

Physiological and anatomical changes during the early ontogeny of the heteroblastic bromeliad, *Vriesea sanguinolenta*, do not concur with the morphological change from atmospheric to tank form

G. ZOTZ^{1,2}, A. ENSLIN³, W. HARTUNG⁴ & H. ZIEGLER⁵

¹Botanisches Institut der Universität Basel, Schönbeinstrasse 6, CH-4056 Basel, Switzerland, ²Smithsonian Tropical Research Institute, Apdo 2072, Balboa, Panama, ³Lehrstuhl für Botanik II der Universität Würzburg, D-97082 Würzburg, Germany, ⁴Lehrstuhl für Botanik I, Universität Würzburg, Julius-von-Sachs-Platz 2, Würzburg, Germany and ⁵Lehrstuhl für Botanik der Technischen Universität München, Am Hochanger 4, D-85354 Freising-Weihenstephan, Germany

ABSTRACT

Two distinct morphological forms characterize the ontogeny of many epiphytic bromeliads. Smaller plants exhibit an atmospheric habit, while larger plants form water-impounding tanks. The study of the functional significance of heteroblasty in epiphytes is severely hampered by considerable size-related variation in morphological, anatomical and physiological parameters. To overcome this problem, plants of varying size of both atmospheric and tank form were included in the present study with *Vriesea sanguinolenta*. The results show that virtually all morphological, anatomical and physiological characteristics vary during ontogeny, but changes were rarely directly related to the step change in gross morphology. Changes were either: (1) gradual from smallest atmospheric to small tank (e.g. leaf divergence angles, reduction in photosystem II efficiency during drought, speed of recovery after drought); (2) there was no change between atmospheric and small tank, but a gradual or step change *within* the tank form (stomatal density, relationship of leaf N and specific leaf area); or (3) developmental patterns were more complicated with decreases and increases during ontogeny (photosynthetic capacity, carbon isotope ratios, abscisic acid levels during drought). Although the comparisons *between* ontogenetic phases were always confounded by size differences, a hypothetical small tank plant is expected to suffer higher water loss than a real atmospheric, whereas a hypothetical, large atmospheric plant would show reduced access to resources, such as nutrients, in comparison with the real tank. The present results are consistent with the notion of heteroblasty as an adaptation of early ontogenetic stages to drought, but highlight that size-related variation greatly modifies any difference directly associated with the step change from atmospheric to tank.

Key-words: abscisic acid; heteroblasty; intraspecific variability; leaf nitrogen; ontogenetic drift; photosynthesis; phyllotaxis; specific leaf area; vascular epiphytes; water relations.

INTRODUCTION

Conspicuous changes in the vegetative phenotype during ontogeny, termed heteroblasty, are found in many cormophytic plant taxa (Jones 1999) and also in algae (Lockhart 1979), but the functional significance of changes in morphological characteristics as diverse as shoot orientation, leaf size, leaf shape, internode length, or the production of adventitious roots is not always clear: attempts to relate these genetically programmed changes in form to function frequently failed. For example, early stages of a suite of heteroblastic woody plants did not show a higher resistance to drought (Darrow *et al.* 2002) or to frost (Darrow *et al.* 2001) as deduced from the environmental conditions experienced by smaller and larger individuals in their native New Zealand habitat. Similarly, Winn's (1999) study with the herb, *Dicerandra linearifolia* (Lamiaceae), provided no evidence that the production of alternative leaf types is adaptive under current ecological conditions. On the other hand, the maintenance of discrete phases of development during evolution suggests some selective advantage and, indeed, some reports do show that phase-specific differences can be adaptive (e.g. Kearsley & Whitham 1997).

Heteroblasty occurs in many epiphytic tillandsioids (Benzing 2000). In these plants, there is a conspicuous shift between two phases with small juveniles having morphological characteristics of atmospherics (i.e. possess non-impounding rosettes of small, linear leaves, which are densely covered by the shields of foliar trichomes), whereas larger conspecifics form tanks (i.e. feature broad leaves, which overlap basally forming water-filled chambers). Although depicted as an adaptation of juveniles to drought for decades (e.g. Schulz 1930), Adams and Martin were the

first to provide experimental evidence for this notion (Adams & Martin 1986b, c). Studying heteroblastic *Tillandsia deppeana*, they showed that morphological and anatomical differences between 'juvenile' atmospherics and 'adult' tanks were accompanied by pronounced differences in a number of physiological characteristics. For example, tank forming individuals of *T. deppeana* showed significantly higher rates of CO₂ exchange and transpiration when well-watered, while suffering a much stronger reduction in gas exchange after the onset of drought than smaller, atmospheric conspecifics. Adams & Martin (1986a) concluded that atmospheric juveniles were crucial to the establishment of this species in the epiphytic habitat.

The typological approach taken by Adams & Martin (1986b, c), i.e. treating individuals *within* a phase as homogeneous groups, has recently been challenged by Schmidt & Zotz (2001). Schmidt & Zotz tested the hypothesis that size-related variation in morphological and physiological characteristics is so pronounced in vascular epiphytes (for a review see Zotz, Hietz & Schmidt 2001a) that a comparison of two supposedly distinct phases should conceal considerable morpho-anatomical and physiological differences *within* each phase and, consequently, may lead to erroneous conclusions as to the ecological significance of heteroblasty in epiphytic bromeliads. Indeed, Schmidt & Zotz (2001) were able to show for *Vriesea sanguinolenta* that ontogenetic changes during the tank phase were at least as pronounced as the step change from juvenile atmospheric to tank. Their study, however, dealt mostly with variation during the tank phase and provided only limited information on the morphology and the physiology of atmospherics. The present communication focuses specifically on the atmospheric phase, on plants in transition and on the smallest tank forms. This approach allowed us to address the question whether ontogenetic changes in morphology, anatomy, and physiology were primarily related to differences in plant size or rather to a step change from atmospheric to tank form. Considering the pivotal role of irregular water supply for growth and survival of vascular epiphytes (Benzing 1990), we particularly emphasized morphological and physiological parameters related to drought tolerance.

MATERIALS AND METHODS

Plant material

Vriesea sanguinolenta Cogn. & Marchal (syn. *Werauhia sanguinolenta*, Grant 1995), is a tank-forming bromeliad with a geographic distribution from Costa Rica to Colombia, including various Caribbean islands. In central Panama, seed dispersal during the late dry season is followed by germination in the early wet season in May. The average size after one year is about 1 cm LL (= length of longest leaf) (Zotz 2004): plants of this size were the smallest included in the present study. The entire atmospheric phase lasts approximately 5 years (Zotz 2004), while tanks reach ages of several decades (Schmidt & Zotz 2002). We either used plants collected in the Barro Colorado Nature Mon-

ument (BCNM, 9°10' N, 79°51' W), Republic of Panama, or plants grown from seeds collected in BCNM. The natural vegetation of this biological reserve is classified as tropical moist forest (Holdridge *et al.* 1971). Mean annual rainfall is approximately 2600 mm with a pronounced dry season from late December to late April. During these 4 months completely rainless periods regularly extend for several weeks (Windsor 1990). A number of measurements were done in Panama. Plants used in experiments at the University of Würzburg had been collected a month earlier in BCNM and had been kept under the following conditions in the Würzburg greenhouse: 24–26 °C air temperature, 60–80% relative humidity, approximately 10 mol m⁻² d⁻¹ photon flux density (PFD) (these conditions closely resemble those at typical growing sites in Panama, compare Schmidt & Zotz 2001). Plants were watered rigorously twice daily, no fertilizer was applied.

Morphometry and anatomy

In 45 field-grown plants ranging in size from 2.5 to 58 cm LL we determined the average divergence angle of neighbouring leaves. From a similar set of 24 well-watered plants (atmospherics with LL = 1, 2 and 3 cm, intermediate forms, tank forms with LL = 5, 30 cm), hand-made leaf cross-sections from the central portion of a mature leaf were studied. Tissue thickness was determined under a light microscope, and leaf thickness was calculated as the sum of all tissue layers. Control measurements with a calliper yielded similar results of total leaf thickness. Leaf width was measured in the middle of the leaf blade. Additional cross sections from the basal, central, and apical leaf portions allowed the calculation of surface to volume ratios by treating the entire leaf as a number of frustums. Leaves of *V. sanguinolenta* are hypostomatous, therefore stomatal densities refer only to the abaxial side. The number of trichomes, in contrast, was related to a bi-layered leaf surface. Trichome and stomatal densities were also determined near the middle of a leaf blade.

O₂ exchange

As described in detail earlier (Stuntz & Zotz 2001), O₂ exchange was measured at 30 °C with two LD2 Leaf Disc Electrodes (Hansatech Ltd, King's Lynn, Norfolk, UK) in combination with LS2 light sources and neutral density filters. The electrode chambers were charged with water vapour-saturated air containing about 5% CO₂. All plant material was collected in the field between 0800 and 1000 h and measurements were completed within a few hours. When leaves were big enough, discs were used (area: 10 cm²), otherwise several leaves were put into the chamber and leaf area was later determined using a digitizing tablet (Sigma Scan; Jandel Scientific, Corte Madera, CA, USA). Photosynthetic induction was obtained by alternating periods of darkness (5 min) and light (5 min) of a PFD of approximately 600 μmol m⁻² s⁻¹. Normally, the rate of oxygen evolution was constant after three to four of these

cycles. Then the rate of dark respiration was determined followed by a stepwise increase of PFD until a maximum of approximately $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$. The photosynthetic capacity was then calculated following Smith (1937). Samples were dried at 60°C for 2 weeks to determine the dry mass (DM), which allowed the calculation of specific leaf area (SLA) and mass-related photosynthetic capacity. Later, the nitrogen content was determined at the University of Würzburg using a CHN-O Element Analyser (Heraeus, Hanau, Germany).

Carbon isotope ratios

Samples of mature leaves from 21 plants differing in size from 1.8 to 69 cm LL were collected in BCNM. All were growing in close proximity to each other in the canopy of three *Annona glabra* trees. Samples were dried at 60°C for 7 d and ground to fine powder. As described in detail elsewhere (Zotz & Ziegler 1997), samples were analysed at the GSF (Neuherberg, Germany) using a Heraeus CHN analyser connected on-line to a trapping-box-gas-isotope-mass-spectrometer system (Delta 5; Finnigan MAT, Bremen, Germany). The error of determinations is $\pm 0.1\%$ external precision.

Physiological changes during drought

After several weeks under well-watered conditions in the greenhouse, irrigation was discontinued. In a series of experiments we monitored the subsequent changes in plant water content, net CO_2 exchange (NP), maximum photochemical yield of photosystem II (PSII), and plant abscisic acid (ABA) content in plants of four different size classes. During the duration of the experiments, plants remained in the greenhouse.

The reduction of net CO_2 exchange after the onset of drought was studied in a first experiment with a 'mini-cuvette system' (Walz, Effeltrich, Germany). Artificial, saturating illumination provided a PFD of approximately $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, cuvette temperature was 25°C , relative humidity was approximately 90%. All CO_2 measurements were done using BINOS infrared gas analysers (Rosemount, Hanau, Germany) operating in differential mode. An additional BINOS in absolute mode was used to determine the CO_2 concentration of the air flowing through the system ($380\text{--}420 \mu\text{L L}^{-1}$). Differences in the CO_2 concentration could be measured to within 0.1 p.p.m. Depending on plant size, up to five plants were put in wire-mesh baskets (3.8 cm diameter, 1–1.5 cm height, mesh width 1.6 mm), placed into the gas exchange cuvette, and NP was measured as soon as equilibrium was reached. Then the samples were removed and weighed on an electronic balance (PM 460; Mettler, Giessen, Germany; accuracy: 1 mg). After measurements with well-watered plants this procedure was repeated at 1, 3 and 8 d after the onset of drought.

A second drought experiment lasted for 4 weeks. For a subset of plants we determined plant dry mass, and established an allometric relationship between plant size (LL)

and plant dry mass. This allowed the estimation of plant water content during the experiment under the assumption that all subsequent weight changes were attributable to water loss. Maximum photochemical yield of PSII was measured as variable chlorophyll fluorescence of dark-adapted leaves at room temperature ($F_v/F_M = (F_M - F_0)/F_M$; Kitajima & Butler 1975) with a PAM-2000 fluorometer (Walz). Using fibre optics (Walz), which were held perpendicular to each rosette, minimum fluorescence (F_0) was excited at 655 nm and 600 Hz modulation frequency, and maximum fluorescence (F_M) was measured with 100-kHz modulation frequency. The F_M was elicited by saturating flashes of 0.8-s duration from a built-in miniature halogen lamp. A subset of these plants (three to seven individuals per size class) were re-watered after 1, 3, 8 and 20 d of drought to study recovery. After 1, 2 and 3 d of recovery, we determined both plant fresh weights and F_v/F_M ratios. Data from the third day were used to quantify the level of recovery.

An additional number of plants, which were subjected to the same experimental conditions, were sampled at regular intervals to allow the extraction and analysis of free ABA. Entire shoots (leaves and 'stems') were freeze-dried, homogenized, and extracted with 80% aqueous methanol. After centrifugation, the supernatant was purified by passing through SEP-PAK C_{18} -Cartridges (Millipore Corp., Milford, MA, USA) and eluted with 70% aqueous methanol. The methanol was evaporated, and the residue was re-suspended in water and partitioned three times against an equal volume of ethyl acetate at pH 3. The organic fraction was reduced to dryness, taken up with $300 \mu\text{L}$ of Tris-buffered saline buffer (Tris HCl: 50 mM Tris, 150 mM NaCl, 1 mM MgCl_2 , pH 7.8) and analysed immunologically by enzyme-linked immunosorbent assay (ELISA) as described by Weiler (1986).

Data analysis

Data were analysed with STATISTICA (Statsoft Inc., Tulsa, OK, USA). When necessary, data were transformed before analysis (Sokal & Rohlf 1995). When this procedure did not yield normal distributions and homoscedasticity, we used a non-parametric test (Kruskal–Wallis-ANOVA).

RESULTS

Morphology and anatomy

Leaves of seedlings were almost opposite, but leaf divergence angles decreased continuously with plant size, approaching the ideal angle of 137.5° in plants ≥ 20 cm LL (Fig. 1). By definition, leaves of atmospherics are relatively slender and also absolutely thicker than those of small tanks (Table 1), and have higher trichome densities (Fig. 2). This gross morphological variation between atmospherics and tank forms was, however, not reflected in the majority of the other morphological and anatomical differences that we studied (Fig. 2). For example, shield size did not vary with size. Stomata density remained also constant in atmo-

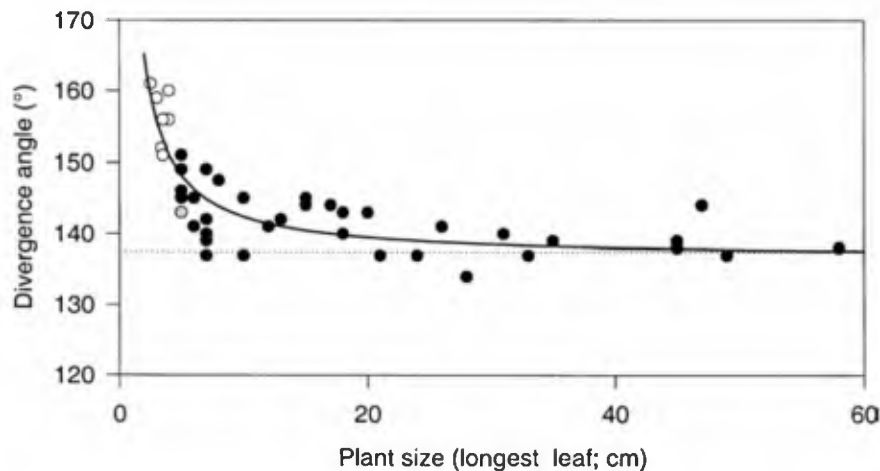


Figure 1. Changes in divergence angles in *V. sanguinolenta* rosettes of different size. Open circles symbolize atmospheric, closed symbols are tanks, intermediate forms have grey symbols. The regression line is: divergence angle = $137 + 57 \text{ LL}^{-1}$; $r^2 = 0.69$.

spherics and small tanks, but increased in larger tanks. Similarly, the relative proportions of the chlorenchyma did not vary in atmospheric and small tanks, but increased in large tanks, while the opposite trend was found in the relative thickness of the hydrenchyma.

O₂ gas exchange, leaf N, and specific leaf area

Leaf photosynthetic capacity (PC) as determined with an oxygen electrode changed significantly with plant size (one-way ANOVA, $F_{7,40} = 10.44$; $P < 0.001$; Fig. 3). Most of this size-related variation could be explained by differences in leaf-N [PC = $0.41 + 0.055$ leaf N (%), Pearson product moment correlation; $r^2 = 0.71$, $n = 34$]. The relationship between leaf N and PC was not affected by the transition from atmospheric to tank form. There was, however, a step change in the relationship of SLA and leaf N (Fig. 4), but this change did not concur with the phase change: atmospheric, transition forms and small tanks followed the same relationship, whereas larger tank forms followed a different one, which was shifted to the left with similar slope ($P > 0.05$).

Size-related changes in carbon isotope ratios

The $\delta^{13}\text{C}$ ratios of the leaves of 21 plants from natural populations varied from -25.4 to -29.7‰ with a significant

trend towards more negative $\delta^{13}\text{C}$ ratios in larger plants in both atmospheric and tank forms (Fig. 5). The smallest atmospheric and the smallest tanks had similarly high $\delta^{13}\text{C}$ ratios.

Changes in CO₂ exchange and F_v/F_M ratios under drought

Consistent with the results of the oxygen electrode measurements, NP of entire plants was highest in A_{1cm} plants under well-watered conditions (Fig. 6). Drought affected the gas exchange of the smallest plants very quickly. After only 1 d without water, gas exchange had ceased in the smaller atmospheric. The largest atmospheric and the smallest tanks had their stomata closed after 3 d.

In well-watered plants, the efficiency of PSII (F_v/F_M) increased significantly with plant size from 0.68 ± 0.01 (means \pm SE, $n = 14$) in the smallest atmospheric (A_{1cm}) to 0.74 ± 0.01 in small tanks (T_{5cm}; $n = 21$) (one-way ANOVA, $F_{3,70} = 6.11$, $P < 0.001$). After 3 d without irrigation, relative plant water content (RWC) had decreased by 34–47%, whereas F_v/F_M changed only by 6–13% (Fig. 7). Even after 4 weeks of drought, when RWC had dropped to 23–30% of initial values, the decrease in F_v/F_M was still rather moderate. Although large atmospheric and small tanks tended to have much higher F_v/F_M ratios, the differences between size classes in the efficiency of PSII were not significant (one-

Table 1. Morphological changes in leaves during the early ontogeny of *V. sanguinolenta*

Parameter	Size class				Analysis of variance
	A _{1cm}	A _{2cm}	A _{3cm}	T _{5cm}	
Leaf length/width	7.0 ± 1.5^a	8.4 ± 0.3^{ab}	6.2 ± 1.6^{ac}	3.1 ± 0.4^d	$F_{3,16} = 19.5$, $P < 0.001$
Leaf width/thickness	5.5 ± 1.1^a	5.8 ± 1.3^a	9.8 ± 3.2^b	24.4 ± 3.1^c	$F_{3,16} = 67.4$, $P < 0.001$
Leaf thickness (μm)	451 ± 23^{ab}	564 ± 61^a	501 ± 19^{ab}	394 ± 36^b	$F_{3,12} = 3.6$, $P < 0.05$
Surface to volume ratio (mm^{-1})	3.7 ± 0.1^a	3.1 ± 0.1^b	3.0 ± 0.1^b	3.4 ± 0.1^{ab}	$F_{3,16} = 6.3$, $P < 0.01$

Given are means \pm SE for each category. Different letters indicate significant differences in *post hoc* comparisons (Newman–Keuls test, $P < 0.05$). Leaf length refers to the green portion only.

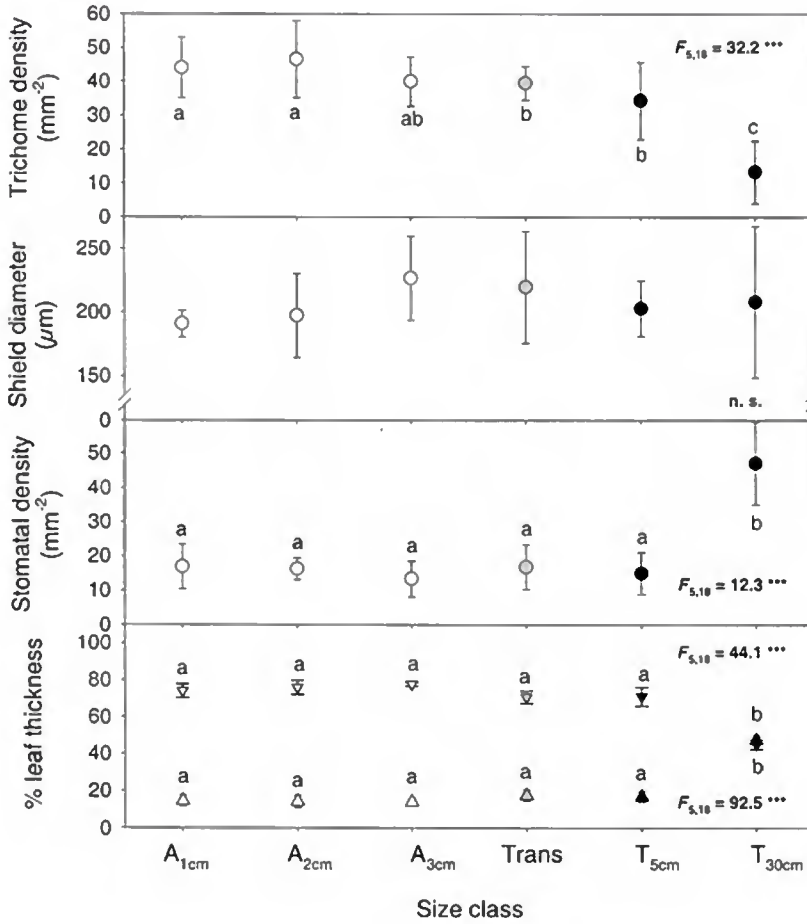


Figure 2. Anatomical and morphological changes during ontogeny in *V. sanguinolenta*. Shown are scale density, scale size, stomatal density, and relative tissue thickness of the chlorenchyma (Δ) and the hydrenchyma (∇) for plants of varying size. Data are means \pm SE, $n = 4$. Atmospheric (A, open symbols), intermediate forms (Trans, grey symbols), and tanks (T, closed symbols). Different letters indicate significant differences between size classes (one-way ANOVA, Newman-Keuls test, $P < 0.05$).

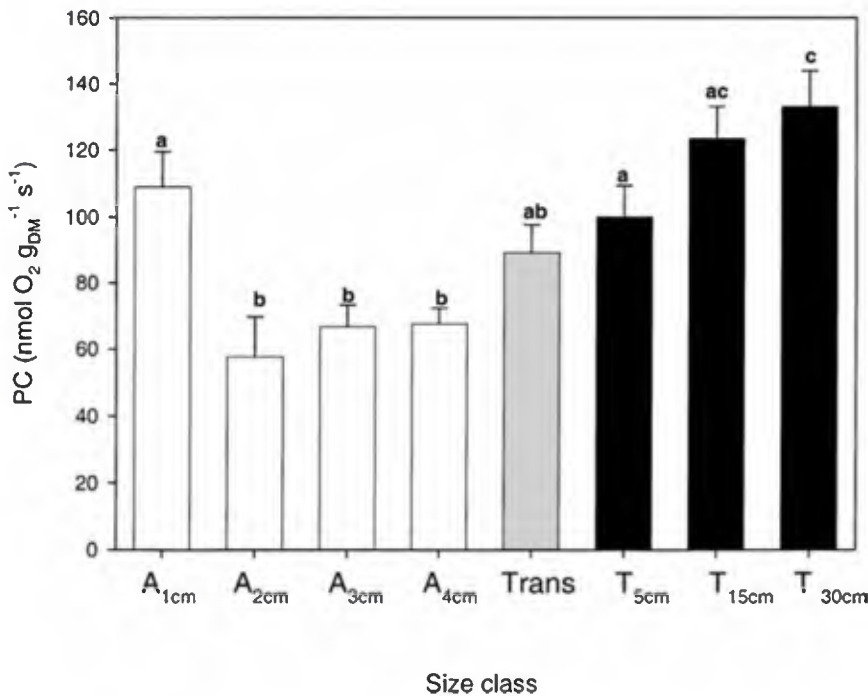


Figure 3. Changes in photosynthetic capacity (in $\text{nmol O}_2 \text{g}_{\text{DM}}^{-1} \text{s}^{-1}$) with plant size in *V. sanguinolenta*. Data are means \pm SE, $n = 5-6$. Open bars are atmospheric, grey bars are intermediate forms, and black bars are tanks. Different letters indicate significant differences between size classes (one-way ANOVA, Newman-Keuls test, $P < 0.05$).

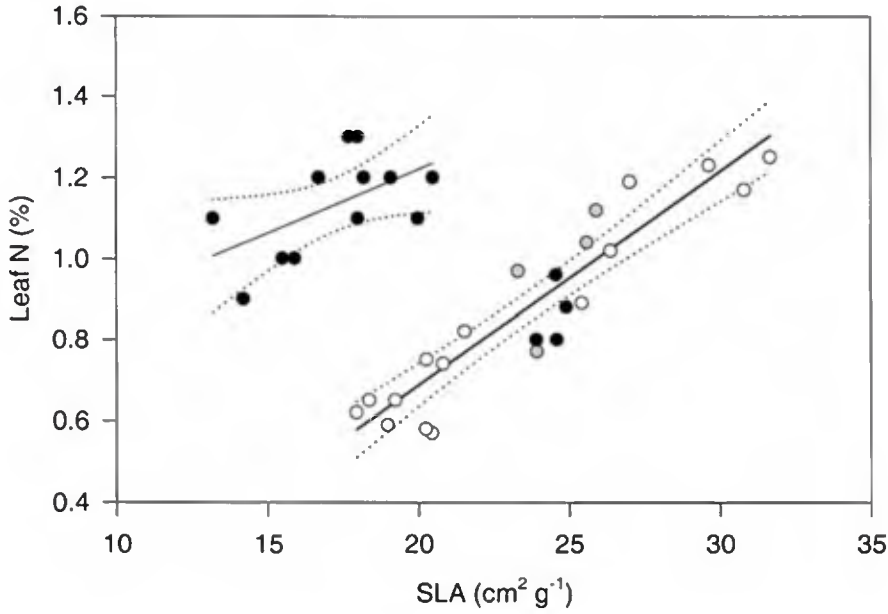


Figure 4. Relationship of leaf N and SLA in *V. sanguinolenta*. Open circles are atmospheric, grey circles are intermediate forms, and closed circles are tanks. Two distinct regression lines (solid lines) and 95% confidence intervals (dotted lines) are given. The regressions are: leaf N = $-0.37 + 0.052 \text{ SLA}$; $r^2 = 0.86$, $P < 0.001$, $n = 22$ (atmospherics, intermediate forms, tanks $\leq 5 \text{ cm LL}$), and leaf N = $0.59 + 0.031 \text{ SLA}$; $r^2 = 0.34$, $P < 0.05$, $n = 12$ (tanks of 15–35 cm LL).

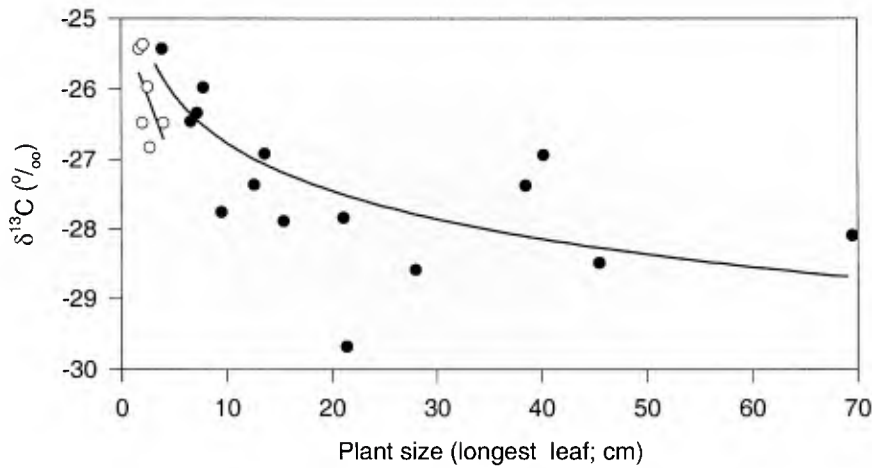


Figure 5. Relationship of carbon isotope ratios ($\delta^{13}\text{C}$ PDB standard) and plant size in *V. sanguinolenta*. The regressions are: $\delta^{13}\text{C} = -25.1 - 0.41 \text{ LL}$ (atmospherics, open circles, $r^2 = 0.30$); $\delta^{13}\text{C} = -25.1 - 1.89 \log(\text{LL})$ (tanks, closed circles, $r^2 = 0.36$).

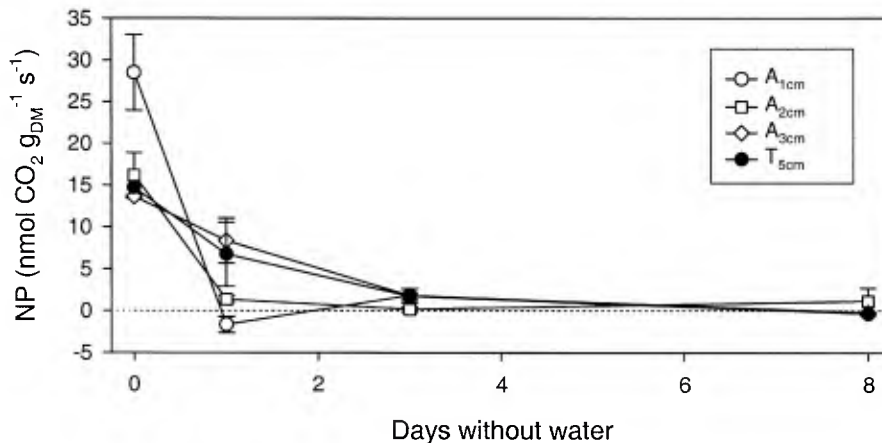


Figure 6. Reduction in net photosynthesis (NP, in $\text{nmol CO}_2 \text{ g}_{\text{DM}}^{-1} \text{ s}^{-1}$) after the onset of drought in *V. sanguinolenta*. Data are means \pm SE, $n = 4$.

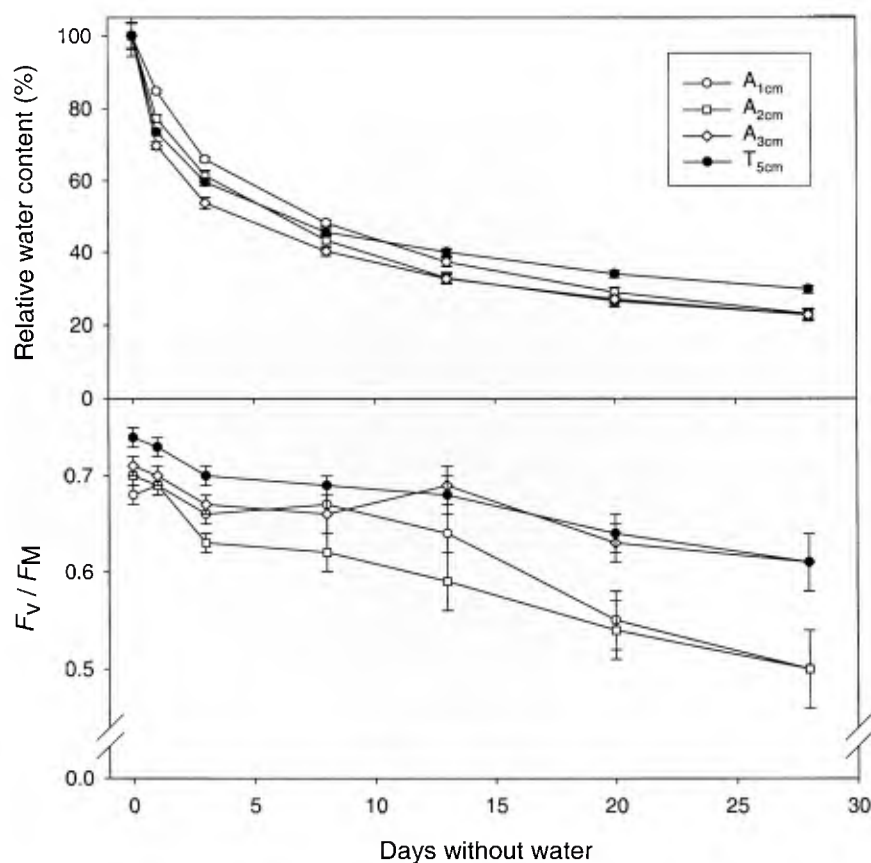


Figure 7. Changes in plant relative water content (upper panel) and the ratio of variable to maximum fluorescence (F_v/F_M , lower panel) after the onset of drought in *V. sanguinolenta*. Data are means \pm SE, $n = 4$.

way ANOVA, $P = 0.10$). Recovery after short-term drought was fast and complete: all size classes reached their original plant fresh weights and F_v/F_M ratios after 1 d (Table 2). Recovery after long-lasting drought, however, was incomplete in particular in smaller plants. Even after 3 d under well-watered conditions, smaller atmospherics (A_{1cm}) had reached less than 80% of pre-treatment F_v/F_M ratios. Their fresh weight remained reduced by almost 30%, but this was mostly due to biomass loss associated with leaf mortality during the preceding drought.

Changes in ABA contents during drought

Even under well-watered conditions, ABA contents were quite high in all size classes, in particular in the smallest atmospherics (A_{1cm}) and the smallest tanks (T_{5cm}). In all atmospherics, there was a significant increase in ABA content after the onset of drought, but this was most pronounced in the smallest size class (Fig. 8a). Small tanks showed no increase in ABA content with time. The highest ABA contents were observed in small atmospherics after

Table 2. Recovery of plant fresh weight and the efficiency of photosystem II (F_v/F_M) after variable lengths of drought

Duration of drought	Size class			<i>P</i> -value
	A_{1cm}	A_{2cm}	T_{5cm}	
Part 1: Fresh weight				
1 d	106 \pm 3 (6)	114 \pm 1 (3)	109 \pm 1 (3)	0.25
3 d	103 \pm 54 (5)	108 \pm 2 (3)	104 \pm 11 (3)	0.29
8 d	94 \pm 4 (4)	90 \pm 11 (3)	100 \pm 9 (3)	0.61
20 d	74 \pm 5 (7)	96 \pm 7 (6)	99 \pm 3 (7)	< 0.001
Part 2: F_v/F_M				
1 d	98 \pm 2 (6)	95 \pm 2 (3)	103 \pm 1 (3)	0.10
3 d	95 \pm 5 (5)	101 \pm 3 (3)	100 \pm 4 (3)	0.67
8 d	89 \pm 4 (4)	88 \pm 7 (3)	99 \pm 2 (3)	0.11
20 d	78 \pm 6 (7)	81 \pm 5 (6)	92 \pm 3 (7)	0.03

Data are percentages of pre-stress values (determined after 3 d of recovery) and are means \pm SE (n). *P*-values refer to Kruskal–Wallis analyses of variance conducted separately for each drought duration.

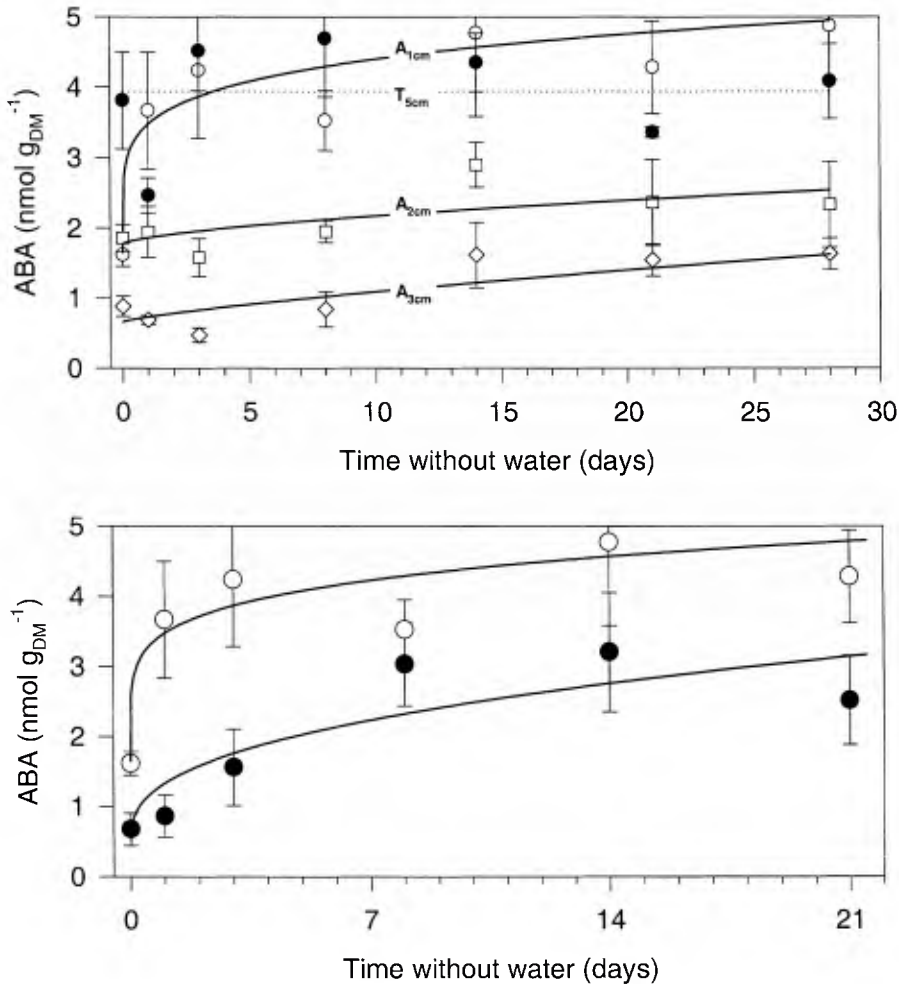


Figure 8. Changes in ABA content of *V. sanguinolenta* plants during drought. Data are means \pm SE, $n = 5$. The upper panel compares field-grown plants of variable size ($A_{1\text{cm}}$ open circles, $A_{2\text{cm}}$ open squares, $A_{3\text{cm}}$ open rhombus, $T_{5\text{cm}}$ closed circles). Regressions (solid lines) are power functions (r^2 between 0.45 and 0.86), the dotted line represents the average ABA content of $T_{5\text{cm}}$ plants. The lower panel compares plants of similar size ($A_{1\text{cm}}$) that were either collected in the field (open circle) or had germinated in the greenhouse (closed circles). Solid lines are power functions ($r^2 = 0.77$ and 0.86).

4 weeks of drought with almost $5 \text{ nmol g}^{-1} \text{ DM}$. Among atmospherics, ABA contents were inversely related to plant size (two-way ANOVA on log-transformed data, Newman–Keuls test, $P < 0.05$).

All plants in the previous experiment had been collected in the field and had thus probably experienced drought stress repeatedly. To explore a possible carry-over effect, we conducted a similar drought experiment with a cohort of plants that had germinated in the greenhouse and had been kept under continuously well-watered conditions until reaching a size of approximately 1 cm LL. In comparison with field-grown plants of similar size (Fig. 8a & b), the initial ABA contents of these plants were much lower (0.68 ± 0.27 versus $1.6 \pm 0.17 \text{ nmol g}^{-1} \text{ DM}$). Overall, the average ABA content found in the two groups differed almost two-fold (Fig. 8b and two-way ANOVA, $F_{1,56} = 16.0$, $P < 0.01$).

DISCUSSION

Size-related changes in morphology were superimposed on the step change from atmospheric to tank form during the ontogeny of *V. sanguinolenta*. Apart from the conspicuous morphological differences in leaf shape and relative leaf

thickness (Table 1), few morphological, anatomical and physiological characteristics were directly affected by the shift between ontogenetic phases. For example, small tanks did not differ from atmospherics in stomatal or trichome morphology, or in the proportional thickness of different tissue types (Fig. 2). Most other studied parameters showed a gradual change with size, again irrespective of ontogenetic phase: leaf divergence angles decreased from 170° in small atmospherics to 137° in tanks of 20 cm LL (Fig. 1), and the efficiency of PSII (F_v/F_m) under well-watered conditions slightly increased from smallest atmospherics to small tanks (Fig. 7). Other parameters showed more complicated patterns. For example, $\delta^{13}\text{C}$ values declined from smallest to largest atmospheric, but increased again in small tanks (Fig. 5). The mass-based photosynthetic capacity (PC) of the smallest atmospherics ($A_{1\text{cm}}$) exceeded that of other atmospherics almost twofold, but was similar to that of small tanks (Fig. 3). Photosynthetic capacity and leaf nitrogen contents are usually closely correlated (Field & Mooney 1986). The high nitrogen content in $A_{1\text{cm}}$ plants, and thus their high PC (Fig. 3) and NP (Fig. 6), are probably directly related to the high N contents of *V. sanguinolenta* seeds. This suggestion is based on the following calculation: the total nitrogen content of a seed of $18 \mu\text{g N}$ (average dry

mass: 0.51 mg, Zotz 2004; average N content $3.6 \pm 0.1\%$, $n = 4 \times 100$ seeds; Zotz, unpubl. data) is equivalent to more than 60% of the total nitrogen content of $30 \mu\text{g N}$ of a $A_{1\text{cm}}$ plant (average dry mass: 2.5 mg, Zotz 2004; average N concentration: 1.2%, Fig. 4). After a drop in N content and PC during the later atmospheric phase, both PC and leaf N gradually increased in tanks, which was expected considering the improved nutrient availability in these impounding structures (Reich *et al.* 2003).

The strongly improved nutrient availability in larger tanks, but also major changes in leaf structure that occur *within* the tank phase, may explain the step change in the relationship of leaf N and SLA (Fig. 4). The low SLA in larger tanks can be related to an increase in leaf thickness and tissue density (Dijkstra 1989). We did not measure density directly, whereas the increase in thickness has been documented for *V. sanguinolenta* by Schmidt & Zotz (2001). A physiological function of these structural changes is indicated by the relative increase of the chlorenchyma at the expense of the hydrenchyma in larger tanks (Fig. 2): area-related PC increases significantly with size (Schmidt & Zotz 2001). On the other hand, structural changes reflected in SLA may also be related to mechanical functions. Leaves of *V. sanguinolenta* reach a length of more than 100 cm. Since the deflection of a leaf is proportional to the cube of its length (Niklas 1999), increased investment in stiff tissues is essential to avoid buckling under its own weight, let alone under the external forces associated with tropical rainstorms.

As expected, drought-affected smaller individuals more than larger ones, but again the observed differences seemed mostly related to plant size than to life form. Consistent with this statement, CO_2 exchange ceased first in the smallest atmospheric ($A_{1\text{cm}}$), last in small tanks ($T_{5\text{cm}}$, Fig. 6), relative water loss during drought was greatest in $A_{1\text{cm}}$ and smallest in $T_{5\text{cm}}$ (Fig. 7), and recovery after drought was also a function of size (Table 2). We also expected and found an inverse relationship between plant size and ABA contents in atmospheric plants (Fig. 8). In small tanks, however, ABA levels were almost as high as in the smallest atmospheric. If ABA levels reflect the magnitude of previous drought stress in these plants, then the smallest tanks must have been affected as much by drought as the smallest atmospheric in their natural habitat.

The production and accumulation of endogenous ABA reached very high levels for a homoiohydric species (Fig. 8) and were comparable to those found in desiccated tissues of resurrection plants such as *Chamaegigas intrepidus* or *Craterostigma plantagineum* (Hartung, Schiller & Dietz 1998). There is only one other epiphyte species in which abscisic acid production under drought has been studied, the orchid *Dimerandra emarginata* (Zotz, Thomas & Hartung 2001b). In both cases, ABA accumulation was only partly related to stomatal physiology (compare Figs 6 & 8), i.e. ABA continued to rise substantially after stomatal closure. As suggested earlier (Zotz *et al.* 2001b), the function of these increasing levels of ABA in desiccating tissue may be related to drought tolerance via the synthesis of dehy-

drins and other protective polypeptides (Bray 1991; Ingram & Bartels 1996; Chaves, Maroco & Pereira 2003). Although not measured directly, the recovery after stress release must be rather slow. This can be deduced from the high ABA contents of plants after weeks of irrigation (Fig. 8a). These plants, which had been collected in the field, had obviously experienced several drought cycles before (Schmidt & Zotz 2001) and only partly reduced their ABA contents under greenhouse conditions. By comparison, plants that had never experienced drought stress in their life had significantly lower ABA levels (Fig. 8b). A similar phenomenon has been observed in the desiccation tolerant liverwort *Exormothea holstii* (Hellwege *et al.* 1994). Rehydrated thalli that had been kept under optimal unstressed conditions for a longer period in the greenhouse lost their desiccation tolerance and exhibited a low ABA content. Those non-hardened thalli had to be re-hardened slowly or pre-incubated with solutions of high ABA concentrations for desiccation tolerance to recover.

Does the lack of a sudden change in most morphological and physiological characteristics with the transition from atmospheric to tank form contradict the widely held notion that the atmospheric phase of heteroblastic bromeliads is an adaptation to drought (Schulz 1930; Adams & Martin 1986a; Benzing 2000)? Atmospheric are more succulent and have a higher trichome density than tanks (Table 1, Fig. 2). These features alone should make them less susceptible to drought compared to a hypothetical tank form of, say, $LL = 1 \text{ cm}$ or $LL = 2 \text{ cm}$. Conversely, a hypothetical, large atmospheric plant should have reduced access to resources, particularly nutrients, compared to a real tank plant. Small tanks are known to be quite inefficient in bridging rainless periods and accumulating organic material as nutrient source (Zotz & Thomas 1999). The greater risk of dehydration of the smallest tanks compared to the largest atmospheric (due to the higher surface/volume ratios; Table 1) is reflected in the relative increase in $\delta^{13}\text{C}$ ratios (Fig. 5), and in absolute ABA levels comparable to those of $A_{1\text{cm}}$ plants (Fig. 8). The effects of improved resource supply, on the other hand, became only detectable in tanks of larger size (e.g. Fig. 4, see also Schmidt & Zotz 2001). This suggests that the transition from atmospheric to tank form is a typical life-history bottleneck (Loveless, Hamrick & Foster 1998; Lotze, Worm & Sommer 2001), which is also consistent with the finding that mortality rates in *V. sanguinolenta* plants immediately after the transition are increased compared to large atmospheric (Zotz 2004).

In conclusion, although most studied functional changes were not directly associated with the transition from atmospheric to tank form, our results are consistent with the notion that the atmospheric stage is broadly associated with increased drought tolerance, whereas (larger) tanks allow improved access to nutrients. However, our study clearly showed that morphological, anatomical, and physiological changes during the ontogeny of heteroblastic species cannot be studied as 'step changes'. They have to be put in the context of size-related variation.

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