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Are ontogenetic shifts in foliar structure and resource acquisition spatially conditioned in tank-bromeliads?

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The phenotypic plasticity of plants has been explored as a function of either ontogeny (apparent plasticity) or environment (adaptive plasticity), although few studies have analyzed these factors together. In the present study, we take advantage of the dispersal of *Aechmea mertensii* bromeliads by *Camponotus femoratus* or *Pachycondyla goeldii* ants in shaded and sunny environments, respectively, to quantify ontogenetic changes in morphological, foliar, and functional traits, and to analyze ontogenetic and ant species effects on 14 traits. Most of the morphological (plant height, number of leaves), foliar (leaf thickness, leaf mass area, total water content, trichome density), and functional (leaf $\delta^{13}\text{C}$) traits differed as a function of ontogeny. Conversely, only leaf $\delta^{15}\text{N}$ showed an adaptive phenotypic plasticity. On the other hand, plant width, tank width, longest leaf length, stomatal density, and leaf C concentration showed an adaptation to local environment with ontogeny. The exception was leaf N concentration, which showed no trend at all. *Aechmea mertensii* did not show an abrupt morphological modification such as in heteroblastic bromeliads, although it was characterized by strong, size-related functional modifications for CO_2 acquisition. The adaptive phenotypic variation found between the two ant species indicates the spatially conditioned plasticity of *A. mertensii* in the context of insect-assisted dispersal. However, ant-mediated effects on phenotypic plasticity in *A. mertensii* are not obvious because ant species and light environment are confounding variables. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, 175, 299–312.

ADDITIONAL KEYWORDS: *Aechmea mertensii* – *Camponotus femoratus* – French Guiana – leaf traits – mutualistic ants – natural stable isotopes – ontogeny – *Pachycondyla goeldii* – phenotypic plasticity – plant morphology.

INTRODUCTION

Plant development may be viewed as a succession of different ontogenetic phases (e.g. embryonic, juvenile,

adult vegetative, and adult reproductive), each characterized by morphological, anatomical, and physiological changes (Poethig, 2003; Barthélémy & Caraglio, 2007). These changes, which are the result of a developmentally programmed growth trajectory, have been referred to as 'apparent plasticity' (Weiner,

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2004). For most species, this apparent plasticity is relatively subtle and gradual ('homoblastic' species), although there are also species with an abrupt change ('heteroblastic' species; Leroy & Heuret, 2008; Fadzly *et al.*, 2009; Zotz, Wilhelm & Becker, 2011). In Bromeliaceae, many epiphytic C₃ photosynthetic pathway tillandsioids are characterized by a conspicuous shift between two (Benzing, 2000; Schmidt & Zotz, 2001; Zotz *et al.*, 2004) or even three (Meisner & Zotz, 2011) ontogenetic phases. Small juveniles have morphological characteristics typical of atmospheric tillandsioids (i.e. non-impounding rosettes with small, linear leaves, densely covered by the shields of absorbent trichomes), whereas larger bromeliads form tanks or phytotelmata (i.e. interlocking leaves forming water-filled chambers). This change in life form implies morphological, anatomical, and physiological differences (Adams & Martin, 1986a, 1986b; Reinert & Meirelles, 1993; Schmidt & Zotz, 2001). However, recent studies have shown that most of these changes are more pronounced within a life form than between life forms (Schmidt, Stuntz & Zotz, 2001; Schmidt & Zotz, 2001; Zotz *et al.*, 2004; Meisner, Winkler & Zotz, 2013).

As a result of a sessile life-style, plants respond to different environmental conditions through changes in their phenotypes. Subsequently, plants may show considerable morphological and physiological variations during their life cycle. Such an 'adaptive phenotypic plasticity' is rather frequent in tank-bromeliads, which grow in a broad range of environmental conditions, especially in terms of light environments and flooding regimes (Lee *et al.*, 1989; Scarano *et al.*, 2002; Freitas, Scarano & Biesboer, 2003), as well as nutrient availability (Zotz & Asshoff, 2010; Gonzalez *et al.*, 2011). Besides abiotic factors, biological interactions with other organisms may also play a significant role in plant plasticity. For example, herbivory can modify the morphological traits of plants (Agrawal, 1998; Ohgushi, 2005; Heil, 2010) and insect-assisted seed dispersal can influence the shape and size of the associated plants through the distribution of the seedling along gradients of incident light (Leroy *et al.*, 2009a, 2012). Hence, associations of plants with other organisms that result in predictable spatial distribution gradients form particularly suitable model systems for disentangling the relative contributions of the ontogenetic (apparent plasticity) and environmental (adaptive plasticity) determinants of plant phenotypes.

The epiphytic tank-bromeliad *Aechmea mertensii* Schult.f. occurs only in association with arboreal ants that build 'ant-gardens' (AGs; Orivel & Leroy, 2011). In French Guiana, the AGs are initiated by either *Camponotus femoratus* or *Pachycondyla goeldii* ants. As seed dispersers and protective agents for this

bromeliad, *C. femoratus* and *P. goeldii* directly and/or indirectly influence its vegetative (Leroy *et al.*, 2009a) and reproductive (Leroy *et al.*, 2012) traits by determining the location of the seedling, from sun-exposed to partially shaded areas, respectively. These studies dealing with mature *A. mertensii* showed that the variation in some traits (i.e. the size and shape of the bromeliad) was related to abiotic environmental acclimation processes, whereas others (i.e. leaf thickness, leaf mass area, number of flowers and fruits) were related to nutrient-stressed environments linked to the species of the associated ant.

Earlier studies conducted on ontogenetic (i.e. plant size) or environmental factors (Laube & Zotz, 2003; Cavallero, Lopez & Barberis, 2009) showed that the morphological and physiological plasticity of bromeliads can be seen as a result of both ontogeny and adaptive plasticity. In the present study, we took advantage of the dispersal of *A. mertensii* bromeliads by *C. femoratus* or *P. goeldii* in shaded and sunny environments, respectively, to carry out a joint analysis of ontogenetic and habitat effects on phenotypic traits in bromeliads. Specifically, we predicted that the apparent and adaptive plasticity of bromeliads is spatially conditioned related to the spatial distribution (i.e. shaded or sunny environments) of the plants by the two ant species. To test this hypothesis, we quantified ontogenetic changes by measuring a set of key morphological, foliar, and functional traits (Table 1) of the tank-bromeliad *A. mertensii* associated either with *C. femoratus* or *P. goeldii* in habitats ranging from shaded understories to fairly sun-exposed areas.

First, we quantified the effect of ant species and ontogeny on plant growth and architecture by measuring plant height, plant and tank width, number of leaves, and the longest leaf length. Because mature plants are significantly larger when associated with *C. femoratus*-AGs than when associated with *P. goeldii*-AGs (Leroy *et al.*, 2009a), we hypothesized that growth is higher when *A. mertensii* is associated with the former ant (Table 1). Next, we used foliar traits such as leaf thickness, leaf mass area, total water content, and the density of the stomata and trichomes to quantify the resource-use strategy. Here, we also expected higher values for these traits during ontogeny for bromeliads associated with *C. femoratus*. Specifically, we assessed the CO₂ uptake strategy by using the leaf $\delta^{13}\text{C}$ and percentage of leaf C. Because previous studies recorded a decrease in leaf $\delta^{13}\text{C}$ with ontogeny for C₃ bromeliad species (Zotz *et al.*, 2004), we hypothesized that the crassulacean acid metabolism (CAM) photosynthetic pathway would have the same pattern in *A. mertensii*. Finally, by using the natural abundance of nitrogen stable isotope ratios ($\delta^{15}\text{N}$) and percentage of leaf N, we established the

Table 1. List of the 14 measured traits with their biological functions and the expected effects of ontogeny and ant species on phenotypic plasticity

| Traits | Units | Functions | Expected effects | |
|--|----------------------------|-----------------------------|------------------|------|
| | | | Ontogeny | Ants |
| Plant height | cm | Plant growth/architecture | ↗ | + |
| Plant width | cm | Plant growth/architecture | ↗ | + |
| Tank width | cm | Plant growth/architecture | ↗ | + |
| Number of leaves | nb | Plant growth/architecture | ↗ | + |
| Longest leaf length (LL _{max}) | cm | Plant growth/architecture | ↗ | + |
| Leaf thickness | µm | Resource-use strategy | ↗ | + |
| Leaf mass area (LMA) | g m ⁻² | Resource-use strategy | ↗ | + |
| Total water content (TWC) | % | Resource-use strategy | ↗ | + |
| Stomatal density | Number per mm ² | Resource-use strategy | ↗ | + |
| Trichome density | Number per mm ² | Resource-use strategy | ↗ | + |
| Foliar ¹³ C composition (δ ¹³ C) | ‰ | CO ₂ acquisition | ↘ | + |
| Foliar carbon | % | CO ₂ acquisition | ↗ | + |
| Foliar ¹⁵ N composition (δ ¹⁵ N) | ‰ | Nitrogen acquisition | ↗ | + |
| Foliar nitrogen | % | Nitrogen acquisition | ↗ | + |

The arrows indicate either an expected increase (↗) or a decrease (↘) of trait values with ontogeny.

nitrogen sources and acquisition strategy, respectively. Assuming that its association with *C. femoratus* provides *A. mertensii* with more nutrients than does its association with *P. goeldii* (Leroy *et al.*, 2009a; 2013) and that the leaf δ¹⁵N increases with plant ontogeny (Reich *et al.*, 2003), we hypothesized that large plants associated with *C. femoratus* would have both a strong ant species and ontogenetic pattern with higher N acquisition.

MATERIAL AND METHODS

STUDY SITE AND SPECIES CHARACTERISTICS

Field sampling was conducted in February 2011 in pioneer growths along forest edges near the field station at Petit Saut, Sinnamary, French Guiana (05°03'30.0"N; 52°58'34.6"W) along the 'Crique Plomb' dirt road. The climate is moist tropical, with 3500 mm of annual precipitation distributed over 280 days. A major drop in rainfall occurs between July and November (dry season) and there is another shorter, more irregular, dry period in March. The maximum monthly temperature averages approximately 33.5 °C, and the monthly minimum is approximately 20.3 °C.

Aechmea mertensii Schult.f. (Bromeliaceae) is a CAM species of subfamily Bromelioideae. This bromeliad has tightly interlocking leaves forming wells that collect rainwater and organic detritus. These tanks, or phytotelmata ('plant-held water'), provide a habitat for aquatic micro- and macro-organisms and vertebrates (Richardson, 1999; Carrias, Cussac & Corbara,

2001; Brouard *et al.*, 2011). *Aechmea mertensii* grows on the arboreal carton nests of AGs inhabited by the ants *C. femoratus* and *Crematogaster levior* and by *Pachycondyla goeldii*. *Camponotus femoratus* (subfamily Formicinae) is a polygynous (multiple queens), arboreal species living in a parabioc association with *C. levior* (Myrmicinae; Orivel, Errard & Dejean, 1997; Vantaux *et al.*, 2007). Its large, polydomous (multiple nests) colonies and aggressiveness identify it as a territorially dominant species in Neotropical rain forest canopies. *Pachycondyla goeldii*, in contrast, is a monogynous (single queen) arboreal ponerine species with smaller populations, although the colonies may be polydomous (Corbara & Dejean, 1996; Dejean *et al.*, 2000).

SAMPLING PROCEDURE

All of the bromeliads studied were located on easily accessible AGs (37 *C. femoratus*-AGs and 13 *P. goeldii*-AGs). From these 50 AGs, we studied a total of 83 individuals of *A. mertensii* of different sizes, rooted on *C. femoratus*- (*N* = 53) or *P. goeldii*- (*N* = 30) AGs. Thus, for a given ant species, the bromeliads originated either from separate or the same AGs.

Because we did not know the age of the bromeliads, we used the number of wells as a proxy for plant size and therefore ontogenetic stage, with the aim of including the entire size range, from the smallest (seedlings) to the largest (reproductive phase) bromeliads encountered in the natural habitat. Also, the relationship between the number of leaves and the

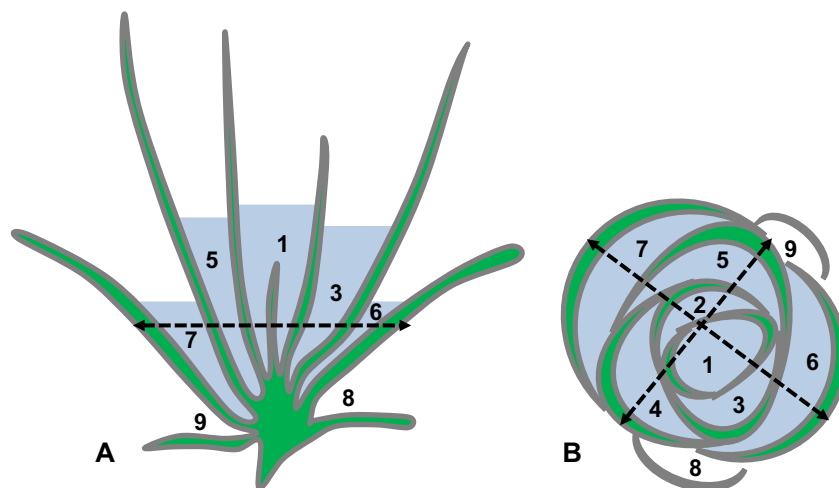


Figure 1. Schematic representation of longitudinal (A) and transversal (B) sections of a tank-bromeliad. The tank (the phytotelm) was considered as the entire combination of wells filled with water. In this example, seven wells constitute the functional tank and two wells without water form a terrarium and were not taken into account in the measurement of the width of the tank. Dotted lines indicate tank width measurements (two measurements at 90°). As a result of the small size of the youngest leaf and the water level, wells 1 and 2 constitute a single well; marked 1 in (A).

number of wells is highly significant for *C. femoratus*- and *P. goeldii*-associated bromeliads (Spearman correlations, $\sigma = 0.941$, $P < 0.0001$ and $\sigma = 0.939$, $P < 0.0001$, respectively).

In the field, the percentages of total incident radiation above all of the AGs were calculated using hemispherical photographs and an image processing software (GAP LIGHT ANALYZER, version 2.0; Frazer, Canham & Lertzman, 1999), as described by Leroy *et al.* (2009a). We measured plant height as the distance from the bottom of the body to the top of the crown and plant width as the maximum distance between the tips of the leaves (mean of two measurements at 90°). Tank width (mean of two measurements at 90°; Fig. 1) and the total number of leaves and distinct wells filled with water constituting the tank were also recorded. Finally, the longest leaf length (LL_{\max}) was measured for all of the bromeliads.

One young, fully developed leaf was collected from all of the bromeliads studied. For each of these 83 sampled leaves, we sampled approximately 5 cm² of leaf tissue from (1) the basal part, corresponding to the part that forms the tank and (2) the aerial part, corresponding to the top of the green part of the leaf blade. All of the samples corresponding to each leaf portion were separated into two pieces.

First, one piece was fixed in FAA (5% formaldehyde, 5% acetic acid, 50% ethanol) for 2 weeks, and then transferred to 70% ethanol for long-term storage. Stomatal and trichome densities were determined for the abaxial and adaxial surfaces of each aerial and basal part of the leaves from imprints made using transparent nail varnish. The imprints were observed

using an inverted microscope (Olympus-BX51). Images were acquired with a digital camera (Lumiera) and processed using image analysis software (IMAGE-PROPLUS, Media Cybernetics). The number of stomata and trichomes per mm² were then recorded for each sample from five randomly selected areas of 1 mm² each. Hand-cut cross sections (ten replicates) were taken from these fixed materials to measure lamina thickness using IMAGE-PROPLUS software. Hereafter, only the abaxial surfaces were analyzed because no stomata were found on the adaxial surfaces and the trichome density did not significantly vary between the two surfaces (data not shown).

Second, several discs of various diameters, depending on the size of the leaf, were collected from the middle of the aerial and basal parts of the leaves. After measuring their fresh weight, the discs were vacuum-dried to calculate percentage total water content [(fresh weight – dry weight)/dry weight × 100] and the dry mass per unit area [leaf mass area (LMA), g m⁻²].

On a subsample representative of each category of plant size ($N = 26$ for both ant species), the dried upper parts of the leaves were ground into a homogeneous powder using a mixer mill. In each case, approximately 1 g was analyzed for its N, C, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ concentrations. Stable isotope analyses were conducted at the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, AZ, USA) using a Thermo-Finnigan Delta^{Plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. The natural abundances of ^{15}N and ^{13}C were calculated as:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where X is the element of interest, and R_{sample} and R_{standard} are the molar ratios (i.e. $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) of the sample and the standard, respectively (DeNiro & Epstein, 1978). The standards were Pee Dee Belemnite for C and atmospheric N_2 for N (Dawson & Brooks, 2001).

STATISTICAL ANALYSIS

Preliminary tests showed that incident radiation was not normally distributed (Shapiro Wilk's test) even after transformation. Thus, the nonparametric Mann–Whitney test was used to test differences in light incidence between ant species. The correlation between incident radiation and bromeliad individuals was determined using Spearman's rank correlations.

Because the bromeliads were sampled alone in an AG or together with other bromeliads from the same AG, we analyzed the relationships between all measured morphological, foliar, and functional traits with ontogeny, ant species, and leaf parts using linear mixed-effect modelling with individual bromeliads nested within AGs as a random factor. More specifically, full models of the morphological and functional traits (dependent variables) were regressed against ontogeny, ant species (independent variables), and their interactions, whereas full models of foliar traits also included leaf parts (independent variable) and the associated interactions. Morphological and foliar traits were log-transformed to fit a normal distribution, whereas functional traits remained untransformed. The results of analysis of variance F-tests for all terms of the full models were reported. Then, we selected the final model by removing the nonsignificant terms and we reported t - and P -values associated with each regression slope estimate of the final models. Departures from homoscedasticity and the normality of the residual errors were evaluated graphically for each full model. All statistical analyses were evaluated under a 95% confidence level and were conducted using R, version, 2.14.1 (R Development Core Team, 2010) and the associated packages for linear mixed effect modelling (nlme).

RESULTS

INCIDENT LIGHT

Camponotus femoratus-AGs received on average significantly less transmitted light than *P. goeldii*-AGs (mean \pm SE, $31.63 \pm 3.17\%$ versus $45.99 \pm 4.59\%$, respectively; Mann–Whitney $U = -5.49$, $P < 0.001$). The correlation between transmitted light and the size of the bromeliads was not significant for *C. femo-*

ratus- or *P. goeldii*-associated bromeliads (Spearman correlations: $\sigma = -0.2574$, $P = 0.062$ and $\sigma = 0.2021$, $P = 0.284$, respectively).

MORPHOLOGICAL TRAIT VARIATION

Bromeliads associated with *C. femoratus* had a maximum of 13 wells forming the tank, whereas those associated with *P. goeldii* had a maximum of 10 wells. All traits related to morphology increased linearly and significantly with ontogeny and did not differ between ant species, except for plant width (Fig. 2, Table 2). Finally, only tank width and LL_{max} were positively correlated with the ontogeny–ant species interaction ($t = 2.08$, $P = 0.044$ and $t = -3.22$, $P = 0.003$, respectively).

FOLIAR TRAIT VARIATION

Leaf thickness and the LMA increased with ontogeny ($t = 7.53$, $P < 0.0001$ and $t = 6.57$, $P = 0.012$, respectively) but did not vary significantly with the ant species ($t = 0.52$, $P = 0.604$ and $t = 0.451$, $P = 0.546$, respectively) (Fig. 3A, B, Table 3). Moreover, leaf thickness and the LMA were higher for the aerial parts of the leaves compared to the basal ones ($t = 3.51$, $P = 0.007$ and $t = 2.52$, $P = 0.013$, respectively). Leaf thickness was also positively correlated with the ontogeny–leaf part interaction ($t = 5.38$, $P < 0.0001$). Conversely, the TWC significantly decreased as a function of ontogeny ($t = -5.64$, $P < 0.0001$) (Fig. 3C, Table 3) and did not vary with ant species ($t = -0.15$, $P = 0.877$). The aerial portions of the leaf had a higher TWC compared to the basal ones ($t = 2.71$, $P = 0.008$) and this was positively correlated with the ontogeny–leaf part interaction ($t = 2.49$, $P = 0.014$). For stomatal density, all of the independent variables and the ontogeny–leaf part interaction were significant (Fig. 3D, Table 3). The stomatal densities were higher for bromeliads associated with *P. goeldii* than for those associated with *C. femoratus* ($t = 2.94$, $P = 0.0052$). The number of stomata per mm^2 was significantly different between the two leaf parts ($t = 2.15$, $P = 0.034$), with decreasing and increasing values for basal and aerial parts, respectively, with ontogeny ($t = -5.62$, $P < 0.0001$). The stomatal density was also positively correlated with the ontogeny–leaf part interaction ($t = 2.49$, $P = 0.014$). Finally, if the trichome density did not vary with ant species ($t = -1.34$, $P = 0.185$) (Fig. 3E, Table 3), basal leaf parts have significantly higher trichomes than aerial leaf portions ($t = -4.26$, $P < 0.0001$) and both were positively correlated with ontogeny ($t = 4.85$, $P < 0.0001$). Seedlings with no wells were devoid of trichomes; once the first wells

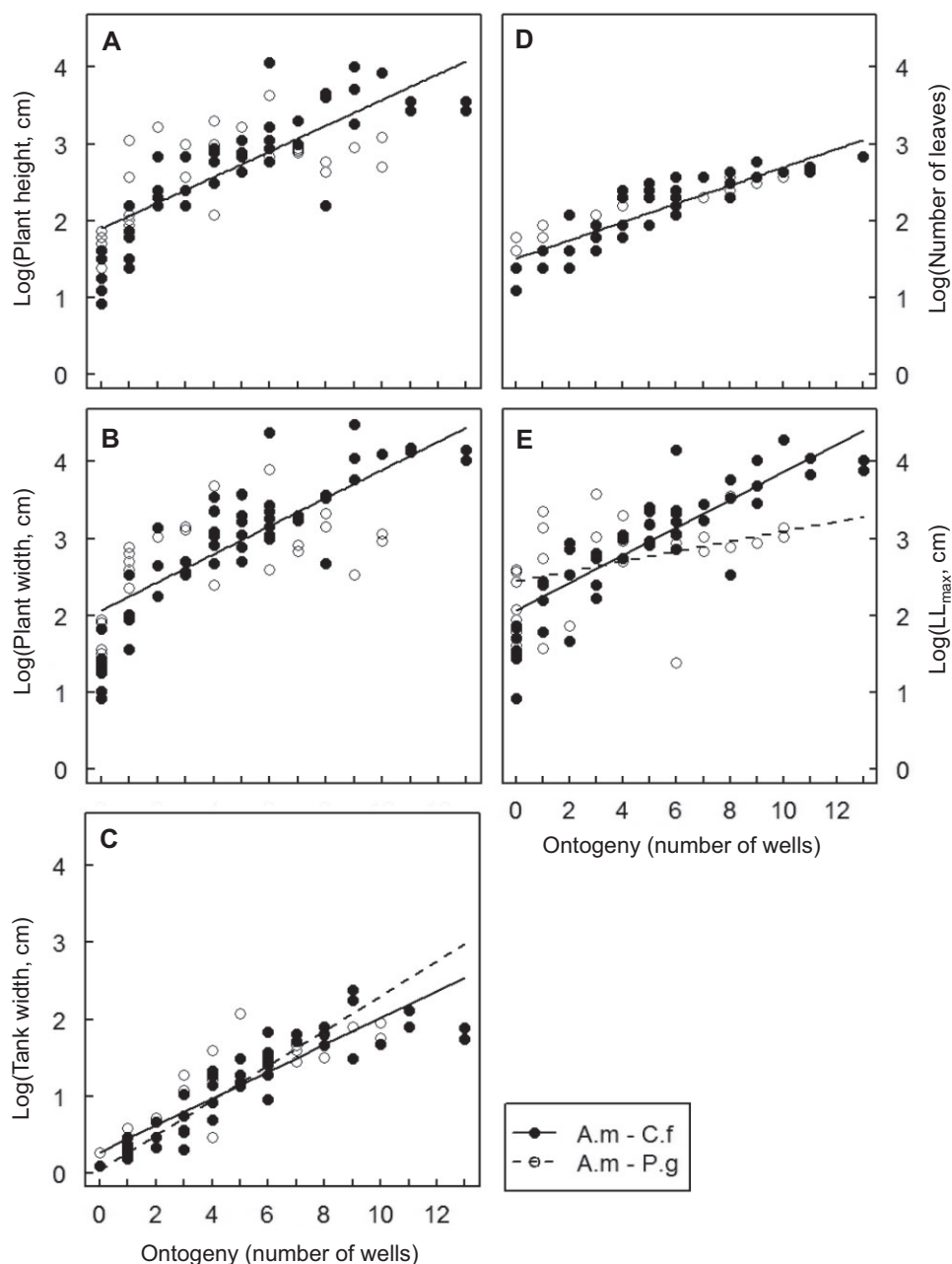


Figure 2. Variation in plant height (cm) (A), plant width (cm) (B), tank width (cm) (C), number of leaves (D), and longest leaf length (LL_{max} , cm) (E) in relation to ontogeny (i.e. number of wells forming the tank) for *Aechmea mertensii* associated with *Camponotus femoratus* (filled circles) or *Pachycondyla goeldii* (open circles). Final linear mixed-effect model predictions are shown as solid lines for *C. femoratus*-associated *A. mertensii* and as dotted lines for *P. goeldii*-associated *A. mertensii* (for more information on the models, see Table 2). In the case of nonsignificant ant species terms, we have only included a single regression line.

appeared, the trichome density increased from 5 to 10 per mm^2 on average.

FUNCTIONAL TRAIT VARIATION

Leaf $\delta^{13}C$ did not vary with ant species ($t = 0.91$, $P = 0.372$) but did vary from -25% to -16% with a

significant trend toward more negative values in seedlings compared to mature bromeliads ($t = 4.17$, $P = 0.0009$) (Fig. 4A, Table 4). Leaf C concentration varied with ontogeny ($t = 3.68$, $P = 0.0025$) and ant species ($t = 2.45$, $P = 0.0194$) (Fig. 4B, Table 4). By contrast, leaf $\delta^{15}N$ did not vary with ontogeny ($t = -2.12$, $P = 0.0522$) but varied significantly with

Table 2. Models describing morphological traits with ontogeny (i.e. number of wells per tank), ant species, and the different interactions involved

| | Estimates, mean \pm SE | d.f. | <i>F</i> | <i>P</i> | SDRa | SDRe |
|----------------------|------------------------------------|--------------|---------------|--------------------|------|------|
| Plant height | | | | | 0.3 | 0.13 |
| Intercept | 1.9 \pm 0.14 | 1, 43 | 1981.49 | < 0.0001 | | |
| Ontogeny | 0.17 \pm 0.02 | 1, 36 | 90.07 | < 0.0001 | | |
| Ant species | | 1, 43 | 1.63 | 0.21 | | |
| Ontogeny–Ant species | | 1, 36 | 0.29 | 0.6 | | |
| Plant width | | | | | 0.3 | 0.13 |
| Intercept | 2.13 \pm 0.16 | 1, 43 | 1905.53 | < 0.0001 | | |
| Ontogeny | 0.19 \pm 0.02 | 1, 36 | 87.83 | < 0.0001 | | |
| Ant species | | 1, 43 | 4.92 | 0.03 | | |
| Ontogeny–Ant species | | 1, 36 | 0.16 | 0.69 | | |
| Tank width | | | | | 0.17 | 0.08 |
| Intercept | 0.27 \pm 0.1 | 1, 43 | 791.58 | < 0.0001 | | |
| Ontogeny | 0.17 \pm 0.02 | 1, 36 | 267.62 | < 0.0001 | | |
| Ant species | –0.23 \pm 0.16 | 1, 43 | 0.01 | 0.91 | | |
| Ontogeny–Ant species | 0.05 \pm 0.02 | 1, 36 | 4.35 | 0.04 | | |
| Number of leaves | | | | | 0.15 | 0.06 |
| Intercept | 1.46 \pm 0.06 | 1, 43 | 6892.54 | < 0.0001 | | |
| Ontogeny | 0.13 \pm 0.01 | 1, 36 | 264.09 | < 0.0001 | | |
| Ant species | | 1, 43 | 0.00 | 0.98 | | |
| Ontogeny–Ant species | | 1, 36 | 1.33 | 0.26 | | |
| Longest leaf length | | | | | 0.28 | 0.12 |
| Intercept | 2.07 \pm 0.14 | 1, 43 | 2249.9 | < 0.0001 | | |
| Ontogeny | 0.18 \pm 0.02 | 1, 36 | 61.16 | < 0.0001 | | |
| Ant species | 0.38 \pm 0.23 | 1, 43 | 3.72 | 0.06 | | |
| Ontogeny–Ant species | –0.12 \pm 0.04 | 1, 36 | 10.41 | 0.003 | | |

The full models are presented, although only variables and interactions with $P < 0.05$ were interpreted as being statistically significant. The lines, highlighted in bold, indicate variables or interactions that appear in the final model. SDRa, standard deviation of the random effect (plants nested within ant gardens); SDRe, standard deviation of residuals.

ant species ($t = -4.54$, $P = 0.0001$) and with the ontogeny–ant species interaction ($t = 2.23$, $P = 0.0420$) (Fig. 4C, Table 4). *Aechmea mertensii* associated with *C. femoratus* had higher leaf $\delta^{15}\text{N}$ values than bromeliads associated with *P. goeldii* (2.60 ± 0.94 versus -0.38 ± 0.94 , respectively; Mann–Whitney $U = -8.34$, $P < 0.0001$). Finally, leaf nitrogen concentration did not show any trend related to ontogeny ($t = -1.01$, $P = 0.329$) or ant species ($t = -0.28$, $P = 0.777$) (Fig. 4D, Table 4).

DISCUSSION

All measured traits showed either an apparent or an adaptive plasticity or a combination of both, except for leaf N concentration, which showed no pattern. Plant height, number of leaves, leaf thickness, LMA, trichome density, and leaf $\delta^{13}\text{C}$ increased with ontogeny, whereas TWC decreased and, in contrast to expectations, none of these variables differed with ant species. Conversely, leaf $\delta^{15}\text{N}$ varied with ant species but not with ontogeny. Plant width, stomatal density,

and leaf C concentration for bromeliads growing in two different AGs remained constant along a size gradient (i.e. no interaction between ant species and ontogeny), whereas tank width and the longest leaf length varied along the size gradient (i.e. significant interaction between ant species and ontogeny). Thus, some of the morphological, foliar, and functional traits were the result of an adaptation to local environmental conditions (related to ant species and local habitat) and also varied with ontogeny.

As dispersal agents of *A. mertensii*, *C. femoratus* and *P. goeldii* ants determine the location of the seedlings from partially-shaded/high nutrient to sun-exposed/low nutrient sites, respectively (Leroy *et al.*, 2009a, b). If the first phases of growth appear to favour, from a morphological point of view, the bromeliads associated with *P. goeldii* compared to those associated with *C. femoratus*, after reaching vegetative maturity, the opposite trend was observed. This was amplified because mature *C. femoratus*-associated bromeliads were larger than those associated with *P. goeldii*. We can hypothesize that

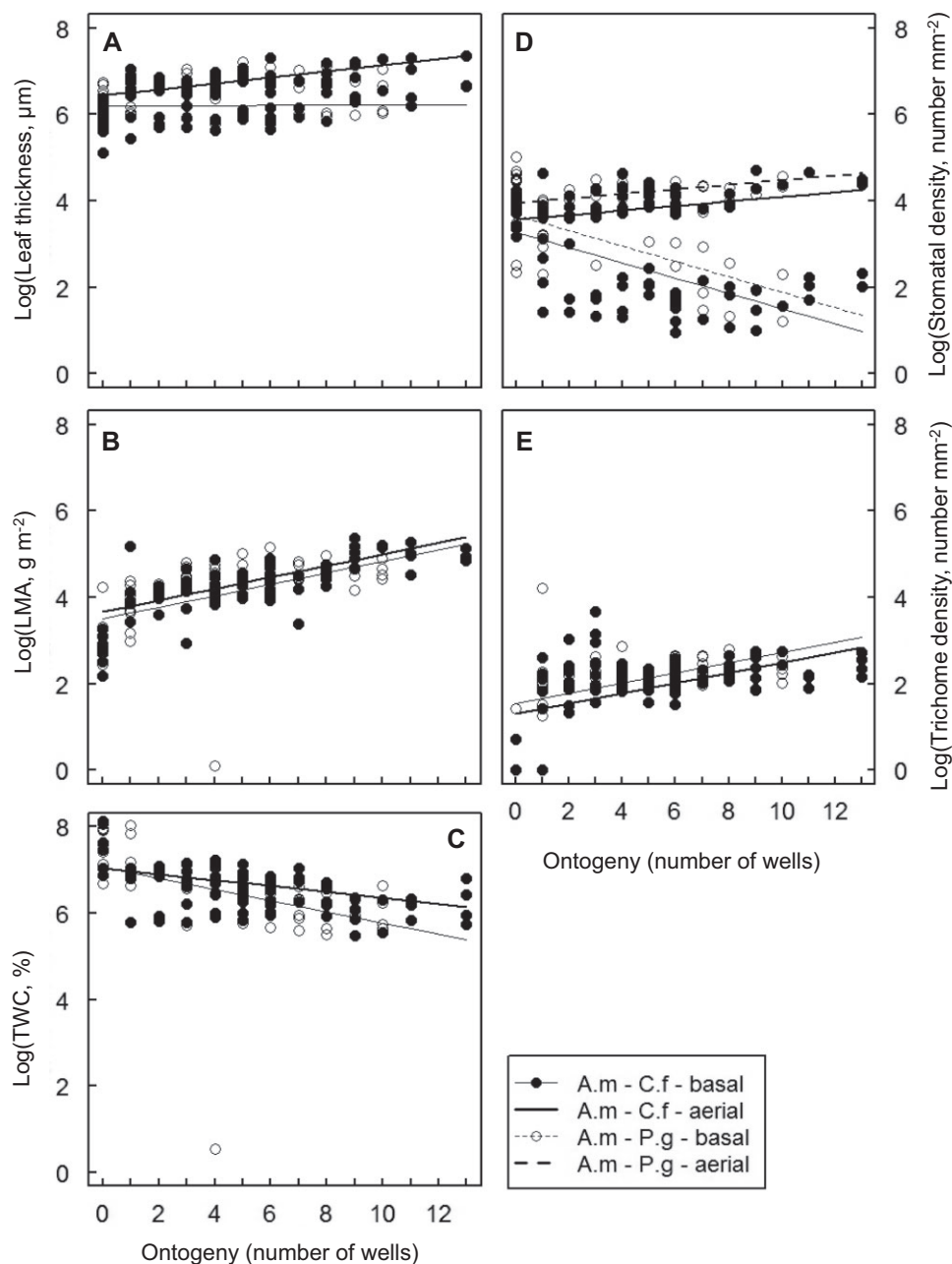


Figure 3. Variation in leaf thickness (μm) (A), leaf mass area (LMA; g m^{-2}) (B), total water content (TWC; %) (C), stomatal density (number per mm^2) (D), and trichome density (number per mm^2) (E) in relation to ontogeny (i.e. number of wells forming the tank) for *Aechmea mertensii* associated with *Camponotus femoratus* (filled triangles) or *Pachycondyla goeldii* (open triangles). Final linear mixed-effect model predictions are shown as solid lines for *C. femoratus*-associated *A. mertensii* and as dotted lines for *P. goeldii*-associated *A. mertensii* (for more information on the models, see Table 3). In the case of nonsignificant ant species terms, we have only included a single regression line.

A. mertensii living in large *C. femoratus*-AGs can intercept larger amounts of nutrients (i.e. nutrients leached from the overhanging trees or obtained through the decaying of trapped leaf litter and dead terrestrial arthropods) and are less limited by space than those associated with *P. goeldii* in small AGs.

Although it is difficult to establish the causes underlying some of the observed trends, others are apparent and may have an essential functional significance. The higher stomatal density and leaf C concentration in *P. goeldii*-associated bromeliads compared to those associated with *C. femoratus* are typically a response

Table 3. Models describing foliar traits with ontogeny (i.e. number of wells per tank), ant species, leaf part, and the different interactions involved

| | Estimates, mean \pm SE | d.f. | <i>F</i> | <i>P</i> | SDRa | SDRe |
|-----------------------|------------------------------------|--------------|---------------|--------------------|---------|------|
| Leaf thickness | | | | | 0.11 | 0.28 |
| Intercept | 6.17 \pm 0.09 | 1, 80 | 33423.1 | < 0.0001 | | |
| Ontogeny | 0.01 \pm 0.01 | 1, 36 | 12.04 | 0.002 | | |
| Ant species | | 1, 43 | 1.66 | 0.2 | | |
| Leaf part | 0.25 \pm 0.08 | 1, 80 | 158.04 | < 0.0001 | | |
| Ontogeny–Ant species | | 1, 36 | 2.03 | 0.16 | | |
| Ontogeny–Leaf part | 0.07 \pm 0.01 | 1, 80 | 29.56 | < 0.0001 | | |
| Ant species–Leaf part | | 1, 80 | 0.00 | 0.95 | | |
| Leaf mass area | | | | | 0.25 | 0.41 |
| Intercept | 3.62 \pm 0.15 | 1, 80 | 4604.64 | < 0.0001 | | |
| Ontogeny | 0.11 \pm 0.02 | 1, 36 | 54.07 | < 0.0001 | | |
| Ant species | | 1, 43 | 0.58 | 0.45 | | |
| Leaf part | 0.11 \pm 0.12 | 1, 80 | 6.58 | 0.01 | | |
| Ontogeny–Ant species | | 1, 36 | 0.94 | 0.34 | | |
| Ontogeny–Leaf part | | 1, 80 | 2.59 | 0.11 | | |
| Ant species–Leaf part | | 1, 80 | 1.85 | 0.18 | | |
| Total water content | | | | | 0.44 | 0.39 |
| Intercept | 7.01 \pm 0.17 | 1, 80 | 9817.02 | < 0.0001 | | |
| Ontogeny | –0.11 \pm 0.03 | 1, 36 | 28.39 | < 0.0001 | | |
| Ant species | | 1, 43 | 0.13 | 0.72 | | |
| Leaf part | 0.02 \pm 0.16 | 1, 80 | 7.8 | 0.007 | | |
| Ontogeny–Ant species | | 1, 36 | 1.46 | 0.23 | | |
| Ontogeny–Leaf part | 0.06 \pm 0.02 | 1, 80 | 6.18 | 0.02 | | |
| Ant species–Leaf part | | 1, 80 | 0.44 | 0.51 | | |
| Stomatal density | | | | | 4.3e–05 | 0.71 |
| Intercept | 3.13 \pm 0.17 | 1, 80 | 2582.87 | < 0.0001 | | |
| Ontogeny | –0.16 \pm 0.03 | 1, 36 | 10.75 | 0.002 | | |
| Ant species | 0.74 \pm 0.25 | 1, 43 | 9.92 | 0.003 | | |
| Leaf part | 0.44 \pm 0.2 | 1, 80 | 136.64 | < 0.0001 | | |
| Ontogeny–Ant species | | 1, 36 | 1.06 | 0.31 | | |
| Ontogeny–Leaf part | 0.22 \pm 0.03 | 1, 80 | 51.0 | < 0.0001 | | |
| Ant species–Leaf part | | 1, 80 | 2.31 | 0.13 | | |
| Trichome density | | | | | 0.23 | 0.54 |
| Intercept | 1.7 \pm 0.2 | 1, 80 | 569.35 | < 0.0001 | | |
| Ontogeny | 0.09 \pm 0.03 | 1, 36 | 23.15 | < 0.0001 | | |
| Ant species | | 1, 43 | 0.15 | 0.7 | | |
| Leaf part | –0.18 \pm 0.11 | 1, 80 | 18.15 | < 0.0001 | | |
| Ontogeny–Ant species | | 1, 36 | 3.16 | 0.08 | | |
| Ontogeny–Leaf part | | 1, 80 | 0.34 | 0.56 | | |
| Ant species–Leaf part | | 1, 80 | 0.42 | 0.52 | | |

The full models are presented, although only variables and interactions with $P < 0.05$ were interpreted as being statistically significant. The lines, highlighted in bold, indicate variables or interactions that appear in the final model. SDRa, standard deviation of the random effect (plants nested within ant gardens); SDRe, standard deviation of residuals.

to high light levels. Indeed, any increase in carbohydrate concentrations with higher light incidence can increase the differentiation of the stomata (Gay & Hurd, 1975), which improves CO₂ uptake (Evans, 1999). By contrast, the lower leaf $\delta^{15}\text{N}$ for bromeliads associated with *P. goeldii* rather reflects the nutrient availability linked to the mutualistic ant species

(Leroy *et al.*, 2009a, 2012). Considering these morphological and functional differences, further ecophysiological studies are needed to provide greater insight into the plastic response of *A. mertensii* as a function of ontogeny and mutualistic ant species.

Our results also clearly show that the traits studied change gradually but do not shift between two dis-

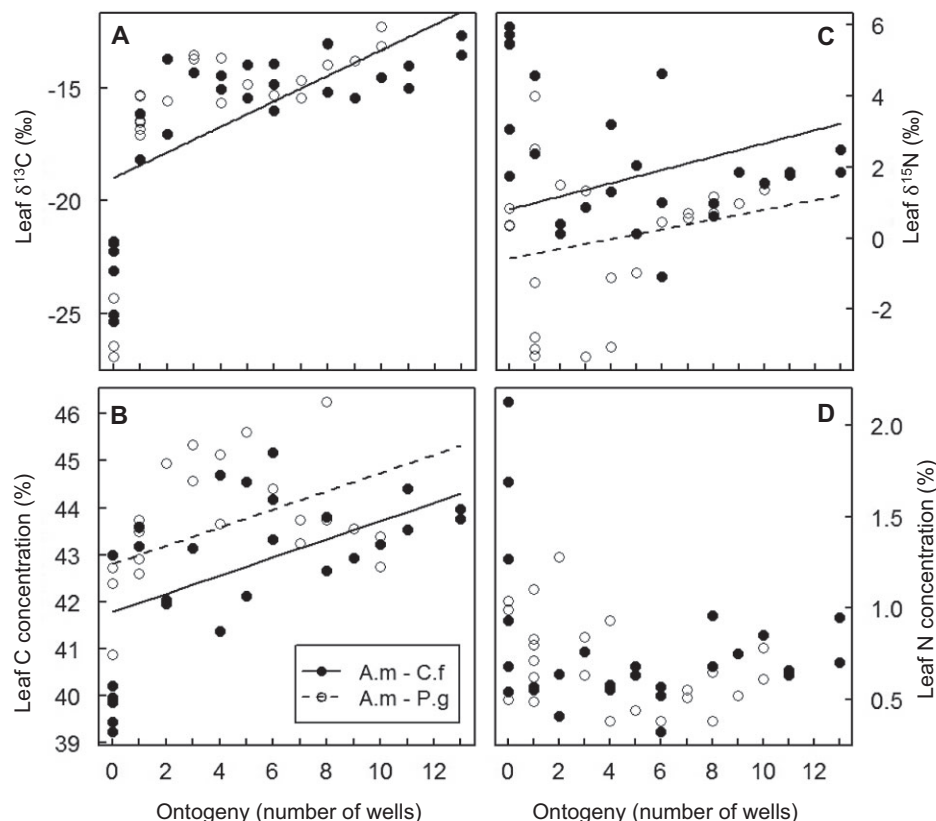


Figure 4. Variation in leaf $\delta^{13}\text{C}$ (‰) (A), leaf C concentration (%) (B), leaf $\delta^{15}\text{N}$ (‰) (C), and leaf N concentration (%) (D) in relation to ontogeny (i.e. number of wells forming the tank) for *Aechmea mertensii* associated with *Camponotus femoratus* (filled circles) or *Pachycondyla goeldii* (open circles). Final linear mixed-effect model predictions are shown as solid lines for *C. femoratus*-associated *A. mertensii* and as dotted lines for *P. goeldii*-associated *A. mertensii* (for more information on the models, see Table 3). In the case of nonsignificant ant species terms, we have only included a single regression line.

tinct ontogenetic phases, as was described in earlier studies on heteroblastic tillandsioid tank-bromeliads. Thus, *A. mertensii* might be qualified as a homoblastic bromeliad species. However, as recently noted by Meisner *et al.* (2013), a change from an atmospheric to a tank-bromeliad rarely concurs with changes in morphological, anatomical, and physiological traits. The low efficiency of small tanks may be the ultimate reason why many tank-bromeliads possess an atmospheric juvenile stage, which is assumed to be more drought-tolerant (Adams & Martin, 1986a, 1986b; Zotz & Hietz, 2001). Meisner & Zotz (2012) also suggested that heteroblasty might reduce self-shading in juveniles. If the juvenile tillandsioids are characterized by leaves densely covered by trichomes, the juveniles were totally devoid of them in the present study. Even without trichomes, however, *A. mertensii* seedlings did not suffer from drought because the total water content was high compared to the larger bromeliads. Water and nutrients were thus undoubtedly assimilated by the roots at the seedling

stage. This is consistent with the fact that, when a young *A. mertensii* seedling grows on a young AG (at the AG initiation stage), its roots not only have a role in anchoring the plant, but also in wrapping and reinforcing the walls of the ant nest (Corbara & Dejean, 1996). These walls are built with a 'carton', which may be considered as a hanging soil that provides nutrients to the roots. When the seedling grows on a mature AG (already harbouring mature epiphytes), its roots no longer play an essential role in the nest architecture; they anchor the young *A. mertensii* on the carton/soil of the AG where they can absorb water and nutrients. Moreover, in contrast to that found for other bromeliad species, leaf $\delta^{15}\text{N}$ values did not differ as a result of plant size, which also supports the idea that water and nutrients are absorbed by the roots of young *A. mertensii*. Indeed, the ontogenetic shift in N isotopic values from atmospheric juveniles to tank individuals was the result of N sources from atmospheric N deposition to soil-derived or tank-derived N (Hietz & Wanek, 2003;

Table 4. Models describing functional traits with ontogeny (i.e. number of wells per tank), ant species and the different interactions involved

| | Estimates, mean \pm SE | d.f. | <i>F</i> | <i>P</i> | SDRa | SDRe |
|----------------------------|------------------------------------|--------------|--------------|---------------|------|------|
| Leaf $\delta^{13}\text{C}$ | | | | | 0.98 | 0.57 |
| Intercept | -19.44 ± 0.92 | 1, 34 | 1244.04 | < 0.0001 | | |
| Ontogeny | 0.61 ± 0.14 | 1, 14 | 25.42 | 0.0002 | | |
| Ant species | | 1, 34 | 1.04 | 0.31 | | |
| Ontogeny–Ant species | | 1, 14 | 0.06 | 0.79 | | |
| Leaf C concentration | | | | | 0.87 | 0.35 |
| Intercept | 41.53 ± 0.42 | 1, 34 | 43286.94 | < 0.0001 | | |
| Ontogeny | 0.24 ± 0.06 | 1, 14 | 13.43 | 0.0025 | | |
| Ant species | 1.7 ± 0.69 | 1, 34 | 5.71 | 0.0225 | | |
| Ontogeny–Ant species | | 1, 14 | 1.53 | 0.23 | | |
| Leaf $\delta^{15}\text{N}$ | | | | | 1.73 | 0.43 |
| Intercept | 3.20 ± 0.56 | 1, 34 | 20.30 | 0.0001 | | |
| Ontogeny | -0.18 ± 0.08 | 1, 14 | 0.90 | 0.36 | | |
| Ant species | -3.73 ± 0.82 | 1, 34 | 18.02 | 0.0002 | | |
| Ontogeny–Ant species | 0.32 ± 0.14 | 1, 14 | 5.01 | 0.042 | | |
| Leaf N concentration | | | | | 0.18 | 0.09 |
| Intercept | 0.82 ± 0.11 | 1, 34 | 188.63 | < 0.0001 | | |
| Ontogeny | | 1, 14 | 1.88 | 0.19 | | |
| Ant species | | 1, 34 | 0.47 | 0.49 | | |
| Ontogeny–Ant species | | 1, 14 | 0.02 | 0.88 | | |

The full models are presented in the table, although only variables and interactions with $P < 0.05$ were interpreted as being statistically significant. The lines, highlighted in bold, indicate variables or interactions that appear in the final model. SDRa, standard deviation of the random effect (plants nested within ant gardens); SDRe, standard deviation of residuals.

Reich *et al.*, 2003; Zotz *et al.*, 2004). In the present study, the juveniles, which had similar leaf $\delta^{15}\text{N}$ to the adults, may obtain N from the plant fibres and ant remains in the AGs (Leroy *et al.*, 2009b). As the bromeliads grew, the number of wells and leaf trichomes increased, whereas the $\delta^{15}\text{N}$ remained constant. Assuming that roots of mature bromeliads are used to maintain position and do not play a significant role in plant nutrition (Nadkarni & Primack, 1989; Winkler & Zotz, 2009), we hypothesized that nutrient uptake might shift from the roots to the leaves once an individual of *A. mertensii* has reached a sufficient size enabling it to retain water and aquatic organisms. The differential morphological and functional capacities between the basal and apical leaf portions are progressively established as the plant grows. The basal part is involved in water and nutrient uptake (as indicated by the presence of trichomes), whereas the upper part is devoted to CO_2 acquisition and photosynthesis (as indicated by the presence of stomata; Freschi *et al.*, 2010).

Aechmea mertensii seedlings showed a lower C isotope composition (with C_3 -like $\delta^{13}\text{C}$ values) compared to adult tank-bromeliads (with CAM $\delta^{13}\text{C}$ values). However, $\delta^{13}\text{C}$ measurements alone are not sufficient to determine the photosynthetic pathway.

Other parameters, such as day/night fluctuations in titratable acidity, CAM enzyme activity, and diurnal patterns of gas exchange, are needed (Pierce, Winter & Griffiths, 2002; Silvera, Santiago & Winter, 2005; Winter, Garcia & Holtum, 2008). Thus, we cannot correctly determine whether the juvenile phase of *A. mertensii* is a C_3 pathway or a facultative CAM. Facultative CAM plants, also known as C_3 -CAM plants, have been identified in various CAM species (Griffiths & Smith, 1983; Lüttge, 1999). These plants are capable of conducting C_3 photosynthesis under favourable growth conditions and switching to a CAM mode when challenged by environmental constraints, such as limited water availability or excessive light incidence (Griffiths & Smith, 1983; Lüttge, 2006; Winter & Holtum, 2007; Freschi *et al.*, 2010). If these studies have demonstrated that the induction of CAM is exclusively under environmental control, the present study has allowed us to show, for the first time, an ontogenetic induction of CAM in Bromeliaceae. The shift from C_3 photosynthesis to CAM as leaves mature has also been detected in other families such as Aizoaceae (Adams *et al.*, 1998), Clusiaceae (Winter *et al.*, 2008), and Polypodiaceae (Martin, Allen & Haufler, 1995). For Adams *et al.* (1998), CAM must be viewed as a

developmental process for which the initiation, speed of transition and magnitude are governed by the environment. In *A. mertensii*, the shift from a C₃-like to a CAM pathway was abrupt and appears to occur with the development of the first tank-wells. Overall, this result provides additional evidence that C₃ and CAM need to be seen as a continuum, instead of two distinct states (Zotz, 2002).

The phenotypic plasticity of *A. mertensii* takes place in an ecological context in which the bromeliads are exposed to multifactorial environmental conditions (e.g. light, water, nutrients) and simultaneous interactions with many species (e.g. mutualistic ants, phytotelm-dwelling organisms). If *A. mertensii* did not show an abrupt morphological modification, as do heteroblastic plants, we found strong size-related functional modifications for CO₂. The adaptive phenotypic variation between the two possible associations with ants indicated the spatially conditioned plasticity of *A. mertensii* in the context of insect-assisted dispersal. However, ant-mediated effects on *A. mertensii* phenotypic plasticity were not obvious because ant species and the light environment are two confounding variables. Thus, future reciprocal transplants and cultivation in a controlled environment will be needed to improve our understanding of which biotic and abiotic factors might be the most relevant force driving phenotypic plasticity.

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