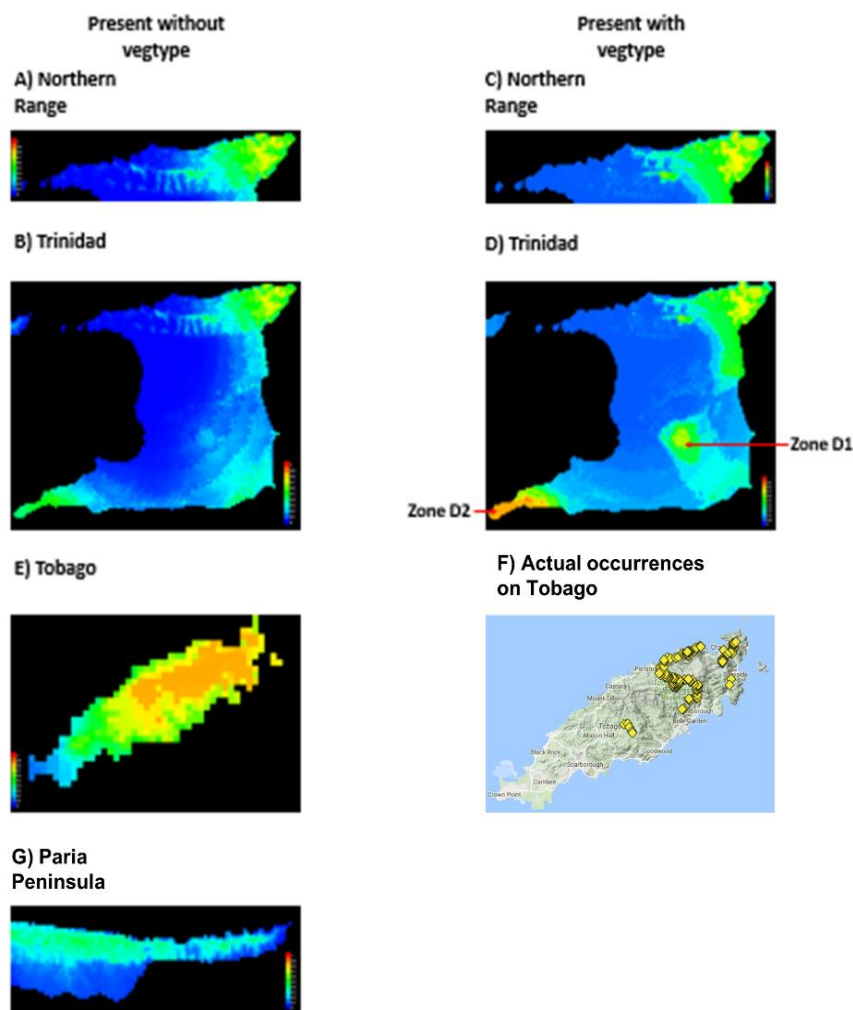


Figure 6.3.3. Maxent probability distribution grid maps and comparative Tobagonian distributional data for *Ae. dichlamydea* under present-day climate for: a) the Northern Range (model without vegtype); b) Trinidad (model without vegtype); c) the Northern Range (model with vegtype); d) Trinidad (model with vegtype); e) Tobago (model without vegtype); f) actual occurrences recorded on Tobago; g) the Paria Peninsula (model without vegtype).

Projected P_{occ} was high (≥ 0.60) across over three-quarters of Tobago under present climatic conditions (Fig. 6.3.3e), with P_{occ} of 0.90 being assigned to the whole of the Main Ridge. Low P_{occ} (0.20-0.30) was projected to occur only in the drier southwestern tip of the island. These projections were supported by ground-truthing, which showed that *Ae. dichlamydea* var. *dichlamydea* was extremely abundant across the Main Ridge but was rare at low elevations in the northeast and absent in the southwest (Fig. 6.3.3f. Projection assigned moderate P_{occ} for *Ae. dichlamydea* (~ 0.50) to large areas of the Paria Peninsula (Fig. 6.3.3g), particularly at high elevations towards the western end of the peninsula, where the subspecies *Ae. dichlamydea* var. *pariaensis* Pittendr. Is known to occur.

Aechmea fendleri

Models with and without vegtype reflected the observed distribution of *Ae. fendleri* across the Northern Range (Fig. 6.3.4a,c). When the model for *Ae. fendleri* trained on presence data from the Northern Range was projected across the whole of Trinidad, P_{occ} of 0.40 was projected around the highest summits of the Central Range (Tamana and Mount Harris), but otherwise P_{occ} was projected to be near-zero across those parts of the island outside the Northern Range (Fig. 6.3.4b). No major differences were observed between PDG maps generated with models exclusive (Fig. 6.3.4a-b) and inclusive (Fig. 6.3.4c-d) of the vegtype layer. Low P_{occ} for *Ae. fendleri* was projected for the whole of Tobago (Fig. 6.3.4e), except for a few patches of the Main Ridge, which is the coolest and wettest part of the island. Consistent with previous reports, no evidence was found for the presence of *Ae. fendleri* on Tobago in this investigation. Several areas of high P_{occ} (≥ 0.80) for *Ae. fendleri* occurred in the PDG projected onto the Paria Peninsula, particularly towards its eastern seaward tip (Fig. 6.3.4f). No systematic surveys of the spatial distribution of bromeliads on the Paria Peninsula has been performed, but there are references to the occurrence of *Ae. fendleri* at high elevations in the peninsula's mountain range.

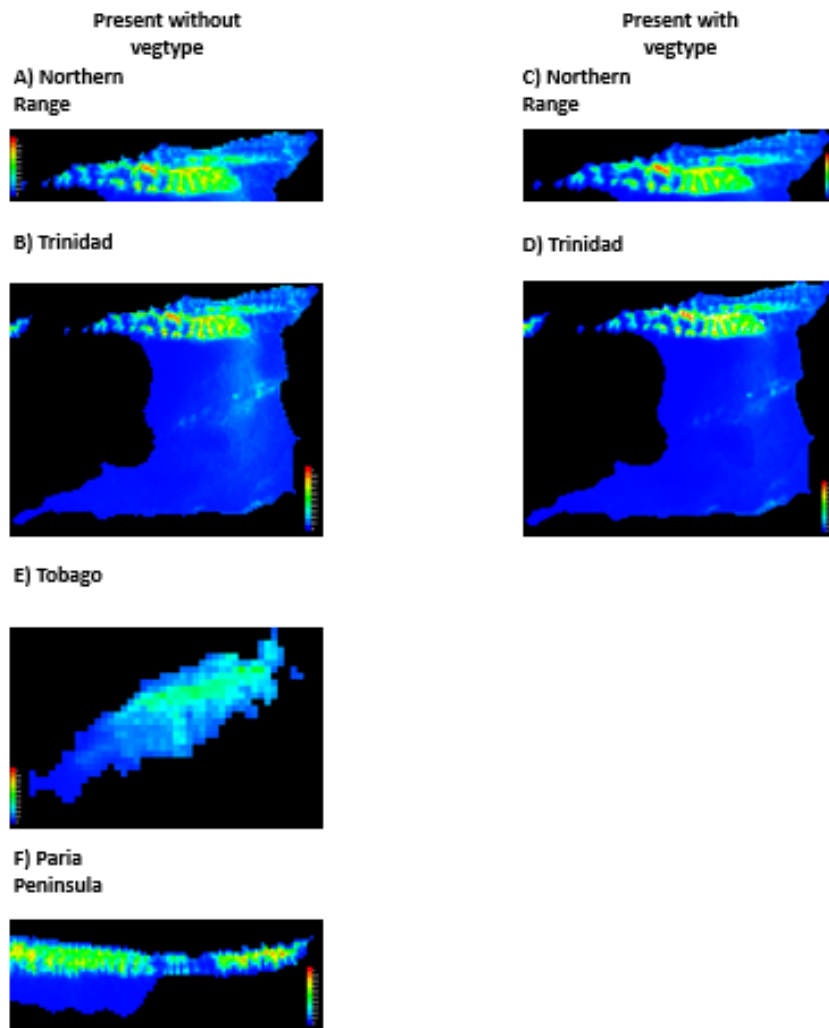


Figure 6.3.4. Maxent probability distribution grid maps for *Ae. fendleri* under present-day climate for: a) the Northern Range (model without vegtype); b) Trinidad (model without vegtype); c) the Northern Range (model with vegtype); d) Trinidad (model with vegtype); e) Tobago (model without vegtype); f) the Paria Peninsula (model without vegtype).

Aechmea nudicaulis

Projections for Trinidad under present-day climate, exclusive (Fig. 6.3.5a-b) or inclusive (Fig. 6.3.5c-d) of the vegtype layer, produced near-homogenous PDG maps for the whole of the island, with a ~50% probability of occurrence in all areas. The same was true of the PDG maps projected for Tobago (Fig. 6.3.5e), where *Ae. nudicaulis* was observed to occur in various climatic zones and vegetation types but at lower abundance than in Trinidad (Fig. 6.3.5f). The PDG map projected for the Paria Peninsula was also consistent with an even distribution of P_{occ} across the peninsula (Fig. 6.3.5g). These results are suggestive of the potential for near-ubiquitous occurrence of *Ae. nudicaulis*, although they are based on a model of minimal discriminatory power and factors not in

evidence in the Northern Range of Trinidad could be important in determining the likelihood of occurrence elsewhere.

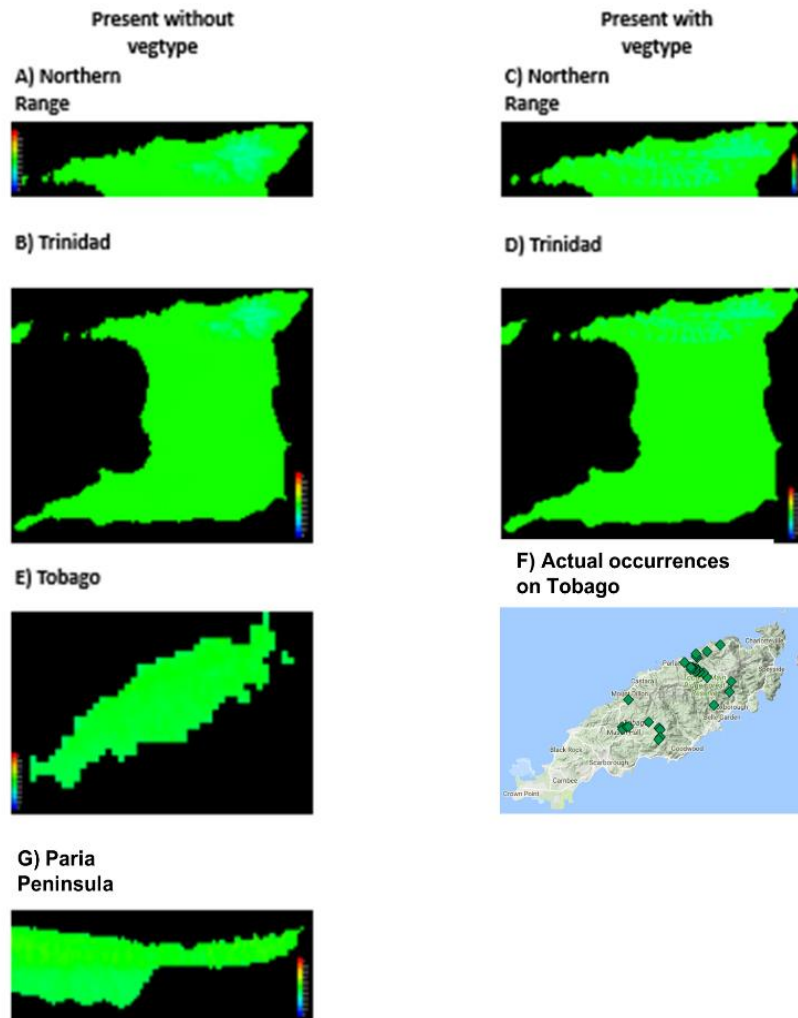


Figure 6.3.5. Maxent probability distribution grid maps and comparative Tobagonian distributional data for *Ae. nudicaulis* under present-day climate for: a) the Northern Range (model without vegtype); b) Trinidad (model without vegtype); c) the Northern Range (model with vegtype); d) Trinidad (model with vegtype); e) Tobago (model without vegtype); f) actual occurrences recorded on Tobago; g) the Paria Peninsula (model without vegtype).

Forecasting of future potential distributions under an aggressive 2070 climate scenario was also performed, and is reported in **Appendix 6.7**.

6.3.3 Leaf anatomical characterisation

Abundant foliar anatomical differentiation between *Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis* was apparent. Data are summarised in Table 6.3.3. The mean thickness of the leaf blade was greater in *Ae. nudicaulis* than in either of the other species. This was driven both by a thicker chlorophyllous mesophyll and water-storage hydrenchyma in *Ae. nudicaulis*. The structure of hydrenchyma differed between species. In *Ae. aquilega* it consisted of 2-3 layers of hexagonal cells measuring approximately 200 μm in diameter, while in *Ae. fendleri* it comprised 2 layers of hexagonal cells of diameters of approximately 150 μm . The structure of the hydrenchyma in *Ae. nudicaulis* was more complex, with small cuboidal cells near the adaxial epidermis grading into tall (375 μm) columnar cells. In *Ae. nudicaulis*, the chlorenchyma was differentiated into distinct spongy and biseriate palisade layers, whereas this differentiation was not observed in *Ae. aquilega* or *Ae. fendleri*. The palisade cells of *Ae. nudicaulis* were columnar and approximately 150 μm in height. The spongy cells were globose and slightly smaller in *Ae. nudicaulis* than in the other two species, meaning that a greater number of cell layers occurred between vein bundle sheaths and the abaxial epidermis. Bulk IAS was slightly lower in *Ae. nudicaulis* than in the other species. However, the mesophyll of all three species was traversed by longitudinal aerenchyma of similar dimensions, which occurred in alternation with the vascular bundles. Stellate cells were observed within the aerenchyma of all species, and the air spaces were frequently continuous with substomatal cavities. The lower IAS of *Ae. nudicaulis* may therefore simply be due to the higher proportion of hydrenchyma in the leaves of this species, a tissue which contains very little air space. Cell wall thicknesses were comparable in all species, and were over twice as thick in chlorenchyma as in hydrenchyma in *Ae. fendleri* but three times thicker in the chlorenchyma of *Ae. aquilega* and *Ae. nudicaulis* than in the hydrenchyma of those species. Epidermal features of the three species differed considerably. With a mean diameter of 467 μm , the trichomes of *Ae. nudicaulis* were larger than those of the other species, particularly *Ae. fendleri* ($d_{\text{trichome}} = 367 \mu\text{m}$). The ratio of trichome density to stomatal density was substantially higher in *Ae. nudicaulis* than in the other two species. Vascular architecture showed a certain amount of interspecific differentiation, with the vascular plane being set deeper into the leaf in *Ae. nudicaulis* than the other species, a difference which underlay a slightly lower IVD: VED ratio in this species.

| Species | Leaf thickness (μm , \pm SE)* | Hydrenchyma cell shape* | $d_{\text{cell,hyd}}$ (μm , \pm SE)* | Number of cell layers in hydrenchyma* | Hydrenchyma thickness (μm , \pm SE)* | $T_{\text{cw,hyd}}$ (μm \pm SE)* |
|---------------------------|---|-------------------------|--|---------------------------------------|--|--|
| <i>Aechmea aquilega</i> | 754 \pm 16 | Hexagonal | 209 \pm 4 | 2-3 | 298 \pm 6 | 0.10 \pm 0.01 |
| <i>Aechmea fendleri</i> | 503 \pm 19 | Hexagonal | 150 \pm 3 | 2 | 167 \pm 5 | 0.13 \pm 0.02 |
| <i>Aechmea nudicaulis</i> | 961 \pm 27 | Cuboidal-columnar | 375 \pm 5 | 4-7 | 552 \pm 8 | 0.11 \pm 0.02 |

| Species | Chlorenchyma cell shape* | $d_{\text{cell,chlor}}$ (μm , \pm SE)* | Cells between BS and abaxial epidermis* | $T_{\text{cw,chlor}}$ (μm \pm SE)* | Chlorenchyma thickness (μm , \pm SE)* | IAS (% , \pm SE) |
|---------------------------|--------------------------|--|---|--|---|--------------------|
| <i>Aechmea aquilega</i> | Globose | 123 \pm 2 | 4 | 0.31 \pm 0.04 | 456 \pm 7 | 11.58 \pm 0.34 |
| <i>Aechmea fendleri</i> | Globose | 131 \pm 4 | 3 | 0.28 \pm 0.03 | 336 \pm 5 | 10.26 \pm 0.86 |
| <i>Aechmea nudicaulis</i> | Globose | 97 \pm 2 | 5 | 0.37 \pm 0.04 | 409 \pm 6 | 8.32 \pm 0.38 |

| Species | d_{trichome} (μm , \pm SE)* | Number of cell layers in trichome shield* | TD (mm^{-2} , \pm SE)* | SD (mm^{-2} , \pm SE) | TD: SD* | LMA (g m^{-2} , \pm SE) |
|---------------------------|--|---|------------------------------------|-----------------------------------|---------|-------------------------------------|
| <i>Aechmea aquilega</i> | 433 \pm 5 | 10 | 63.66 \pm 3.05 | 29.44 \pm 1.31 | 2.16 | 171.0 \pm 5.76 |
| <i>Aechmea fendleri</i> | 367 \pm 3 | 6 | 83.69 \pm 4.02 | 35.62 \pm 1.22 | 2.35 | 189.7 \pm 6.91 |
| <i>Aechmea nudicaulis</i> | 467 \pm 6 | 7 | 86.40 \pm 3.31 | 26.43 \pm 1.99 | 3.27 | 209.5 \pm 5.99 |

| Species | IVD (μm , \pm SE) | VED (μm , \pm SE) | IVD: VED |
|---------------------------|---------------------------------|---------------------------------|----------|
| <i>Aechmea aquilega</i> | 336.7 \pm 3.5 | 350.0 \pm 5.8 | 0.96 |
| <i>Aechmea fendleri</i> | 313.3 \pm 3.8 | 327.5 \pm 7.7 | 0.96 |
| <i>Aechmea nudicaulis</i> | 320.0 \pm 5.8 | 392.5 \pm 5.8 | 0.82 |

Table 6.3.3. Leaf anatomical characteristics of three *Aechmea* species. For all traits measured for this chapter (designated with an asterisk, *), $n \geq 20$ leaves from ≥ 6 individuals per species. Trait key: $d_{\text{cell,hyd}}$ = diameter of hydrenchyma cells; $T_{\text{cw,hyd}}$ = thickness of hydrenchyma cell walls; $d_{\text{cell,chlor}}$ = diameter of chlorenchyma cells; $T_{\text{cw,chlor}}$ = thickness of chlorenchyma cell walls; IAS = internal air space fraction; d_{trichome} = diameter of trichomes; TD = trichome density; SD = stomatal density; LMA = leaf mass per unit area; IVD = interveinal distance; VED = vein-epidermis distance.

6.3.4 Physiological characterisation

6.3.4.1 Gas exchange and titratable acidity

Ae. aquilega, *Ae. fendleri*, and *Ae. nudicaulis* all showed CAM gas exchange profiles with Phase I (nocturnal assimilation) dominating carbon uptake (Fig. 6.3.6a-f). There was a small amount of Phase IV fixation (late afternoon stomatal opening with direct fixation of CO₂ by RuBisCO) in *Ae. fendleri*.

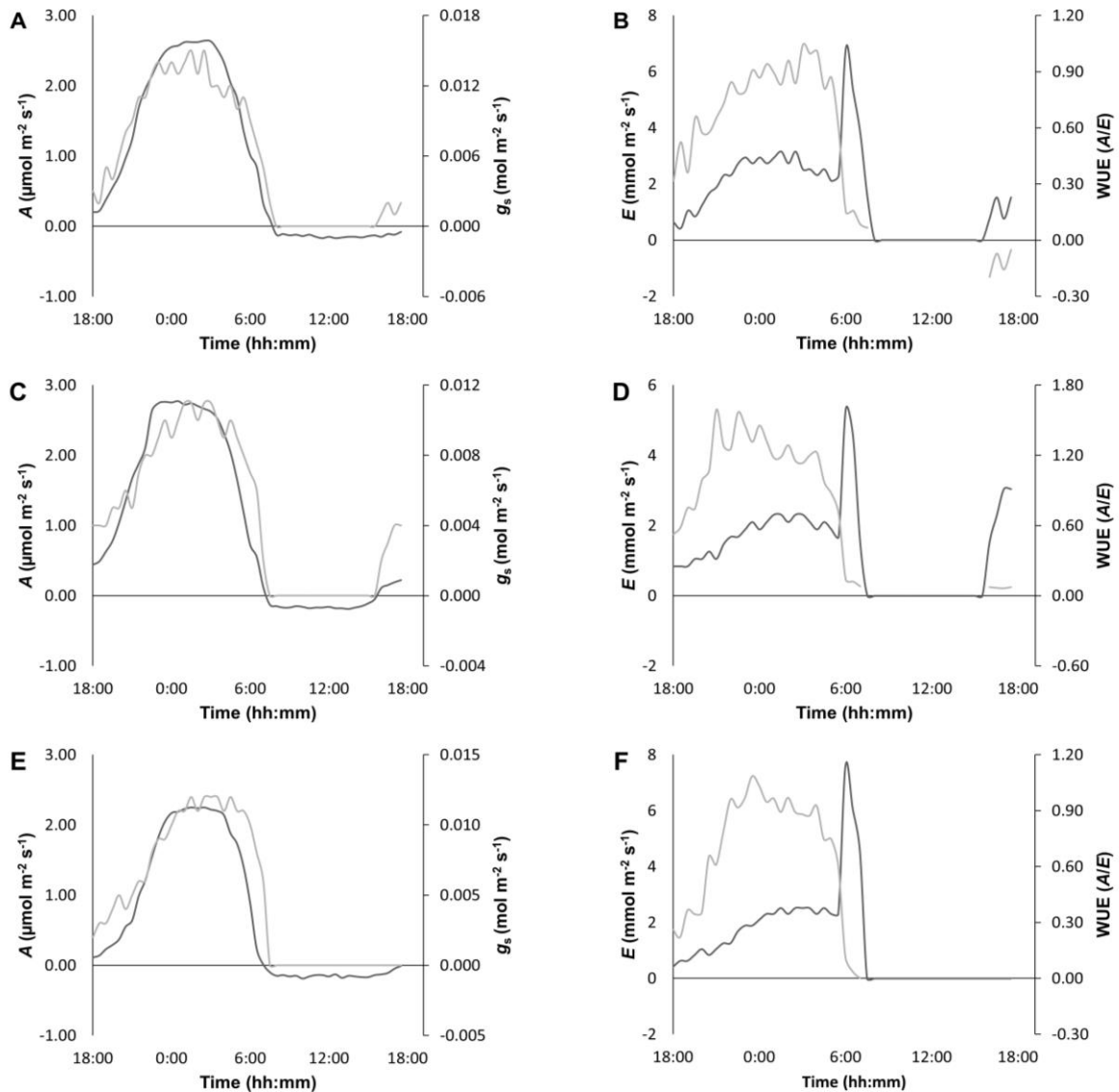


Figure 6.3.6. Representative day-night gas exchange profiles for *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis* based on spot measurements of parameters logged at 30 min intervals. a) A (dark line) and g_s (light line) for *Ae. aquilega*; b) E (dark line) and WUE (light line) for *Ae. aquilega*; c) A (dark line) and g_s (light line) for *Ae. fendleri*; d) E (dark line) and WUE (light line) for *Ae. fendleri*; e) A (dark line) and g_s (light line) for *Ae. nudicaulis*; f) E (dark line) and WUE (light line) for *Ae. nudicaulis*.

Under well-watered conditions, area-specific dusk-dawn ΔH^+ was relatively high in all three species. The mean (\pm SE) values were 221 ± 13 mmol m⁻² for *Ae. aquilega*, 235 ± 13 mmol m⁻² for *Ae. fendleri*, and 199 ± 13 mmol m⁻² for *Ae. nudicaulis*.

6.3.4.2 Leaf economic and hydraulic traits

A combination of data reported in **Chapter 3** and newly-collected data highlighted anatomical and physiological similarities and differences among the three *Aechmea* species (Table 6.3.4). All species showed similar values of Ψ_{tip} , suggesting that they were roughly equally sensitive to drought at the cellular level. Interestingly, *Ae. fendleri* showed marginally higher C_{FT} than the other species, but the lowest value of SWC. When this result is compared with the anatomical data, it is apparent that the greater thickness and density of the leaves of *Ae. nudicaulis* is determined in part by increased water storage in more succulent chlorenchyma cells relative to hydrenchyma. The reduced accessibility of this water may account for the lower value of C_{FT} in this species. A_{max} was relatively low in all species, but highest in *Ae. fendleri*, despite the lowest values of N_{leaf} , g_s and K_{leaf} also occurring in this species. *Ae. fendleri* also showed the greatest sensitivity to leaf water potential in terms of both stomatal conductance (P_{50s}) and leaf hydraulic conductance (P_{50L}). Meanwhile S_s was lowest in *Ae. fendleri*, suggesting this species has the lowest capacity for adjustment of water-use in response to microclimatic dynamics.

| Species | Ψ_{tip} (MPa) | C_{FT} (mol m ⁻² MPa ⁻¹) | ϵ (MPa) | SWC* | A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | N_{leaf} (%) |
|---------------------------|---------------------------|--|------------------|-------------|---|-----------------------|
| <i>Aechmea aquilega</i> | -1.22 ± 0.07 | 5.79 ± 0.44 | 6.86 ± 0.05 | 4.19 ± 0.12 | 2.63 ± 0.13 | 1.48 ± 0.03 |
| <i>Aechmea fendleri</i> | -1.18 ± 0.11 | 6.11 ± 0.11 | 6.50 ± 0.12 | 4.01 ± 0.08 | 2.81 ± 0.18 | 0.60 ± 0.03 |
| <i>Aechmea nudicaulis</i> | -1.21 ± 0.10 | 5.86 ± 0.34 | 5.86 ± 0.34 | 4.82 ± 0.10 | 2.26 ± 0.18 | 0.92 ± 0.02 |

| Species | K_{leafmax} (mmol m ⁻² s ⁻¹ MPa ⁻¹) | g_{smax} (mol m ⁻² s ⁻¹) | S_s (mol m ⁻² s ⁻¹ kPa ⁻¹) | P_{50L} (MPa) | P_{50S} (MPa) |
|---------------------------|--|--|--|-----------------|-----------------|
| <i>Aechmea aquilega</i> | 0.22 ± 0.02 | 0.017 ± 0.001 | 0.66 ± 0.01 | -1.27 ± 0.07 | -0.55 ± 0.05 |
| <i>Aechmea fendleri</i> | 0.16 ± 0.02 | 0.011 ± 0.001 | 0.42 ± 0.01 | -1.20 ± 0.08 | -0.52 ± 0.04 |
| <i>Aechmea nudicaulis</i> | 0.23 ± 0.02 | 0.013 ± 0.001 | 0.51 ± 0.02 | -1.23 ± 0.08 | -0.62 ± 0.06 |

Table 6.3.4. Mean values (\pm SE) of leaf economic and hydraulic properties of *Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis* retrieved from data in **Chapter 3** or newly-collected for this chapter. Trait key: Ψ_{tip} = leaf water potential at turgor loss point; C_{FT} = leaf capacitance at full turgor; ϵ = leaf bulk elastic modulus; SWC = saturated water content; A_{max} = maximum photosynthetic capacity; N_{leaf} = leaf nitrogen content; K_{leafmax} = maximal leaf hydraulic conductance; g_{smax} = maximal stomatal conductance; S_s = instantaneous stomatal sensitivity to leaf-air vapour pressure deficit; P_{50L} = leaf water potential at 50% loss hydraulic conductance; P_{50S} = leaf water potential at 50% stomatal closure.

6.3.4.3 Temperature relations

| Species | T_{15} (°C) PM | T_{50} (°C) PM | T_{15} (°C) AM | T_{50} (°C) AM | ΔT_{15} (°C) PM-AM | ΔT_{50} (°C) PM-AM |
|---------------------------|------------------|------------------|------------------|------------------|----------------------------|----------------------------|
| <i>Aechmea aquilega</i> | 36.9 ± 0.1 | 45.9 ± 0.1 | 34.0 ± 0.1 | 44.1 ± 0.1 | 2.9 | 1.8 |
| <i>Aechmea fendleri</i> | 32.5 ± 0.2 | 42.1 ± 0.2 | 30.4 ± 0.1 | 38.7 ± 0.1 | 2.1 | 3.5 |
| <i>Aechmea nudicaulis</i> | 38.1 ± 0.1 | 49.4 ± 0.2 | 34.8 ± 0.2 | 44.8 ± 0.1 | 3.3 | 4.7 |

Table 6.3.5. Thermal sensitivity of ΦPSII during early (AM) and late (PM) parts of the photoperiod. Sensitivity quantified as the mean leaf temperature (°C, \pm SE) at which 15% or 50% of initial ΦPSII had been lost (T_{15} and T_{50} respectively).

Data encapsulating the thermal sensitivity of photochemistry in the *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis* are presented in Table 6.3.5. *Ae. nudicaulis* showed the highest thermal tolerance, with values of T_{15} and T_{50} several degrees higher than those displayed by *Ae. fendleri*. *Ae. aquilega* showed intermediate values. This suggests that high leaf temperatures can impair photosynthetic

capacity in the montane species, *Ae. fendleri*, whereas this negative effect is less pronounced in the exposure-seeking species. In all species, the values of T_{15} PM and T_{50} PM were consistently higher than T_{15} AM and T_{50} AM, when the acid pool is still high.

Nocturnal acid accumulation (ΔH^+) was sensitive to night-time temperature in all species (Table 6.3.6). In *Ae. aquilega*, ΔH^+ declined steeply at night-time temperatures below 18°C, and increased slightly at higher night-time temperatures. A similar pattern occurred in *Ae. nudicaulis*, except that the sensitivity to lower temperatures was less pronounced and the positive response to higher temperatures was stronger. *Ae. fendleri* showed the smallest decline in ΔH^+ between 18°C and 13°C, although ΔH^+ did then decrease more substantially by 8°C. At night-time temperatures above 18°C, ΔH^+ declined steeply in this species, consistent with biochemical adaptation to lower night-time temperatures.

| Species | Night-time temperature (°C) | $\Delta H^+ \pm SE$ (mmol m ⁻²) | % deviation from 18°C |
|---------------------------|-----------------------------|---|-----------------------|
| <i>Aechmea aquilega</i> | 8 | 76 ± 3 | -65% |
| | 13 | 131 ± 5 | -40% |
| | 18 | 220 ± 5 | n/a |
| | 23 | 255 ± 4 | +16% |
| | 28 | 262 ± 5 | +19% |
| <i>Aechmea fendleri</i> | 8 | 103 ± 3 | -56% |
| | 13 | 201 ± 5 | -14% |
| | 18 | 235 ± 6 | n/a |
| | 23 | 221 ± 4 | -6% |
| | 28 | 141 ± 4 | -40% |
| <i>Aechmea nudicaulis</i> | 8 | 112 ± 5 | -44% |
| | 13 | 167 ± 4 | -16% |
| | 18 | 199 ± 6 | n/a |
| | 23 | 261 ± 5 | +31% |
| | 28 | 283 ± 6 | +42% |

Table 6.3.6. Effect of night-time temperature on mean nocturnal acid accumulation (ΔH^+) in *Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis*, showing % deviation from ΔH^+ at standard night-time temperature of 18°C. n = five leaves per treatment per species.

6.3.4.4 Effect of surface wetting

Experimental wetting of leaf surfaces aimed at simulating the effect of occult precipitation had a strong, species-specific impact on photosynthetic activity. Representative day-night profiles of assimilation rate with and without surface wetting are displayed in Fig. 6.3.7. In *Ae. aquilega*, total

assimilation during Phase I (calculated by integrating the area under the gas exchange curve) was reduced by surface-wetting by an average of 81%, and in *Ae. fendleri* the mean value was 78%. For *Ae. nudicaulis*, only a 69% reduction occurred.

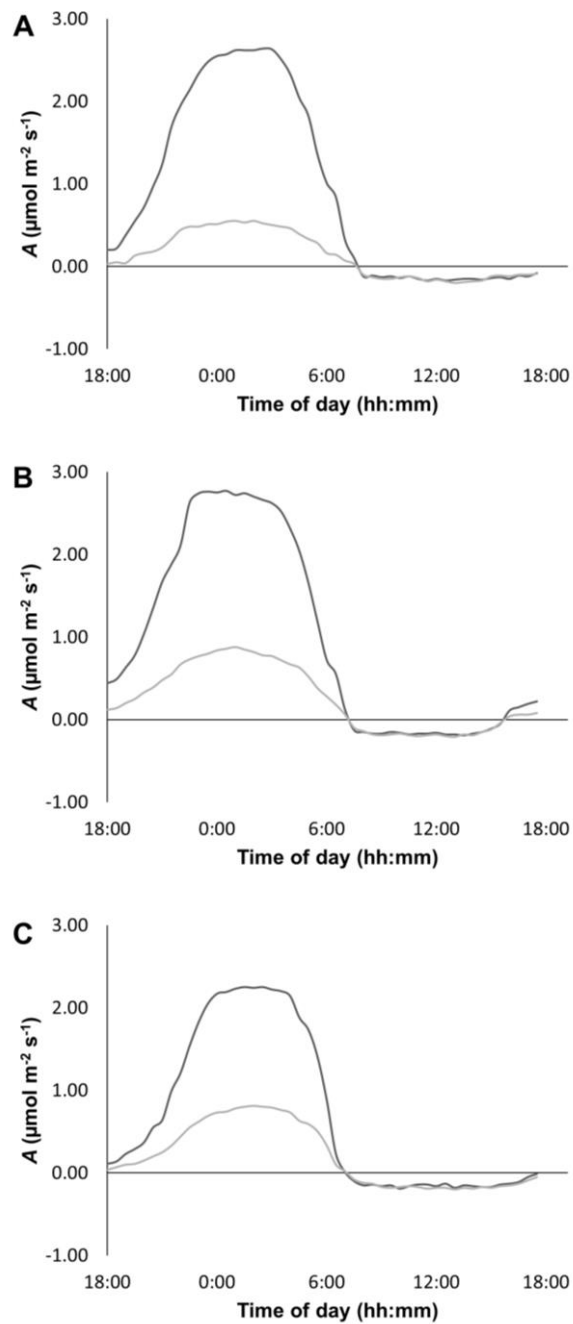


Figure 6.3.7. Effect of surface wetness on net assimilation rate (A) in a) *Ae. aquilega*, b) *Ae. fendleri* and c) *Ae. nudicaulis*. Dark lines show representative day-night profiles of net assimilation rate for non-wetted leaves; light lines show representative profiles for surface-wetted leaves.

The effect of surface-wetting on nocturnal acid accumulation was assessed simultaneously. Surface-wetting led to a mean reduction in ΔH^+ of 41% ($\pm 3.3\%$ SE) for *Ae. aquilega*, a 20% ($\pm 1.6\%$ SE) reduction in *Ae. fendleri*, and an 11% ($\pm 0.7\%$ SE) reduction in *Ae. nudicaulis*. The effect of surface-wetting on nocturnal acid accumulation was therefore weaker than its effect on gas exchange, consistent with a proportional increase in CO₂ recycling. This increased dependency on CO₂ recycling was less apparent in *Ae. aquilega* than the other two species, suggesting that it has less flexibility in responding to surface wetness.

6.3.4.5 Vein- and trichome-mediated rehydration kinetics

Time-constants for both the fast and slow phases of vein-mediated rehydration were lowest in *Ae. nudicaulis* and highest in *Ae. fendleri* (Table 6.3.7). Fast- and slow-phase pool sizes appeared to be coordinated across the three species, indicating that they do not differ drastically in hydraulic design (see **Chapter 4** for further discussion of the relationships between leaf anatomy and hydraulic design). The time-constant for trichome-mediated water uptake was lowest in *Ae. nudicaulis*, despite the fact that this species also displayed the largest pool size. These results are suggestive of a gradient of water acquisition and distribution capacity across the three species.

| Species | Fast-phase time-constant (s) \pm SE | Fast-phase pool size (g) \pm SE | Slow-phase time-constant (s) \pm SE | Slow-phase pool size (g) \pm SE | Trichome time-constant (s) \pm SE | Trichome pool size (g) \pm SE |
|---------------------------|---------------------------------------|-----------------------------------|---------------------------------------|-----------------------------------|-------------------------------------|---------------------------------|
| <i>Aechmea aquilega</i> | 722 \pm 22 | 0.215 \pm 0.01 | 7952 \pm 267 | 1.406 \pm 0.11 | 18,979 \pm 544 | 1.122 \pm 0.10 |
| <i>Aechmea fendleri</i> | 788 \pm 31 | 0.204 \pm 0.01 | 8133 \pm 394 | 1.341 \pm 0.13 | 19,680 \pm 286 | 0.965 \pm 0.12 |
| <i>Aechmea nudicaulis</i> | 704 \pm 18 | 0.304 \pm 0.02 | 7894 \pm 502 | 1.561 \pm 0.12 | 18,332 \pm 199 | 1.844 \pm 0.11 |

Table 6.3.7. Fitted time-constants and pool sizes for fast and slow phases of rehydration in *Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis*. Data for *Ae. fendleri* and *Ae. nudicaulis* are from **Chapter 4**.

6.3.4.6 Tank water storage

Tank surface area was considerably lower in *Ae. nudicaulis* than in the other two species (Table 6.3.8). This, combined with a relatively large tank volume, provided a tank morphology that appeared to be adapted for maximising water storage while minimising evaporative water loss. At the opposite end of the spectrum, tanks in *Ae. fendleri* showed the largest surface area and lowest volume, meaning that this species could hold comparatively little water and was most susceptible to evaporative loss.

| Species | Tank surface area (cm ²) | Tank volume (mL) | Tank surface area: volume |
|---------------------------|--------------------------------------|------------------|------------------------------|
| <i>Aechmea aquilega</i> | 73.01 ± 4.1 | 297.22 ± 5.6 | 0.25 |
| <i>Aechmea fendleri</i> | 89.14 ± 5.0 | 261.31 ± 6.4 | 0.34 |
| <i>Aechmea nudicaulis</i> | 31.89 ± 1.8 | 294.06 ± 6.2 | 0.11 |

Table 6.3.8. Tank water storage characteristics for *Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis* ($n = 5$ plants per species).

6.3.4.7 Drought course

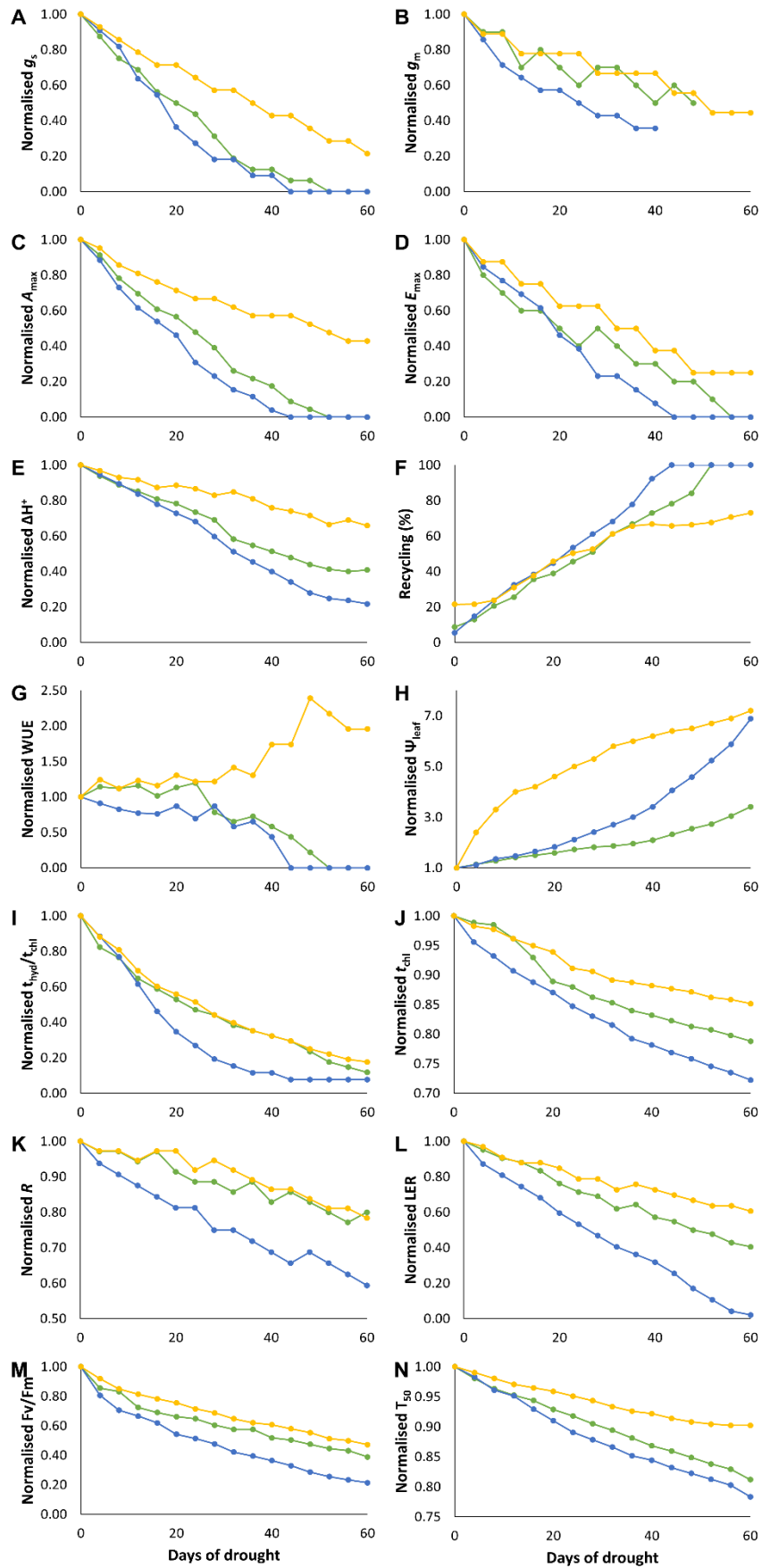


Figure 6.3.8 (overleaf). Physiological responses to experimental drought in *Ae. aquilega* (green lines), *Ae. fendleri* (blue lines) and *Ae. nudicaulis* (yellow lines). Plots show changes in the following variables, normalised by initial values at the beginning of the drought period: a) stomatal conductance to CO₂, g_s ; b) mesophyll conductance to CO₂, g_m ; c) photosynthetic capacity, A_{max} ; d) transpiration rate at maximum photosynthetic rate, E_{max} ; e) nocturnal acid accumulation, ΔH^+ ; f) CO₂ recycling; g) water-use efficiency, WUE; h) leaf water potential, Ψ_{leaf} ; i) ratio of hydrenchyma to chlorenchyma thickness, t_{hyd}/t_{chl} ; j) chlorenchyma thickness, t_{chl} ; k) respiration rate, R_L ; l) leaf elongation rate, LER; m) maximum quantum yield, F_v/F_m ; n) thermal sensitivity of Φ_{PSII} , T_{50} .

Across 60 d of experimental drought, individuals of the three *Aechmea* species displayed contrasting physiological responses to water limitation. Absolute data for all traits are available in **Appendix 6.8**. The relative decline in g_s was stronger in *Ae. aquilega* and *Ae. fendleri* than in *Ae. nudicaulis* (Fig. 6.3.8a). By 44 d of drought, stomatal conductance (g_s) was 0 in *Ae. fendleri* and by 52 d g_s was 0 for *Ae. aquilega*. Even after 60 d, *Ae. nudicaulis* maintained a g_s of 0.003 mol m⁻² s⁻¹. There was also a decline in mesophyll conductance to CO₂ (g_m) in all species (Fig. 6.3.8b), and this effect was stronger in *Ae. fendleri* than in the other two species. After 40 d, g_m had fallen to 35% of its initial value in *Ae. fendleri*. Photosynthetic capacity (A_{max}) declined most steeply during drought in *Ae. fendleri*, with net assimilation ceasing by 44 d. *Ae. aquilega* showed slightly lower sensitivity, with net assimilation being abolished by 52 d (Fig. 6.3.8c). Meanwhile *Ae. nudicaulis* was still attaining A_{max} of 0.90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after 60 d. Parallel reductions in the transpiration rate occurring at A_{max} (E_{max}) were seen in *Ae. fendleri* and *Ae. aquilega*, whereas in *Ae. nudicaulis* the reduction of E_{max} was stronger than that of A_{max} (Fig. 6.3.8d). *Ae. fendleri* showed the strongest relative decline in nocturnal acid accumulation (ΔH^+) during drought, falling by 78% after 60 d (Fig. 6.3.8e). By this point, ΔH^+ had fallen by 59% in *Ae. aquilega* and only 34% in *Ae. nudicaulis*. From the titratable acidity data and integration of gas exchange curves, it was possible to calculate the percentage of carbon fixation attributable to recycling of respired CO₂ (Fig. 6.3.8f). This percentage increased in all species during drought, from starting points of 5.4% for *Ae. fendleri*, 8.7% for *Ae. aquilega* and 21.5% for *Ae. nudicaulis*. The slope of the increase in the proportion of recycled CO₂ was similar for all species, but in *Ae. nudicaulis* the curve began to plateau after approximately 40 d of drought. Water-use efficiency (WUE) steadily increased during drought in *Ae. nudicaulis*, whereas it declined in the other two species (Fig. 6.3.8g). Initial leaf water potential (Ψ_{leaf}) was least negative (-0.10 MPa) in *Ae. nudicaulis*, with more negative values in *Ae. aquilega* (-0.22 MPa) and *Ae. fendleri* (-0.17 MPa; Fig. 6.3.8h). During the initial stages of drought, *Ae. aquilega* and *Ae. fendleri* showed similar rates of change in relative Ψ_{leaf} . However, they began to diverge after 12 d, with *Ae. fendleri* showing a more rapid decline in Ψ_{leaf} . After 60 d,

Ψ_{leaf} for *Ae. fendleri* was -1.17 MPa compared to -0.75 MPa for *Ae. aquilega*. In *Ae. nudicaulis*, Ψ_{leaf} showed a very different response to drought. The initial decline was much more rapid, but the rate of change then began to decrease such that after 60 d Ψ_{leaf} in *Ae. nudicaulis* was similar (-0.72 MPa) to *Ae. aquilega*. The thickness of the hydrenchyma relative to the chlorenchyma ($t_{\text{hyd}}/t_{\text{chl}}$) declined most rapidly in *Ae. fendleri*, suggesting that the less well-developed storage tissue in this species is depleted faster (Fig. 6.3.8i). The slopes for *Ae. aquilega* and *Ae. nudicaulis* were similar. Meanwhile the decrease in normalised chlorenchyma thickness (t_{chl}) was also steepest for *Ae. fendleri*, in which t_{chl} had declined by 38% after 60 d (Fig. 6.3.8j). By this point, t_{chl} had declined by 21% in *Ae. aquilega* and only 15% in *Ae. nudicaulis*.

The dark respiration rate in the light (R_L) declined most strongly in *Ae. fendleri*- by 41% after 60 d (Fig. 6.3.8k). In both *Ae. aquilega* and *Ae. nudicaulis* it had fallen by only ca. 20% by this stage. Leaf elongation rate (LER) also declined more steeply in *Ae. fendleri* than in the other two species, with growth almost unmeasurably low after 60 d (Fig. 6.3.8l). By contrast, by the same point, LER had declined by 60% in *Ae. aquilega* and only 39% in *Ae. nudicaulis*. Maximum quantum yield (F_v/F_m) showed a very similar pattern, and after 60 d had fallen by 79% in *Ae. fendleri*, 61% in *Ae. aquilega*, and 53% in *Ae. nudicaulis* (Fig. 6.3.8m). Thermal tolerance of the quantum yield of photosystem II (ΦPSII), quantified as T_{50} , again declined most steeply in *Ae. fendleri*, down by 22% after 60 d (Fig. 6.3.8n). In *Ae. aquilega* the effect was similar, with the value of T_{50} falling by 19% after 60 d. However, in *Ae. nudicaulis* T_{50} had dropped by only 10% after 60 d, suggesting that thermal tolerance in this species is less compromised by drought than is the case for the other two species.

Overall, while 60 d of experimental drought did not lead to plant mortality in any species, it had a strong negative impact on the physiological performance of *Ae. fendleri*, while *Ae. nudicaulis* was least affected. This is consistent with the observation that the former species is restricted to high-rainfall zones in Trinidad whereas the latter species displays a broad environmental range encompassing areas of more frequent and intense drought conditions.

6.4 Discussion

6.4.1 Distributions of *Aechmea bromeliads* in Trinidad

Across the Northern Range of Trinidad and adjacent lowland areas, there was clear geographical and altitudinal zonation of the distributions of the focal *Aechmea* species. The new observations presented here were generally consistent with both the historical data available from herbarium collections, and reports in the literature (Pittendrigh et al., 1948; Smith et al., 1986). The observations made in the field regarding the paucity of epiphytic bromeliads and other epiphytes in

forest types dominated by lianas are in accordance with comments made by Beard (1946a), and support a wider consensus that invasion of disturbed forest patches by lianescent species can modify the successional pathway by shifting the structural physiognomy and resource capital of the vegetation (Paul and Yavett, 2011 and refs. therein; Tymen et al., 2016; Ichihasi et al., 2017).

6.4.2 Species distribution modelling

6.4.2.1 Distributions across the Northern Range of Trinidad

Correlative species distribution modelling (SDM) using MaxEnt and the presence data collected during field surveys generated probability distribution grid (PDG) maps that were consistent with expectations and highlighted strongly contrasting potential distributions for the three focal *Aechmea* species and for *Ae. dichlamydea* var. *trinitensis*.

Several important limitations of the SDM approach used here should be borne in mind when interpreting the results. First, the approach was based on the use of gridded global bioclimatic datasets, which do not always accurately reflect fine-scale climatic variation. This is important, since high-quality climate data are needed for accurate model fitting, and fine-scale factors may be of particular importance (Kriticos and Leriche, 2010; Potter et al., 2013; Hannah et al., 2014; Storlie et al., 2014). However, it has been shown elsewhere that these datasets are reasonably reflective of the real distribution of climatic variation across Trinidad (Beharry et al., 2014), and MaxEnt models have been produced for Trinidadian plants using Bioclim data with good results (Maharaj, 2011). Correlative SDMs have often been compared with process-based SDMs, which include mechanistic biological information about species' responses to environmental variables (Morin and Thuiller, 2009; Buckley et al., 2010; Keenan et al., 2011; Dormann et al., 2012).

Despite these possible limitations, modelling output was strongly reflective of observations made in the field and highlighted clear patterns to compare with species' traits in order to infer how plant structure-function relationships shape the distributions of CAM tank-epiphyte bromeliads in Trinidad.

6.4.2.2 Relative importance of environmental factors

Jackknife testing of the importance of predictor variables in MaxEnt SDMs suggested that environmental factors assumed different degrees of importance in determining the distributions of each species across the Northern Range. Whereas moisture-related factors appeared to be particularly important for *Ae. aquilega*, the distribution of *Ae. fendleri* was most strongly affected by

topographic factors, temperature and temperature diurnality. *Ae. dichlamydea* required low temperature diurnality and high dry season rainfall. No environmental factors were strong determinants of the distribution of *Ae. nudicaulis*. Thus the environmental niches of the four *Aechmea* species were clearly segregated and defined by different requirements. The next step in this investigation was to compare these observations against the results of anatomical and physiological characterisation for *Ae. aquilega*, *Ae. fendleri*, and *Ae. nudicaulis* to search for structural and functional drivers of the apparent niche segregation of these species.

6.4.3 Anatomical and physiological determinants of *Aechmea* distributions

Although other factors may be involved (see **Appendix 6.9**), the results of the anatomical and physiological characterisation of *Aechmea aquilega*, *Ae. fendleri* and *Ae. nudicaulis* provided strong evidence for trait-mediated bioclimatic niche determination. Each species will now be considered in turn.

6.4.3.1 *Aechmea aquilega*

Field surveys and correlative SDM showed how *Ae. aquilega* is largely confined to lower elevations in the Northern Range, possibly due to a requirement for relatively low levels of moisture. In these hotter, drier lowland environments, the strong stomatal sensitivity to VPD in *Ae. aquilega* could allow this species to rapidly restrict water loss during episodes of high evaporative demand associated with intense insolation. The drought experiment showed that stomatal and mesophyll conductance and net assimilation could be maintained for a longer time than in *Ae. fendleri*, allowing some growth to continue. This is partly because the more extensive hydrenchyma of *Ae. aquilega* was depleted less rapidly. These factors collectively explain why *Ae. aquilega* can succeed at low elevations. Other results provide insights into why this species does not occur at high-elevation, high-rainfall sites in the Northern Range. Of the three species for which physiological characterisation was performed, *Ae. aquilega* mounted the least effective response to experimental leaf wetting, showing the strongest reduction in gas exchange and the lowest capacity for the engagement of CO₂ recycling. Night-time leaf wetting events would therefore have the strongest negative impact on the carbon balance of *Ae. aquilega*. Other physiological traits tended to show intermediate values for *Ae. aquilega*, consistent with its occurrence between *Ae. fendleri* as a highly environmentally-sensitive species and *Ae. nudicaulis* as a flexible environmental generalist.

6.4.3.2 *Aechmea fendleri*

Pittendrigh (1948), while stressing the importance of light availability in determining the vertical and geographical distributions of bromeliads on Trinidad, acknowledged the special significance of humidity for certain species. He observed that for the (C₃) tank-epiphyte species he classified as 'shade-tolerant', including *Guzmania lingulata* and *Lutheria splendens*, individuals occur under 'a wide range of light intensities to accommodate a primary requirement for high humidity'. In other words, these species are able to acclimate to contrasting light environments provided that they experience sufficiently high humidity levels. The observations of *Ae. fendleri*, the species restricted to cooler, moister parts of the forest in the Northern Range, made in this investigation are broadly consistent with such a description, despite Pittendrigh having included this species in his 'sun' group. Pittendrigh attributed the humidity requirement of *G. lingulata* and *L. splendens* to their relatively limited external (or internal) water storage capacity. *Ae. fendleri* has relatively little hydrenchyma, which could render this species more reliant on frequent refilling of tanks by precipitation than other Trinidadian *Aechmea* species (Smith et al., 1986). Moreover, due to the more open morphology of the rosette in *Ae. fendleri*, the tanks of this species were more susceptible to depletion by evaporation, particularly in comparison with those of *Ae. nudicaulis*. Another piece of evidence that points to a physiological basis for the restriction of *Ae. fendleri* to areas of low evaporative demand is the fact that this species showed the slowest rehydration kinetics of all three species that were physiologically characterised, despite having the lowest pool sizes. Water uptake and distribution is therefore least efficient in *Ae. fendleri*, which could render it less able to cope with water deficit or strongly fluctuating environmental conditions. *Ae. fendleri* also showed the weakest stomatal sensitivity to VPD, which could mean that stomatal restriction of water loss under desiccating conditions would be less efficient in *Ae. fendleri* than in either *Ae. aquilega* or *Ae. nudicaulis*, preventing it from occurring in hot, dry lowland environments. During the drought experiment, *Ae. fendleri* expended the water stored in hydrenchyma tissues most quickly, and showed the strongest decline in stomatal and mesophyll conductances, photosynthetic capacity, and nocturnal acid accumulation, indicative of a systemic physiological sensitivity to drought. The rapid loss of mesophyll conductance may have been underlain by tissue shrinkage (see **Chapter 4**), since the chlorenchyma of *Ae. fendleri* shrank most strongly during drought. As a consequence, this species showed a complete loss of leaf growth before the end of the experimental period. Moreover, droughted *Ae. fendleri* plants became increasingly sensitive to temperature and showed the greatest loss of photochemical efficiency.

Temperature seemed to have a particularly strong effect on the photosynthetic physiology of *Ae. fendleri*, with both photochemistry and acid accumulation being more strongly impaired in this

species at high leaf temperatures than in either *Ae. aquilega* or *Ae. nudicaulis*. Both day- and night-time temperature effects could therefore impact independently on productivity in *Ae. fendleri*. Daytime temperature sensitivity of photochemistry in CAM plants has not often been investigated (Gerwick et al., 1977; Chetti and Nobel, 1987). However, the sensitivity of nocturnal acid accumulation to high temperatures has been reported in many CAM species (Brandon, 1967; Kluge et al., 1973; Medina et al., 1977; Gerwick and Williams, 1978; Kluge and Ting, 1978; Buchanan-Bollig and Kluge, 1981; Medina and Osmond, 1981; Medina, 1982; Buchanan-Bollig et al., 1984; Nobel and Hartssock, 1984; Nobel, 1988; Fetene and Lüttge, 1991; Haag-Kerwer et al., 1992; Carter et al., 1995; Nobel et al., 1998), including pineapple (Neales, 1973; Neales et al., 1980). The sensitivity of nocturnal acid accumulation to temperature has often been attributed to the fluidity of the tonoplast membrane and its permeability to malate. Malate efflux from the vacuole causes the deactivation of PEP carboxylase by feedback inhibition (Friemert et al., 1988; Kluge et al., 1991; Lüttge, 2000). A complicating factor is the tendency for the ratio of accumulated citrate to malate to vary depending on day/night temperature regime and other environmental factors (Borland and Griffiths, 1989; Lüttge, 1990; Miszalski et al., 2013). Capacity for thermal acclimation of the CAM rhythm has been observed in some species (e.g. Grams et al., 1995), but there is also a clear adaptational genetic component to variation in photosynthetic temperature optima (Yamori et al., 2014).

On the highest massifs, there appeared to be an upper elevational limit for *Ae. fendleri*. On Morne Bleu (834 m), *Ae. fendleri* was not observed above 750 m. On El Cerro del Aripo (940 m), there were no records above 770 m but *Ae. aripensis*, which is endemic to Trinidad and the adjacent Paria Peninsula of the Venezuelan mainland, was present in the elfin forest at the highest elevations. On both Chaguaramal (859 m) and El Tucuche (936 m), *Ae. fendleri* occurred almost up to the summits, but this can be explained by the fact that on these peaks the MF zone is restricted to a smaller cap at higher elevation. Leaf wettability has been shown to limit physiological activity by suppressing gas exchange in various plant groups (Smith and McClean, 1989; Hanba et al., 2004). With increasing altitude, community aggregated leaf wettability tends to decline (Aryal and Neuner, 2010). In a survey of bromeliad leaf surface hydrophobicity associated with powders and trichomes, Pierce et al. (2001) determined that both the adaxial and abaxial surfaces of *Ae. fendleri* leaves are hydrophilic, whereas in *Ae. nudicaulis* the abaxial, stomatiferous surface is hydrophobic. Thus in *Ae. nudicaulis* abaxial water repellency could prevent inhibition of gas exchange. Measurements on artificially surface wetted leaves showed that wetting of hydrophilic surfaces caused a reduction in gas exchange rates in all three of the focal *Aechmea* species studied in this investigation, strongly increasing dependence on recycling of CO₂. However, the capacity for recycling activity is relatively

limited in *Ae. fendleri*, particularly when compared with *Ae. nudicaulis* (Griffiths, 1988), suggesting that *Ae. fendleri* could struggle to maintain a favourable carbon balance in montane environments with very high levels of moisture. The reduction in leaf gas exchange caused by surface wetting was considerably higher in *Ae. fendleri* than in *Ae. nudicaulis*, which could explain why *Ae. nudicaulis* continues to be observed at higher elevations in MF than *Ae. fendleri*. *Ae. nudicaulis* had higher trichome density and larger trichomes, which could be more effective at preventing a film of water from blocking stomatal pores (Pierce et al., 2001).

6.4.3.3 *Aechmea nudicaulis*

Unlike *Ae. aquilega* and *Ae. fendleri*, *Ae. nudicaulis* can be considered a 'jack-of-all-trades' on the island of Trinidad. The distribution of *Ae. nudicaulis* across such a wide range of vegetation types and bioclimatic zones suggests that correspondingly broad environmental tolerance should be demonstrable in this species' ecophysiology. The evidence accumulated here strongly supports this case, and highlights the multifactorial basis of the niche width of *Ae. nudicaulis* on Trinidad. Morphologically, *Ae. nudicaulis* is distinct from the other species in the tubular form of the rosette. This architecture dramatically reduces the ratio of tank surface area to tank volume, which likely translates to an enhancement of long-term external water storage (Zotz and Thomas, 1999). *Ae. nudicaulis* also displays the best-developed hydrenchyma of the three species that were anatomically characterised, providing extensive internal capacitance that can be drawn on in the event of the tank emptying. This combination extends the number of days with no rainfall for which *Ae. nudicaulis* has access to a reliable water supply, external or internal, for buffering transpiration. During the drought course, photosynthetic capacity was therefore less strongly negatively affected in *Ae. nudicaulis* than in *Ae. aquilega* and *Ae. fendleri*, enabling higher growth rates (captured in the LER data) to be maintained. When water is available, the trichomes of *Ae. nudicaulis* are more efficient at uptake, and water is more rapidly distributed through the leaf. The vascular architecture of *Ae. nudicaulis*, with its long vein-epidermis pathlength and dense venation, appears to be adapted to the minimisation of water loss and the maximisation of the efficiency of vein-mediated water distribution across the lamina. Gas exchange by *Ae. nudicaulis* shows high levels of WUE, probably due to the presence of a higher fraction of chlorenchyma cells on an area basis generating a stronger draw-down of CO₂. In dynamic canopy microhabitats, the strong stomatal sensitivity to VPD that was measured in this species could be important in maximising photosynthetic carbon gain while maintaining WUE. Should all these mechanisms for preventing a decline in leaf water status fail, *Ae. nudicaulis* still enjoys lower leaf hydraulic vulnerability than *Ae. aquilega* and *Ae. fendleri*, allowing physiological function to continue under a greater level of strain.

The thicker lamina and higher respiration rate in *Ae. nudicaulis* allowed it to engage in more CO₂ recycling (Griffiths, 1988). During drought, recycling was upregulated, but because stomatal gas exchange was maintained for longer in this species than in *Ae. aquilega* and *Ae. fendleri*, recycling did not come to account for the whole nocturnal acid pool as occurred in the other species. The metabolic flexibility afforded by the potential for CO₂ recycling was proposed by Pierce et al. (2002) as a key reason for the success of certain CAM tank-epiphyte bromeliads in competition with C₃ species in perhumid cloud forest environments. Photochemistry was compromised at a significantly higher daytime temperature in *Ae. nudicaulis* than in either of the other two species, suggesting that there may be some biochemical adaptation that broadens temperature tolerance in this species. Furthermore, *Ae. nudicaulis* was tolerant of a wider range of night-time temperatures without eliminating a significant amount of nocturnal carbon uptake. This means that *Ae. nudicaulis* is better primed to maintain carbon gain under a wider range of environmental conditions.

6.4.4 Relating bromeliad distributions to broader vegetational patterns

The results of this investigation can be usefully compared with previous analyses of vegetational and floristic patterns in Trinidad. One of the earliest attempts at vegetation classification on Trinidad was undertaken by Marshall (1934), who used a system based on environmental indices, including 20 years of rainfall data collected at 50 stations. This classification was superseded in the following decade by the hierarchical system of Beard (1946a), which was also based primarily on moisture and dealt only with trees. Beard's system ranked physiognomically distinctive 'associations' as subtypes of a series of major forest 'formations' (e.g. Evergreen Seasonal Forest). While this system has certain important shortcomings, such as the fact that it is based only on trees and assumes that the vegetation is in successional equilibrium (Nelson, 2004), it remains a useful tool for interpreting Trinidadian forest structure and composition (Baksh-Comeau et al., 2016). Helmer et al. (2012) used Beard's scheme as a framework for classifying vegetation types on satellite imagery covering the whole land area of the islands of Trinidad and Tobago. Overlaying the field survey results from this investigation on the forest formations and associations *sensu* Beard (1946a) mapped using satellite imagery by Helmer et al. (2012) reveals the close association of *Ae. fendleri* with the Lower Montane Forest formation (*Byrsonima spicata* - *Licania ternatensis* - *Sterculia pruriens* association) and Montane Forest formation (*Richeria grandis* - *Eschweilera tenax* association). Despite the apparent strength of this association, the inclusion or exclusion of the vegtype layer had little impact on MaxEnt SDM runs for *Ae. fendleri*, suggesting that environmental factors are better predictors of occurrence for this species. Although the other *Aechmea* species did not occur in all forest types, there was no strong evidence of specificity to any particular forest formation or association. This

suggests that the physiognomic or taxonomic characteristics of any particular forest type are not fundamental determinants of species distributions, consistent with observations of occupation of contrasting microhabitats and expression of morphological plasticity. Despite the fact that the distributions of both Beard's forest types and at least some of the *Aechmea* species are strongly delimited by climate relations, the climatic zones occupied by individual forest types and epiphytic bromeliads do not necessarily overlap. This suggests that the distributions of herbaceous species, and particularly vascular epiphytes, may be driven by the same factors as those of woody species, but show rather different spatial patterns. In the case of epiphytes, the reduced geographical specificity of individual species could relate in part to the fact that the edaphic factors that secondarily modulate the distributions of tree species do not directly affect epiphytes (Beard, 1946a).

The only comparable study of the distributions of a group of related angiosperm species across geographic and climatic space in Trinidad is a study of the Trinidadian Araceae, many of which are either hemi- or holo-epiphytic, by Simmonds (1950). Simmonds suggested that different environmental factors could control the distributions of different aroid species. He proposed that rainfall was generally only an important factor in the driest forest types, whereas altitudinal temperature gradients appeared to be stronger predictors of the distributions of many *Anthurium* Schott and *Philodendron* Schott species. In much the same way as differences in altitudinal niche occur among Trinidadian bromeliads, montane aroid species such as *A. guildingii* Schott and *P. venosum* (Willd. ex Schult. & Schult.f.) Croat contrast with the lowland species *P. krugii* Engl. and *P. latifolium* K.Koch and with more widespread species such as *P. giganteum* Schott and *P. hederaceum* (Jacq.) Schott. Some light demanding species e.g. *Monstera* spp. No physiological data are currently available to suggest whether similar functional trait divergences are involved in the apparent niche differentiation observed in these aroids as occur in the *Aechmea* bromeliads, but this could form the basis of an interesting comparative investigation in the future.

6.4.5 *Spatial projections of species distributions*

6.4.5.1 *Tobago*

Trinidad's sister island of Tobago has experienced a distinct geological history, but its flora is nevertheless nevertheless dominated by continental rather than Antillean elements (Beard, 1944). The principal topographic feature on Tobago is the Main Ridge, which is formed primarily of volcanic schist and culminates at Pigeon Peak (550 m; Beard, 1944). The Main Ridge Forest Reserve,

established in 1776, is the oldest legally protected forest in the western hemisphere. The forest experienced extensive damage from hurricane Flora in 1963 (Rooks and Barclay, 2012).

Among the bromeliads of Tobago, many species are shared with Trinidad (see Table 6.1.1), including *Ae. aquilega* and *Ae. nudicaulis*. On Tobago, *Ae. dichlamydea* var. *dichlamydea* replaces var. *trinitensis*. There are no records of *Ae. fendleri* from Tobago. *Ae. aquilega* and *Ae. dichlamydea* var. *dichlamydea* are described by Beard (1944) as being particularly abundant in LRF. Using the SDMs trained and tested on presence data derived from the field surveys on Trinidad, projections of species distributions on Tobago were produced. It was assumed that the bioclimatic envelope of *Ae. dichlamydea* var. *trinitensis* on Trinidad and var. *dichlamydea* on Tobago do not differ significantly. There is some evidence from other taxa which demonstrates that species' environmental niches are not necessarily identical in different geographic regions (Prinzing et al., 2002; Randin et al., 2006; Hájková et al., 2008), and caution must be taken when projecting SDMs onto new geographical areas (Peterson et al., 2007; Bahn and McGill, 2013). However, the projected distributions on Tobago were consistent both with herbarium vouchers (see **Appendix 6.1**) and presence data collected during a brief ground-truthing exercise in April 2017. In particular, the projected transition from dominance of *Ae. aquilega* at lower elevations to that of *Ae. dichlamydea* at higher elevations on the Main Ridge was strongly supported by observations made in the field.

6.4.5.2 Paria Peninsula

The Paria Peninsula of Venezuela, adjacent to the Northern Range of Trinidad, hosts a flora that shares many elements with that of Trinidad (Beard, 1946a,c). Its climate is comparable to that of Trinidad, with high levels of annual precipitation (> 4,000 mm) occurring on the highest mountains (Beard, 1946c). The vegetation of the peninsula is highly disturbed due to agricultural activity, with fragments of primary forest persisting only in the most isolated areas (Beard, 1946c).

Projected species distributions of the four *Aechmea* species based on the SDMs trained and tested on presence data collected in Trinidad were consistent with herbarium records (see **Appendix 6.1**). These projected distributions represent an opportunity for future ground-truthing through field surveys on the Paria Peninsula.

6.5 Conclusions

CAM tank-epiphyte *Aechmea* species differ in their distributional patterns across the Northern Range of the Caribbean continental island of Trinidad. Species distribution modelling (SDM) shows

that environmental variation could explain the distributions of *Ae. fendleri* and *Ae. dichlamydea* var. *trinitensis*, and to some extent *Ae. aquilega*, while the ubiquity of *Ae. nudicaulis* was indicative of exceptionally broad environmental tolerance. The environmental factors exerting the strongest influence on the distributions of each species differed, with moisture-related factors being of variable importance and temperature being especially important in the case of *Ae. fendleri*. Anatomical and physiological characterisation of three focal species (*Ae. aquilega*, *Ae. fendleri* and *Ae. nudicaulis*) demonstrated that there was a multifactorial trait-based explanation for observed divergences in climatic specialisation. Limited capacity for drought avoidance and endurance, combined with strong photosynthetic thermal sensitivity, can explain the restricted niche and range of *Ae. fendleri*. At the other end of the scale, the wide environmental tolerance of *Ae. nudicaulis* was associated with more effective drought responses and very low thermal sensitivity. *Ae. aquilega* may be limited to lower elevations by sensitivity of gas exchange to surface wetting. The SDMs allowed projections to be made of the potential distributions of each species across Trinidad, Tobago and the Paria Peninsula. Overall, the data presented in this chapter provide a strong case for the role of divergences in leaf traits in driving environmental niche segregation among congeneric bromeliad species.

7. General Discussion

7.1 Introduction

The research project reported in this thesis was designed to make use of a range of approaches to investigate the structural-functional basis of adaptive ecological differentiation among bromeliad species at multiple levels of organisation, with particular reference to the water-use strategies known to be central to the ecological diversity of the Bromeliaceae (Males, 2016). In this **General Discussion**, the key findings from **Chapters 2-6** are recapitulated and contextualised. This is followed by further synthesis addressing the involvement of the relationships between structure, function and ecology in the evolutionary diversification of each of the major lineages of the Bromeliaceae. The origins of putative ancestral bromeliad phenotypes are addressed, and possible avenues for future research are identified throughout, and the overall conclusions of this thesis are presented.

7.2 Levels of environmental adaptation in the Bromeliaceae

7.2.1 Hydrological habitat occupancy

The first step in this research project was to establish the extent of variation in environmental relations so that subsequent investigation could attempt to explain this on the basis of trait differentiation. The bromeliads have often been described as diverse with respect to environmental niche (e.g. Benzing, 2000; Givnish et al., 2011). However, the systematic quantification of hydrological habitat positions and ranges for a large set of terrestrial bromeliads presented in **Chapter 2** is the first such survey. While methodological constraints meant that only terrestrial species could be considered, the insights gained into the extent of ecological differentiation among these species were manifold, and some results can be extrapolated to the epiphytic context. Hydrological habitat position varied between both taxonomic and functional groups, and there was evidence of varying types and degrees of specialisation within genera. Some of the variation in hydrological habitat position and range was explicable on the basis of existing qualitative knowledge of life-history, morphology and physiology. Concerted evolutionary changes in leaf shape and internal structure, shoot architecture, and plant size have all been associated with convergent transitions towards more water-limited environments. Origins of CAM, subterranean tubers, and deciduous foliage provide further examples of convergent traits that have enabled adaptation to more arid and seasonal environments. The proliferation of the bromeliads across ecological space is therefore clearly intimately linked with vegetative character change. Despite the robust support for these insights from the natural replication provided by convergent evolution, the need for further

quantitative research into the links between structure-function relationships in bromeliad leaves and species' ecophysiological strategies was highlighted in **Chapter 2**. This provided the catalyst for the trait surveys and analyses reported in subsequent chapters of this thesis.

7.2.2 Leaf trait diversity and coordination

The survey of anatomical and physiological leaf traits across 50 bromeliad species reported in **Chapter 3** demonstrated interspecific variation that was consistent with the expected ecophysiological differences between functional types. These results highlighted the importance of evolutionary change in a large suite of structural-functional traits for the establishment of the morphological and ecological syndromes associated with the functional types. Evolutionary lability in internal anatomy has probably been central to the origins of a wide range of growth-forms in the Bromeliaceae, which differ widely within genera such as *Tillandsia* and are of great ecophysiological significance (Benzing, 2000).

The network of correlations among traits was similar to what has been observed in other plant groups, with evidence being recovered in support of many of the classical leaf economic relationships and the coupling of leaf hydraulics and gas exchange. However, origins of innovations including CAM, tank-epiphytism and neoteny appeared to have caused the rewiring of some relationships. This rewiring of the relationships between leaf traits has likely been an important mechanism for the generation of ecophysiological diversity. Quantification of four key drought resistance traits across a wider sample of species showed that these traits had evolved in a coordinated manner, but are of contrasting relevance to climate relations in different functional types. As has been observed in other plant groups, including epiphytic orchids (Zhang et al., 2016), evolutionary flexibility in the relative importance of different structural-functional traits for environmental specialisation points to another potential mechanism for the evolution of species diversity. Vein placement has been shown to be highly variable across the bromeliads, and this variation is closely linked with species' ecophysiological properties. Considering previous reports of strong evolutionary conservation (Zwieniecki and Boyce, 2014), the extreme evolutionary lability in this trait is striking, and has probably made a key contribution to the generation of diverse water-use strategies and high species diversity. The overall impression of the important role of trait lability in the diversification of the bromeliads echoes observations made in other plant lineages (Ogburn and Edwards, 2015; Fernández-Mazuecos and Glover, 2017).

7.2.3 Anatomical structural differences underpin divergences in physiological function

Work presented in **Chapter 4** and **Chapter 5** highlighted how anatomical traits provide the structural basis for divergences in physiological function among bromeliad species of contrasting functional type. Vascular anatomy, venation architecture, and extra-xylary anatomy were all linked to hydraulic traits, suggesting that concerted change in multiple aspects of leaf anatomy has underpinned adaptive evolution of water-use characteristics across bromeliad species. Specific anatomical features, including bundle sheath extensions (BSEs), were associated with major differences in leaf hydraulic design, consistent with the increasingly generalised consensus understanding of structural determination of leaf hydraulics (Sack et al., 2015). Bromeliads displaying contrasting stomatal complex morphology differed greatly in their stomatal conductance, sensitivity to leaf-air vapour pressure deficit (VPD) and stomatal kinetics. While further empirical and modelling-based investigation of the detailed biomechanical basis of these differences is merited, the observed differences in behaviour between different stomatal complex morphologies are consistent with the distinctive ecophysiological syndromes and water-use strategies of the functional types in which they occur. Overall, it is apparent that evolutionary transitions between functional types in the Bromeliaceae have been associated with concerted changes in several key leaf-level structural-functional trait complexes. A major challenge for future research will be to establish whether contemporaneous shifts in multiple traits can be explained by shared developmental control. For example, common factors (genetic or genomic) might determine the spacing of veins and stomata (Zhang et al., 2012; Brodribb et al., 2013).

7.2.4 Trait-mediated environmental niche segregation in Trinidad

In order to test the applicability of inferences made from results presented in previous chapters to ecological differentiation between species in a natural context, the work described in **Chapter 6** examined the extent of trait-mediated environmental niche segregation among CAM tank-epiphyte *Aechmea* species in Trinidad. It was shown that species differed in their occupancy of habitat space across the Northern Range of Trinidad, with contrasting degrees of environmental specialisation and range limitation. Differences in anatomical leaf traits were associated with differences in physiological rates and sensitivities, which in turn explained divergences in environmental tolerance and species distributions. The results of the case study performed on the *Aechmea* species are probably readily generalisable to other bromeliad groups. For example, even within the same geographical context (the island of Trinidad), it would likely be possible to identify a similar sequence linking anatomical, physiological and ecological traits in tank-epiphyte representatives of the

Tillandsioideae subfamily. These range from the massive *Glomeropitcairnia erectiflora* Mez, which is restricted to Montane Forest near the summits of El Cerro del Aripo and El Tucuche, to the small, exposure-demanding *Vriesea procera* (Mart. ex Schult. & Schult.f.) Wittm., which is common in dry lowland situations. Vertical stratification within canopies could also be approached from the same trait-based perspective (Pittendrigh, 1948; Benzing and Renfrow, 1971; Kelly, 1985; Benzing, 2000; Graham and Andrade, 2004; Isaza et al., 2004; Krömer et al., 2007; Reyes-García et al., 2008; Petter et al., 2016). To summarise, Fig. 7.2.1 highlights how the evolutionary ecology of the bromeliads has is at least partly emergent from characteristics at more fundamental levels of organisation, encapsulating the central theme of this thesis.



Figure 7.2.1. Emergence of species' ecological properties from fundamental traits.

7.2.5 Additional factors in bromeliad structural-functional evolution

While the focus of this project has been on structural-functional traits related to water use, water is of course only one of the environmental resources necessary for plant growth. Bromeliad leaves show various adaptations and acclimatory responses to other environmental factors, such as excess or limitation of light. Indeed, many of the foliar anatomical parameters considered here in the context of hydraulics and water relations are also of relevance to leaf optics. For instance, the size, shape and arrangement of hydrenchyma cells will impact on the tissue's light-scattering properties, which could significantly affect photosynthetic activity in the chlorenchyma. Meanwhile differentiation of the chlorenchyma into palisade and spongy layers could affect leaf hydraulic design, and changes in leaf shape associated with adaptation or acclimation to contrasting light environments could also affect leaf hydraulics and water use through vein placement (**Chapter 4**), boundary layer and thermal effects (Nicotra et al., 2011).

As is true of their water-use strategies, the nutrient-acquisition strategies of the bromeliads are perhaps more diverse than in any other angiosperm family (Benzing, 2000). For many species, the role of foliar trichomes in nutrient uptake is as important as their function in water absorption, while some tank-epiphyte species have been described as proto-carnivorous (Frank and O'Meara, 1984; Givnish et al., 1984; Benzing et al., 1985). Among epiphytes in particular, the necessity of judiciously

managing the limited nutrients available may have been a strong determinant of leaf structure and function. Recent insights into foliar nutrient distribution and complex interactions with signalling processes and CAM expression will no doubt encourage further investigation of the evolution of nutrient relations in the bromeliads (e.g. Rodrigues et al., 2014; Gonçalves et al., 2016; Vanhoutte et al., 2017).

Another potentially limiting resource is CO₂. In this connection, the CO₂-concentrating effect of CAM may have been an important selective advantage of the CAM syndrome during coupled fluctuations in atmospheric CO₂ concentration and climate during the Mid-Late Miocene (Kürschner et al., 2011; Crayn et al., 2015). However, the CO₂-concentrating effect *per se* may be of less significance under present-day atmospheric composition, with rising CO₂ levels. There is also currently no evidence to support the hypothesis that CAM could allow vascular epiphytes to benefit from elevated night-time CO₂ concentrations in tropical forests (Hsu et al., 2006).

More work is also needed on structural-functional properties of other plant organs in the bromeliads, which are less well-studied than leaves. Roots, stolons, caulescent stems, and tubers presumably vary in role and importance for whole-plant hydraulics and other functions between functional types and under different environmental regimes, but rarely figure in ecophysiological investigations in the Bromeliaceae (Males, 2016). Similarly, little is known about the carbon and water costs of reproductive effort in the bromeliads. An integrative approach, encompassing all plant parts and stages of life-history, while being highly labour-intensive, would provide a more complete picture than the snapshot view obtained by measuring a limited set of traits at a single ontogenetic stage (Benzing, 2000).

7.2 Evolutionary synthesis

7.2.1 *Factors affecting bromeliad evolutionary dynamics*

The evolutionary history of the bromeliad family is complex, being characterised by extensive biogeographical structuring and trait innovation (Benzing, 2000; Givnish et al., 2011, 2014). The insights gained through contrasting approaches that are presented in this thesis shed new light on the structural basis of vegetative ecological differentiation in the bromeliads. By consequence, they could also improve our understanding of the evolutionary dynamics of the bromeliad family, which is emerging as a model system for plant evolutionary ecology and physiology (Palma-Silva et al., 2016). Clearer understanding of bromeliad evolution and physiological limitations will also help to explain how these plants have come to make such a major contribution to the ecological networks and hydrological and biogeochemical cycles of Neotropical ecosystems (Nadkarni, 1984; Benzing, 1998;

Gonçalves-Souza et al., 2010; Martinson et al., 2010), and how these ecosystem roles could be affected by global change.

Previous work on the evolutionary dynamics and historical biogeography of the Bromeliaceae has focussed principally on the role of key innovations in driving episodes of elevated net species diversification (Givnish et al., 2014; Silvestro et al., 2014). Even insofar as innovations can be considered in isolation, their impact on lineage diversification is necessarily context dependent, in terms of both the environment and other facets of organismal biology (Jones et al., 2013; Donoghue and Sanderson, 2015; Onstein et al., 2016). Additionally, the innovations that have been previously investigated are typically complex emergent syndromes, and existing analyses have not explored changes in underlying, fundamental anatomical and hydraulic traits that facilitate the functioning of these higher-order syndromes. A range of approaches have been taken in this thesis to combat this deficit, making it possible to compare large and representative datasets on leaf traits, whole-plant traits, bioclimatic relationships and biogeographical ranges with the most recent phylogenetic analyses to build up a robust, process-oriented view of bromeliad evolution.

7.2.2. Trait-mediated ecological differentiation in the evolution of major bromeliad lineages

The divergence and diversification of bromeliad lineages have taken place in the context of a broad geographical region, expanding from the Guiana Shield across South America, Central America, and the Caribbean (Givnish et al., 2011). Across the ca. 100 My of bromeliad evolution, major tectonic and physiographic changes have occurred across this region. Perhaps most important was the orogeny of the Andean cordillera and the generation of highly-dissected, fertile montane habitats (Gentry, 1982; Antonelli et al., 2009; Givnish et al., 2011, 2014; Luebert and Weigend, 2014). Although not yet addressed by specific research projects, changes in the extent and configuration of massive wetlands and drainage systems across northern South America have likely also played a fundamental role in shaping the spatial distributions and adaptive radiations of bromeliads as well as other plant groups (Hoorn et al., 2010; Roncal et al., 2013). Climatic fluctuations (e.g. during the Pleistocene) and corresponding vegetation change have also occurred, impacting on the size and connectivity of habitat patches (van der Hammen and Hooghiemstra, 2000; Pennington et al., 2004; Rull et al., 2008; Hoorn et al., 2010). The geographical theatre for bromeliad evolution has therefore been characterised by high levels of dynamism, providing ever-changing opportunities for the invasion of new ecospace. Landscape complexity continues to interact with organismal traits to influence the connectivity of bromeliad populations (Sarhou et al., 2001; González-Astorga et al., 2004; Barbará et al., 2008; Paggi et al., 2010; Zanella et al., 2011; Lavor et al., 2014), reinforcing

genetic divergences that may ultimately lead to further speciation (Palma-Silva et al., 2009; Versieux et al., 2012).

| Clade | D_{stem} (My ⁻¹) | D_{crown} (My ⁻¹) | # Species | Stem age (My) | Crown age (My) | Reference |
|---|--|---|-----------|------------------|-------------------|---|
| <i>Dyckia</i> (Pitcairnioideae) | 1.77 | 2.85 | 169 | 2.9 | 1.8 | Schütz et al. (2016) |
| Tank-epiphyte Bromelioideae | 1.11 | 1.05 | 629 | 5.8 | 5.5 | Givnish et al. (2014) |
| <i>Tillandsia</i> alliance (Tillandsioideae) | 0.97 | 1.13 | 870 | 7.0 | 6.0 | Givnish et al. (2014); Barfuss et al. (2016) |
| Brazilian Shield Bromelioideae | 0.70 | 0.79 | 753 | 9.4 | 7.5 | Givnish et al. (2014) |
| <i>Cryptanthus</i> - <i>Orthophytum</i> (Bromelioideae) | 0.69 | 0.74 | 149 | 7.2 | 6.8 | Givnish et al. (2014) |
| Bromelioideae | 0.63 | 0.64 | 856 | 10.7 | 9.4 | Givnish et al. (2014) |
| <i>Navia</i> - <i>Brewcaria</i> (Navioideae) | 0.58 | 0.58 | 99 | 7.9 | 6.7 | Givnish et al. (2014) |
| <i>Pitcairnia</i> (Pitcairnioideae) | 0.51 | 0.94 | 408 | 11.8 | 6.4 | Schütz et al. (2016) |
| Puyoideae | 0.50 | 0.50 | 217 | 10.7 | 9.4 | Givnish et al. (2014) |
| Puyoideae + Bromelioideae | 0.49 | - | 1073 | 14.1 | - | Givnish et al. (2014) |
| <i>Greigia</i> (Bromelioideae) | 0.47 | 0.96 | 35 | 7.5 | 3.7 | Silvestro et al. (2014) |
| Core Tillandsioideae | 0.47 | 0.67 | 1236 | 15.2 | 9.6 | Givnish et al. (2014) |
| Xeric Clade (Pitcairnioideae) | 0.45 | - | 170 | 11.3 | - | Givnish et al. (2014) |
| Pitcairnioideae | 0.45 | 0.48 | 587 | 14.1 | 11.9 | Givnish et al. (2014) |
| <i>Bromelia</i> (Bromelioideae) | 0.43 | 3.54 | 70 | 9.8 | 1.2 | Silvestro et al. (2014) |
| Tillandsioideae | 0.42 | 0.42 | 1256 | 16.9 | 15.2 | Givnish et al. (2014) |
| <i>Fosterella</i> (Pitcairnioideae) | 0.31 | 0.47 | 31 | 11.0 | 7.3 | Schütz et al. (2016) |
| Navioideae | 0.29 | 0.38 | 107 | 15.9 | 10.5 | Givnish et al. (2014) |
| Hechtioideae | 0.24 | 0.33 | 52 | 16.3 | 9.9 | Givnish et al. (2014) |
| Lindmanioideae | 0.21 | 0.54 | 45 | 18.1 | 5.8 | Givnish et al. (2014) |
| Brocchionioideae | 0.13 | 0.16 | 20 | 22.7 | 14.1 | Givnish et al. (2014) |
| Bromeliaceae | 0.08 | 0.32 | 3140 | 97.5 | 22.7 | Givnish et al. (2014) |

Table 7.2.1. Stem (D_{stem}) and crown (D_{crown}) diversification rates for bromeliad lineages reported by Givnish et al. (2014) and calculated from time-calibrated phylogenies published in Givnish et al. (2014; cf. topology *sensu* Barfuss et al., 2016), Silvestro et al. (2014) and Schütz et al. (2016), where $D = \ln(\# \text{ spp.})/\text{age}$. Species numbers obtained from Brom Taxon List (Butcher and Gouda, 2017).

Wide variation in net diversification rates occurs across the bromeliad lineages. Table 7.2.1 shows a combination of values reported by Givnish et al. (2014) and values calculated from the time-calibrated phylogeny of the Pitcairnioideae published by Silvestro et al. (2014) and Schütz et al.

(2016). The relationships between landscape evolution, biogeography, trait-mediated ecological differentiation and species diversification are best considered by way of a brief discussion of the evolutionary history of each major bromeliad lineage.

The earliest diverging bromeliad subfamily is the Brocchinioideae, which comprises C₃ mesic terrestrials and some derived C₃ tank-lithophytes, and is restricted to the *tepui*s of the Guiana Shield (Givnish et al., 2011). These isolated 'Lost World' ecosystems represent refugia that have been relatively buffered from climatic change across the evolutionary history of the bromeliads native to them, which explains the prevalence of endemics and the relatively narrow, moisture-demanding hydrological niches and poor drought tolerance measured in most *Brocchinia* spp. (**Chapter 2**; Berry and Riina, 2005; Hopper, 2009). Givnish et al. (1997) documented adaptive radiation in *Brocchinia*, describing the phenomenon in terms of differentiation of nutrient acquisition strategies. These divergences are underpinned by contrasting leaf and whole-plant traits, including leaf shape, foliar trichome absorption capacity, tank morphology, and mesophyll structure, which also determine differences in leaf physiology and hydrological niche. This was demonstrated by the broader hydrological habitat ranges of tank-forming *Brocchinia* species identified in **Chapter 2**, which could not be attributed to other drought resistance traits quantified in **Chapter 3**. Two species, *B. micrantha* (Baker) Mez and *B. paniculata* Schult. & Schult.f., are the only truly arborescent bromeliads, and would make interesting subjects for further structural and physiological characterisation and comparison with other arborescent monocots (e.g. Morales et al., 2015).

Also restricted to the Guiana Shield are the Lindmanioideae. This subfamily of C₃ mesic terrestrial species is very rare in cultivation and therefore no new empirical data were generated on Lindmanioideae in this work. However, the habitat occupancy analysis highlighted the narrow climatic niches of these plants (**Chapter 2**), which, as with the Brocchinioideae, is associated with their continued restriction to a relatively small geographical region (Givnish et al., 2011). Structure-function work on this group, when and where living material should become available, would make for an interesting comparison with other early-diverging bromeliad lineages.

The centre of origin of the Tillandsioideae (C₃ tank-epiphytes, CAM tank-epiphytes and CAM atmospheric epiphytes) is not yet clear (Givnish et al., 2011), but is likely to have been in or adjacent to the northern Andes. Orogenic episodes, alongside coevolution with Andean hummingbirds, have therefore probably been very important in promoting the diversification of this subfamily, and range sizes of many Andean taxa are at least partly determined by the topographic dissection of montane landscapes (Kessler, 2002a,b; Givnish et al., 2014). Recent advances in phylogenetic resolution within the Tillandsioideae (Barfuss et al., 2016) can be fruitfully combined with the output of this

investigation to generate a clearer picture of evolution in this subfamily. It has long been established that the earliest-diverging ('non-core') Tillandsioideae are represented by the species-poor genera *Catopsis* and *Glomeropitcairnia*. Crayn et al. (2015) suggested that one reason for the low diversity in these genera relative to the Core Tillandsioideae is their apparent inability to perform even low levels of CAM. CAM could conceivably be of particular benefit to *Catopsis* species, which inhabit exposed canopy microhabitats. However, this argument rests partly on the assumption that the greater diversification of the Core Tillandsioideae is associated with a widespread capacity for, and benefit from, weak CAM, which has not been adequately demonstrated. Alternative explanations could be put forward based on the divergence in stomatal sensitivity to VPD (and perhaps other environmental factors) and stomatal kinetics between bromeliads with unmodified stomatal complexes (such as the non-Core Tillandsioideae) and modified stomatal complexes (such as the Core Tillandsioideae; **Chapter 5**; Tomlinson, 1969), or on the basis of the surprisingly strong underinvestment in veins in *Catopsis*, which could limit leaf hydraulic conductance (**Chapters 3-4**). It should also be noted that while there may be relatively few *Catopsis* species, those that exist must be considered ecologically successful in terms of geographical range. The factors that make *Catopsis* species well-adapted to their exposed environmental niches should be investigated further, taking into account recent observations of counterintuitive responses of water-use efficiency to wind exposure (Schymanski and Or, 2016).

The Core Tillandsioideae are divided between two tribes: Vrieseae and Tillandsieae. The foliar anatomy and ecophysiology of the C₃ tank-epiphyte Vrieseae (including *Alcantarea*, *Mezobromelia*, *Vriesea*, and *Werauhia*) is similar to that of the *Gregbrownia-Guzmania* clade in the Tillandsieae, where a high internal air space fraction maintains relatively high mesophyll conductance to CO₂ and high photosynthetic rates. Low stomatal density, high stomatal sensitivity to VPD and rapid stomatal movements are all important in maintaining high water-use efficiency, while the loss of leaf xylem vessels in some lineages is associated with reduced hydraulic conductance and water loss (**Chapter 4**). The tank growth-form, in this and other Tillandsioideae groups, provides external hydraulic capacitance that limits the necessity for high internal capacitance, reducing leaf construction costs (**Chapter 3**). Other aspects of leaf anatomy, including underinvestment in veins, can be explained in terms of adaptation to high-humidity, low-light epiphytic microhabitats. However, despite the prevalence of structural and functional adaptation to mesic environments, some more extreme environments have been invaded by representatives of this group. For instance, some *Alcantarea* and *Vriesea* species are associated with very high levels of exposure, often being secondarily terrestrial or saxicolous. Leaf thickness, vein placement, investment in hydrenchyma and osmolyte accumulation all vary across these groups and may be important components of specialisation to

more xeric habitats (**Chapters 3-4**). These habitats, which occur in areas such as the *campos rupestres* of Brazil and the Gran Chaco region, were subject to extensive climatic dynamism throughout the Pleistocene, which could account for the high number of narrow endemics in these regions (Antonelli et al., 2010; Krapp et al., 2014; Gomes-da-Silva et al., 2017). Meanwhile inducible responses to environmental dynamism are critical in exceptional cases like the C₃-CAM species *Guzmania monostachia* (Maxwell et al., 1992, 1994, 1995, 1999).

Within the *Tillandsia* alliance (core Tillandsieae *sensu* Barfuss et al., 2016), the major evolutionary trend is from the tank-epiphyte growth-form towards the morphologically-reduced, possibly neotenic, atmospheric growth-forms characteristic of many *Tillandsia* species. Rosette size varies widely among the lineages within the *Tillandsia* alliance, and further analysis will be required to determine, for example, whether large rosette size has evolved secondarily in *Pseudalcantarea* and *Barfussia* or miniaturisation has evolved convergently in *Lemeltonia*, *Racinaea-Wallisia* and *Tillandsia*. The structure-function basis of the ecophysiological distinctiveness of the atmospheric *Tillandsia* species is clearer. Neoteny is the central feature of the life-histories and growth-forms of these plants (Till, 1992; Benzing, 2000). While the basis of neoteny in other plant groups (e.g. Lemnoideae, Araceae) has been attributed to alterations in miRNA activity (Chuck et al., 2007; Rubio-Somoza and Weigel, 2011; Geuten and Coenen, 2013; Wang et al., 2014), the mechanisms have not been explored in bromeliads. However, the consequences of neoteny are clearer: an increase in mesophyll cell packing and cell size led to a stronger commitment to CAM (Nelson et al., 2005; Nelson and Sage, 2008) and a switch to internal (rather than external, tank-based) hydraulic capacitance (**Chapter 3**). The associated reduction in air spaces also combined with a dramatic decrease in the ratios of leaf surface area to volume and stomata to trichomes and changes in vascular anatomy and architecture to depress leaf hydraulic conductance and transpiration rates (**Chapter 3**). This was presumably of special importance in allowing these plants to invade highly desiccating microhabitats. Extremely high stomatal sensitivity to humidity further enhanced their capacity to cope with fluctuating conditions and to capitalise on brief pulses of moisture availability (**Chapter 4**). Although the hydrological habitat positions and ranges of these species were not quantified in this project, many are very geographically widespread, which can be attributed to high dispersal capability, relatively short generation time, and the environmental resilience conferred by the structural and functional adaptations described here (Garth, 1964; Benzing, 2000; Barve et al., 2014; Givnish et al., 2014).

The CAM terrestrial xerophytes of the genus *Hechtia* (Hechtioideae) are very little-studied, but here it has been shown that they are similar to other succulent xerophytic bromeliads in terms of habitat occupancy (**Chapter 2**) and drought resistance traits (**Chapter 3**). Compared to representatives of

other early-diverging bromeliad subfamilies, Hechtioideae differ in terms of factors such as succulence, vein placement, and osmolyte accumulation, all of which changes were likely important in the invasion of more arid environments (**Chapter 3**). Although no work has yet been performed to investigate the root physiology and seasonal water balances of these plants, it would be surprising if commonalities were not found between *Hechtia* and other characteristic succulent terrestrial monocots of the Central and North American semi-deserts (e.g. *Agave*; Crayn et al., 2015; Males, 2016).

Like the Hechtioideae and Lindmanioideae, the C₃ mesic terrestrial Navioideae are not well represented in the literature or in living collections. In this work, it was only possible to make empirical measurements on two *Navia* species, which scored lowly for drought resistance traits, consistent with their narrow, moisture-demanding hydrological niches (**Chapters 2-3**). As with the Lindmanioideae, unspecialised stomata, a low degree of succulence and the absence of the tank growth-form could all place limitations on the ability of Navioideae species to survive under more water-limited conditions. However, *Navia* is a relatively large genus which has diversified rather rapidly (Table 7.2.1), perhaps due to landscape dissection and dispersal limitation (Givnish, 2015), and therefore warrants further attention (Benzing, 2000; Givnish et al., 2014). Habitat occupancy quantification showed that the Navioideae display a relatively broad overall bioclimatic range, but this is due primarily to the specialisation of the drought-deciduous pyrophyte *Cottendorfia florida*, which has successfully invaded the Cerrado biome where it displays an exceptionally broad hydrological habitat occupancy (**Chapter 2**).

The Pitcairnioideae show a clear progression from C₃ mesic terrestrial *Pitcairnia* species through semi-xeromorphic *Fosterella* species to the CAM terrestrial xerophytes of the Xeric Clade (*Deuterocohnia-Dyckia-Encholirium*). *Pitcairnia* is one of the largest bromeliad genera, and has been afforded opportunities for diversification through both Andean orogeny and Quaternary forest dynamics in northern South America (Givnish et al., 2014; Schütz et al., 2016). The hydrological habitat space occupied by *Pitcairnia* species is very broad, with some species being environmentally specialised and others more generalist (**Chapter 2**). Vegetative specialisation is evident in interspecific variation in many traits in *Pitcairnia*, including leaf morphology, heterophylly, and epidermal features (**Chapter 3**). However, all *Pitcairnia* species are somewhat distinctive among the bromeliads for their relatively high physiological capacity in terms of hydraulics and gas exchange, which is underpinned by large xylem vessels (including vessels with simple end-plates in the root), specialised venation architecture (including a midrib vein), BSEs, a high internal air space fraction, high stomatal density, comparatively high cellular drought tolerance, and a relatively high ratio of hydrenchyma to chlorenchyma (**Chapter 3-4**). The situation in the much smaller genus *Fosterella* is

similar, except that these bromeliads tend to be slightly more xeromorphic, display less diversity in growth-form, and are on average less environmentally specialised than *Pitcairnia* species (**Chapter 2**; Benzing, 2000). *Fosterella* also originated in the Andes, but a disjunct clade occurs in Central America as the result of a long-distance dispersal event (Givnish et al., 2011).

The CAM terrestrial Xeric Clade Pitcairnioideae diverged and diversified under the dry, hot conditions of the 'Dry Diagonal' of South America and arid, often high-altitude regions of the Andes (Givnish et al., 2011; Santos-Silva et al., 2013). It has been suggested that Pleistocene climatic fluctuations may have been important in increasing diversity within this clade, particularly in the genus *Dyckia* (Krapp et al., 2014); Gomes-da-Silva et al., 2017, which displays the highest net species diversification rate of any bromeliad genus (Table 7.2.1). While there is some variation in vegetative morphology and anatomy among *Dyckia* species, which could be of significance for those *Dyckia* species which display an unusual rheophytic habit (Hmeljevski et al., 2011), the very high diversification rate in *Dyckia*- both in absolute terms and relative to the Xeric Clade as a whole- is still largely unexplained. The success of the Xeric Clade genera in highly water-limited environments was contingent upon the origin of CAM and simultaneous concerted changes in leaf anatomical characters, including succulence, internal air space fraction, vein placement, venation architecture, and stomatal density and morphology (**Chapters 3-4**). These changes in turn impacted on leaf physiological function, pushing Xeric Clade species towards the 'slow-growing' end of the leaf economic spectrum but enhancing their capacity for survival in highly stressful habitats. However, each of the Xeric Clade genera displays relatively broad variation in hydrological habitat position (**Chapter 2**), which could relate to as yet unexplored aspects of vegetative specialisation.

The C₃, C₃-CAM and CAM succulent terrestrials of the Puyoideae occupy a wide range of habitats across vicariant regions of the northern and central portions of the Andean cordillera, including montane forest, páramo, and coastal plains adjacent to the mountain ranges (Benzing, 2000). The hydrological habitat space inhabited by *Puya* spp. is correspondingly broad, and species range between broad and narrow habitat ranges (**Chapter 2**). The biogeographic patterns and diversity of this subfamily have therefore been strongly influenced by orogenic pulses in the Andes (Jabaily and Sytsma, 2010, 2013). The success of *Puya* spp. across the various stressful environments they inhabit in the Andes may be largely attributable to their photosynthetic lability and the high degree of leaf-succulence they display (**Chapter 3**). The hydraulic capacitance this affords probably buffers transpiration during periods of soil water deficit, and allows relatively high rates of gas exchange to be maintained (**Chapter 3**). Anatomical specialisation, including the presence of leaf xylem vessels and BSEs, promotes high hydraulic conductance and rapid recharge of hydrenchyma (**Chapters 3-4**). Future research into the functional biology of *Puya* spp. could include more detailed analysis of the

functional implications of leaf anatomical variation, and investigate the hydraulic significance of the large belowground tubers that develop in many species (Benzing, 2000).

The evolution of the Bromelioideae has probably been particularly strongly affected by changes in drainage patterns and forest dynamics across northern South America since the late Miocene (Hoorn et al., 2010). Relationships among the C₃ and CAM terrestrial genera of the early-diverging Bromelioideae have still not been fully resolved (Givnish et al., 2014; Silvestro et al., 2014; Evans et al., 2015). Although these lineages are relatively species-poor when compared with the tank-epiphyte Core Bromelioideae, they are ecologically diverse and occupy a wide range of hydrological habitat space, with individual species also tending to show high hydrological habitat range (**Chapter 2**). The more speciose genera (e.g. *Bromelia*, *Cryptanthus*, *Orthophytum*), however, include a relatively high proportion of climate specialists. Investigations into representatives of the C₃ genera *Fascicularia* and *Ochagavia* suggest that these plants, like *Puya* spp., utilise their hydraulic capacitance to maintain relatively high rates of transpiration and photosynthetic fixation (**Chapter 3**), which may be limited instead by mesophyll constraints and nitrogen content (**Chapter 3**). It is noteworthy that among the strictly C₃ genera of terrestrial Bromelioideae, only one has diversified to any appreciable extent: *Greigia*, which occupied a fairly wide area of hydrological habitat space (**Chapter 2**). Why *Greigia* should appear to have been more successful in this regard than genera such as *Fascicularia* and *Ochagavia* remains unclear, but may have involved a tendency towards environmental specialisation in highly-dissected Andean landscapes. Among the CAM genera of the early-diverging Bromelioideae, there is extensive variation in leaf morphology and anatomy, in terms of succulence, internal air space fraction, and leaf shape and size. This underpins a range of physiological strategies which in turn provide the basis for the ecological diversity of these groups.

The extremely high net species diversification rate in the CAM tank-epiphyte Core Bromelioideae (Table 7.2.1) has been explained by Givnish et al. (2011, 2014) in terms of the ecological opportunities opened up by the origin of epiphytism in conjunction with the operation of CAM and the tank growth-form, and orogenic activity in the Serra do Mar and Andes leading to cooler, wetter conditions in the Atlantic Forest region. The evolution of the tank-epiphyte syndrome in the Core Bromelioideae was contingent on similar (but not identical) concerted adaptive structural-functional changes to those that probably occurred in the origin of tank-epiphytism at the base of the Tillandsioideae (**Chapters 3-4**). This research project has shown that interspecific variation in leaf traits is an important component of the ecological diversity within the CAM tank-epiphyte functional type (**Chapter 3**), and the evolutionary lability of these traits should therefore be recognised as a mechanism by which these plants were able to radiate so successfully into epiphytic ecospace. The case study of the *Aechmea* species of Trinidad presented in **Chapter 6** clearly illustrates the

relevance of interspecific differentiation in anatomical and physiological traits for environmental niche segregation and coexistence across heterogeneous landscapes. In light of recent evidence for selection against CAM in mesic sites (Quezada et al., 2017), the number and nature of reversions to C₃ photosynthesis in the Core Bromelioideae (e.g. in *Nidularium*, *Ronnbergia* and *Wittrockia*; Crayn et al., 2015) requires further investigation. This could provide important insights into the structure-function relationships associated with divergent photosynthetic pathways, and into broader questions of the C₃-CAM adaptive landscape in angiosperm evolution.

7.2.3 Reconstructing the deep history of the bromeliad lineage

The evolutionary origins of the bromeliad lineage and its array of ecological syndromes have tantalised researchers for over a century. Particular focus has been placed on the repeated evolutionary progression towards more advanced forms of epiphytism, and how this may relate to ancestral habitats. Schimper (1888) put forward the hypothesis that vascular epiphytes, including the epiphytic bromeliads, originated from terrestrial floristic elements in humid forests, where environmental conditions in trunk-surface and understorey microhabitats might come closest to mimicking those in adjacent terrestrial situations. By contrast, Tietze (1906) argued that selection for increasingly effective absorptive foliar trichomes in semi-arid environments furnished lineages in the Tillandsioideae and Bromelioideae with an adaptation that could facilitate survival in water-limited epiphytic niches. Pittendrigh (1948) developed these ideas further, but his revisions of Tietze's evolutionary scheme suffered from inaccurate phylogenetic concepts based on misleading floral morphological traits. For example, in the context of the Tillandsioideae, Pittendrigh considered *Glomeropitcairnia* to be the most derived genus and *Tillandsia* the earliest-diverging, whereas modern molecular phylogenetic analyses support the exact inverse of this topology (e.g. Givnish et al., 2011). This led Pittendrigh to contend that the progenitors of the Tillandsioideae 'entered forest primarily as light demanding xerophytes in the uppermost levels', and that those 'species appearing now in lower forest levels have moved downward', apparently in accordance with Tietze's hypothesis. Pittendrigh was more circumspect about phylogenetic relationships within the Bromelioideae, but suggested that xeromorphic terrestrial species with rudimentary tanks and tank-roots (e.g. *Bromelia humilis* Jacq.) could represent intermediate forms between soil-dependent terrestrials and epiphytes. This remains consistent with current understanding of the phylogenetics and evolutionary ecology of the Bromelioideae.

However, the controversy surrounding tillandsioid evolutionary history did not abate. Smith (1989), noting that all extant Tillandsioideae are either epiphytic or saxicolous, and possess absorptive

trichomes, supported a 'Pittendrigh-Tietze' hypothesis. Under this hypothesis, the terrestrial ancestor of the Tillandsioideae evolved absorptive trichomes under exposed conditions (see also McWilliams, 1974; Medina, 1974; Crayn et al., 2004). Benzing (2000) countered that certain facultatively terrestrial, mesomorphic Tillandsioideae (e.g. *Guzmania caricifolia* (André) L.B.Sm. and *G. graminifolia* (André ex Baker) L.B.Sm.) could be expressing atavistic habits and morphologies representative of a terrestrial ancestral stock for the subfamily. Other authors have supported the idea that the extant Tillandsioideae are derived from shade-tolerant mesic terrestrials, more in line with Schimper's (1888) hypothesis (Benzing and Renfrow, 1970; Adams and Martin, 1986b; Medina, 1990; Winkler, 1990). Some terrestrial *Lindmania* spp. (e.g. *L. vinotincta* B.Holst & Vivas) bear a striking visual resemblance to the epiphytic Tillandsioideae, and could provide clues as to the growth-form of the terrestrial ancestor of that subfamily. Meanwhile, Benzing (2000) also remarked on the fact that tillandsioid *Alcantarea* spp. provide an example of a secondary acquisition of the terrestrial habit in a lineage derived from mesic epiphytic stock, with some species (e.g. *A. farneyi* (Martinelli & And.Costa) J.R.Grant and *A. hatschbachii* (L.B.Sm. & Read) Leme) having lost the tank growth-form. Benzing also cites the radiation of the genus *Tillandsia* into environments as divergent as the Atacama Desert and perhumid Amazonian forest understories as a further example of the dramatic transitions in ecophysiological strategy that pepper the bromeliad phylogeny and complicate interpretation of evolutionary trends. Closely-related bromeliad species (and even infraspecific taxa) frequently display strongly contrasting ecological tolerances, indicative of an exceptional level of evolutionary lability. In fact, the numerous instances of habit and habitat transitions within bromeliad lineages render the family so riddled with homoplasy that establishing the characteristics of the ancestral stock of the Bromeliaceae as a whole is a major challenge.

However, in light of the latest phylogenetic developments (e.g. Givnish et al., 2011), the data presented in this thesis can be used to make some novel proposals regarding the structure and ecophysiology of the progenitors of the extant Bromeliaceae. Concrete evidence on this subject has remained elusive, with an extremely poor fossil record for the family (Benzing, 2000). A preliminary point that must be emphasised is that although the Brocchinioideae has been clearly shown to be the earliest-diverging bromeliad subfamily (Givnish et al., 2011), this does not mean that extant *Brocchinia* species are necessarily representative of the common ancestor of all extant Bromeliaceae (BCA). In fact, as discussed above, *Brocchinia* is a remarkably diverse genus, and the Brocchinioideae subfamily have undergone ca. 22.7 My of independent evolution since diverging from the remainder of the family.

In the absence of a definitive, complete phylogeny for the Bromeliaceae, hypotheses regarding the characteristics of the BCA must at present be based on cautious consideration of the distribution of

traits among all early-diverging subfamilies and early-diverging elements of more derived subfamilies, alongside comparison with the family's phylogenetic sister group. Recent phylogenetic analyses have definitively placed Bromeliaceae as sister to the Typhaceae in the order Poales (Bouchenak-Khelladi et al., 2014). Possible synapomorphies between the ancestral stocks of the two families include a rhizomatous, terrestrial or semi-aquatic habit, and erect graminoid foliage. Thus in terms of plant architecture and habit, the BCA was likely a soil-rooted, non-tank-forming, terrestrial, rhizomatous rosette with narrow, linear leaves (Benzing, 2000). This combination of traits persists in many extant species of the earlier-diverging bromeliad subfamilies (e.g. *Brocchinia steyermarkii* L.B.Sm., *Lindmania guianensis* (Beer) Mez), as well as in early-diverging genera of Pitcairnioideae. Given the convergent origins of efficient absorptive trichomes in *Brocchinia*, the Tillandsioideae, and Bromelioideae, it seems likely that the BCA had multicellular, non-absorptive foliar trichomes that could be readily co-opted for absorptive function in coordination with the tank growth-form and under selection pressures imposed by water limitation and oligotrophic growth substrates. Widespread non-absorptive functions of trichomes in extant bromeliads include the reduction of transpirational water loss, pollinator attraction, and protection from herbivores (Benzing, 2000), and any or all of these functions may well have been important in the BCA. The small stomata of unspecialised morphology common to early-diverging subfamilies and elements of the Tillandsioideae and Pitcairnioideae are probably representative of the BCA. This stomatal structure was likely associated with relatively high stomatal conductance and transpiration rates, coupled with intermediate stomatal sensitivity to humidity (see **Chapter 5**). C_3 photosynthesis is plesiomorphic in the Bromeliaceae (Givnish et al., 2014; Crayn et al., 2015), but the repeated origins of CAM throughout the family suggest that the BCA may have possessed anatomical or biochemical traits amenable to co-option for the facilitation of CAM. For example, relatively dense cell-packing may have been a feature of BCA leaf chlorenchyma. Adaxial hydrenchyma, almost ubiquitous in the extant bromeliads, was almost certainly present in the BCA, as were the air channels so prominent in many bromeliad lineages and in the sister family Typhaceae. In terms of hydraulic structure-function traits, the BCA probably had narrow xylem vessels throughout the root and shoot vasculature, with the scalariform end-walls common to Typhaceae and all bromeliads except the roots of *Pitcairnia* spp. This would have facilitated moderate whole-plant and leaf hydraulic conductance relative to extant bromeliads (see **Chapters 3 and 4**). Vein placement probably involved equal interveinal and vein-epidermis distances, or slight underinvestment in veins, with no specialisation for hydraulic recharge or foliar resource foraging (see **Chapter 4**). Meanwhile hierarchical venation architecture may have consisted of primary veins alternating with three secondary veins, as occurs in *Puya*

species and mesic Tillandsioideae, without the midrib unique among extant bromeliads to *Pitcairnia* (see **Chapter 4**).

Historical biogeographic analyses of the Bromeliaceae have placed the geographical location of the BCA on the Guiana Shield in northern South America (Givnish et al., 2011). In terms of bioclimatology, all early-diverging bromeliad lineages occupy high-rainfall, low-seasonality environments, while many groups of terrestrial bromeliads show an evolutionary progression towards more exposed, arid environments (see **Chapter 2**). The BCA probably occupied a similar environmental niche to the early-diverging extant bromeliads, growing like graminoid *Brocchinia* and *Lindmania* species in dense clonal mats as a stress-tolerator in moist but exposed sites and on oligotrophic substrates. From this hypothetical ancestral stock, a series of concerted, frequently convergent trait changes allowed the bromeliads to undergo a series of rapid, nested radiations into disparate regions of geographical, environmental, and ecological space (Fig. 7.2.2).

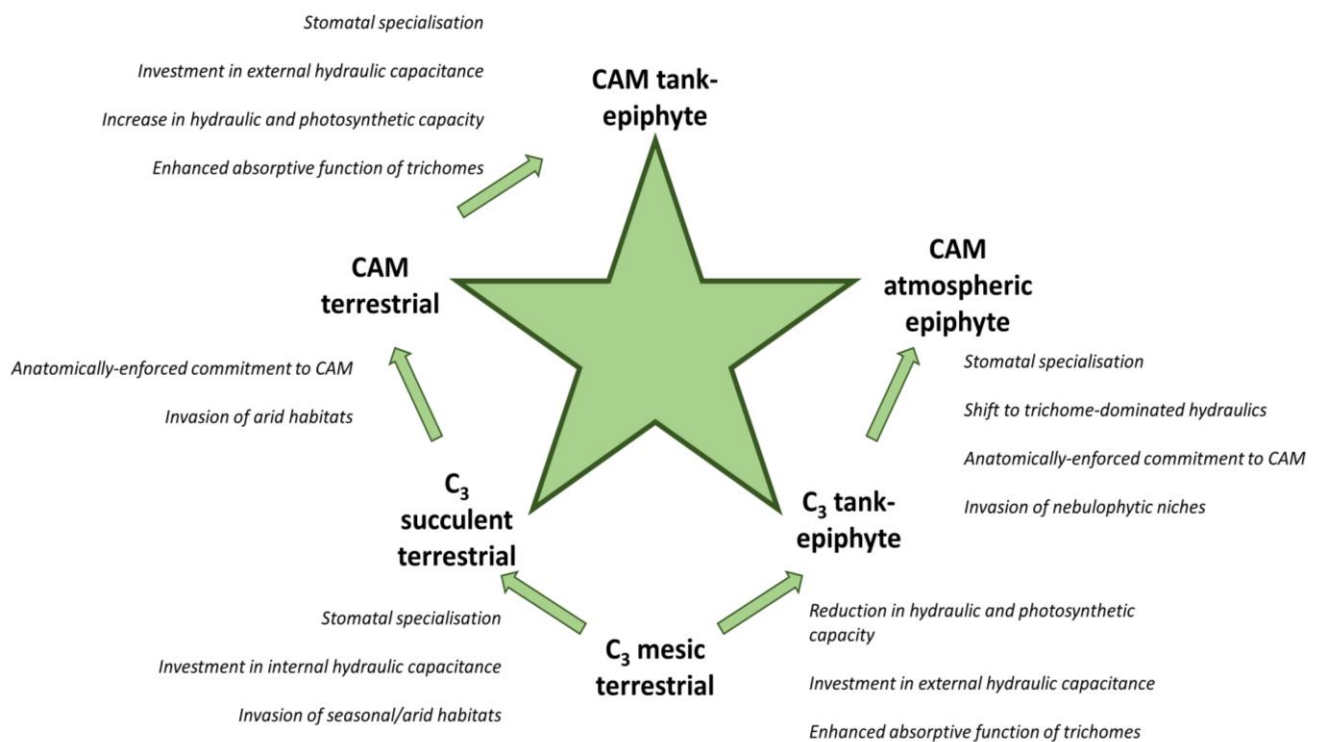


Figure 7.2.2. Summary of major trends in the evolutionary ecology of the Bromeliaceae.

7.3 Overall conclusions

The impressive species diversity of the Bromeliaceae is in part underpinned by extensive ecological differentiation within and between functional types. In the context of the evolutionary history of the family, this process of nested niche differentiation began when bromeliads were first able to

colonise areas beyond the Guiana Shield environments to which the ancestral stock of the Bromeliaceae appears to have been native. This led Givnish et al. (2011) to pose the question: *“What morphological and physiological traits adapted bromeliads for life outside the Guayana Shield?”* Substantial progress has been made in answering this question, most notably in the form of the correlative analyses reported by Givnish et al. (2014), which identified the significance of the tank-growth form, epiphytism, CAM, and other factors in facilitating adaptation to new ecological niches and accelerating net species diversification rates.

This research project has built extensively on these observations by seeking to elucidate some of the key structure-function relationships that have laid the foundations for both the ecological differentiation of the major functional types and interspecific divergences in ecophysiological strategy within these types. Evolutionary change in continuous and discrete anatomical traits has clearly underpinned diversification of hydraulic and photosynthetic characteristics, which have in turn combined to define species’ environmental tolerances and ecophysiological strategies. Ultimately, the cascading effects of anatomical traits on physiological function and plant-environment interactions can be important determinants of species distributions. By facilitating the emergence of contrasting hydrological habitat positions and ranges, evolutionary lability in anatomical traits could therefore have been a critical mechanism (alongside dispersal limitation and environmental dynamism) for the rapid generation of high levels of species diversity in the Bromeliaceae.

This thesis has made use of extensive trait characterisation, with far greater taxonomic coverage than ever previously achieved, providing unprecedented insights into the evolutionary ecology of an important angiosperm radiation. Future work should focus on the remaining enigmatic bromeliad taxa that have eluded sampling, as well as making use of new technologies and methodologies to identify the molecular genetic and genomic features underlying the extraordinary evolutionary lability in bromeliad vegetative anatomy and physiology.

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