

November 2000

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Effects of microhabitat on morphological adaptations of *Pleurothallis sanchoi* (Orchidaceae)

B.J. Labay

Department of Evolution, Ecology, and Behavior, University of Texas at Austin

ABSTRACT

An abiotic gradient of wind, moisture and sun across a tree may directly affect morphological traits adapted for water conservation. In this study, *Pleurothallis sanchoi* was examined in a pasture near the Estación Biológica Monteverde, Costa Rica. I looked at how microhabitats affect morphological adaptations, including leaf thickness, toughness and stomata density. I tested two predictions of my hypothesis that individuals living in harsher conditions would show more adaptations for water conservation. The first is that individuals in an abiotic gradient along a tree will show variance in water conservation adaptations. The second is that in two distinct habitats, full sun, and full shade, I will find individuals in the harsher, full sun habitat to show more strategies against water loss. I found that individuals in trees vary greatly their adaptations for water conservation depending on microhabitat. I found that mean stomata density decreased in harsher, full sun habitats, and that thickness and toughness generally both increased in harsher, full sun habitats. The increase in stomata density and decrease in both thickness and toughness in full shade habitat is attributed to the reduced need to fight desiccation.

RESUMEN

Las condiciones abióticas como el sol, temperatura, nutrientes y agua disponible en un árbol, pueden afectar las estrategias de adaptación de las epífitas. En este proyecto se estudió *Pleurothallis sanchoi* (Orchidiaceae) en un potrero cerca de La Estación Biológica Monteverde, Costa Rica. Mire los microhabitats y como estos afectan las adaptaciones como dureza, grosor y el promedio de estomas. Estudie dos predicciones, que individuos viviendo en condiciones mas extremas van a mostrar más adaptaciones para conservación de agua. El primero es que *P. sanchoi* va a variar las adaptaciones de conservación de agua en partes diferentes del árbol. El segundo es que dos hábitats diferentes, uno en el sol y el otro a la sombra, voy a encontrar *P. sanchoi* mostrando mas adaptaciones para conservación de agua en el hábitat con mas sol. Encontre que los individuos en el árbol varían las adaptaciones dependiendo del microhabitat. Encontre que el número de estomas decrece en el hábitat expuesto al sol y que el grosor y dureza aumentaron en el hábitat expuesto al sol. Mis resultados mostraron que los individuos en microhabitats más drásticos presentan más recursos para conservación de agua.

INTRODUCTION

Pounds et al (1999) states that recent warming has caused changes in species distribution and abundance, but the extent of the effects is unclear. In the highland mountains at Monteverde, Costa Rica, Pounds et al (1999) investigated the biological and climatic patterns that suggested that atmospheric warming has raised the average altitude of the cloudbank, decreasing wind-driven mist during the dry season. Cloud cover and wind driven mist are more important to epiphytes than is rainfall (Grubb et al 1963 in Janzen 1983, Grubb and Whitmore 1966 in Janzen 1983, Sudgen and Robins 1979 in Janzen 1983), and during the dry season, can be an epiphyte's major source of water.

As global warming and human-impacted ecosystems become increasingly important to ecology and diversity, patterns of desiccation in plants have become an indicator for ecologists to assess changes in weather and determine threat of ecosystem decline. Harsher conditions favoring desiccation should favor plants with morphological traits that help prevent excessively high rates of transpiration. This allocation to water conservation may decrease allocation of resources to reproductive efforts (Kinziger 2000), which then could affect population demographics. In this physiological perspective, morphological traits that are linked to temporal variation in the levels of environmental factors can limit plant production (Van Schaik 1993).

Abiotic factors such as wind, precipitation and sunlight may affect morphological strategies of epiphytic orchids. Desiccation is a challenge presented by these abiotic factors that orchids confront with adaptations including pseudobulbs, succulent leaves, velamen, CAM photosynthesis, and mycorrhizal relationships. It has been frequently thought that water availability is both the proximate and ultimate factor controlling the phenologies and morphologies of many tropical forest plants (Reich 1984 in Van Shaik et al. 1993).

The morphology of tropical epiphytes has been shaped by abiotic selective factors. This study considers the Pleurothallidinae, one of the largest sub tribes of orchids and one that dominates the montane cloud forests of Costa Rica. Pleurothallids lack pseudobulbs, and therefore must rely on other means to prevent desiccation. The research here focuses on morphological variation of *Pleurothallis sanchoi* in water conservation strategies to determine how and if abiotic conditions affect individuals in a gradient of microhabitats. In this study it was expected that individuals found in harsher habitats would tend to allocate more resources to water conservation.

METHODS

My research was conducted in Monteverde, Costa Rica in the pasture and surrounding forest behind the Estación Biológica Monteverde from October 19th to November 16th of 2000. Two separate predictions were tested. First I sampled two *Daphnopsis americana* (Thymelaeaceae) trees for *P. sanchoi* in three zones and all compass quadrants. The quadrants were from north to west, north to east, south to west, and south to east on the tree, and I defined these as quadrants one, two, three and four respectively (Figure 1). I defined tree zones in a way to get a high variation in abiotic factors throughout microhabitats. Zone one is the bole of the tree up to the basal part of the first branches, zone two is the basal part of the first branches one foot up, and crotches made by the first major branches. Zone three is the first third from initial branching of the canopy (Figure 2). I sampled zones one through three, making sure to not what quadrant each sample was found. *Daphnopsis americana* is common in pastures and is a mature forest gap species at 1300-1600m (Haber et al 1996). I sampled nine leaves for stomata readings, and six leaves for toughness and thickness in each quadrant in all three zones. I sampled one to two leaves per individual, depending on the abundance of individuals found in quadrants. Only entire, mature leaves with little or no herbivory were sampled. I defined leaf maturity as darker green, larger leaves.

I tested my second prediction by collecting leaves from individuals in extreme conditions designated as complete shade or complete sun. The two categories represent either the individual being in deep shade or full sun for the majority of the day. I collected a single leaf from each of 58 individuals in shade and 47 individuals in sun. Stomata density readings, mean thickness and toughness were measured.

I applied a one-centimeter strip of clear nail polish to the abaxial side of the basal half of each leaf between the mid vein and the outer margin. After the nail polish had dried the peel was removed and viewed under a 40X compound microscope and three density readings were taken for each peel. Density was determined as the number of stomata visible in the field of view of the microscope. Thickness was measured with a caliper in the middle of the leaf. Two measurements were recorded and later averaged. Toughness was measured using a penetrometer, calculating how much weight was required to puncture the leaf.

A two –way ANOVA was used to test for differences in stomata density, thickness, and toughness between quadrants and zones in each tree separately. A chi-squared test was performed to test for habitat preference. An unpaired t-test was used to test for differences in stomata density, leaf thickness and toughness in shade versus sun habitats.

RESULTS

In the methodology of tree microhabitat comparisons, a total of 206 individuals were sampled on two trees, 166 on tree one and 86 on tree two. There was not a significant difference in zone or quadrant preference or frequency of occurrence on either tree, (chi-squared test, tree one: $X^2=0.7724$, $0.995 < p < 0.975$).

Orchids in tree one and two differed in the traits that responded to zone and quadrant differences. On tree one stomata density showed a significant difference in zone and quadrant, but did not have a significant difference in zone*quadrant interactions (Figure 3a). Toughness in tree one did not show a significant difference over zone, quadrant or zone*quadrant interactions (Figure 4a). Leaf thickness in tree one did not differ significantly between quadrants or zone*quadrant interactions, but did differ significantly between zones (Figure 5a). Leaves in zone one were considerably thicker than zone three leaves. In tree two, stomata density did not show a significant difference between zone or quadrant, but did show a significant difference between zone*quadrant interactions (Figure 3b). There was a significant difference between quadrants, but not in zones or zone*quadrant interactions (Figure 4b). Leaf thickness did not differ significantly between zones, quadrants, or zone*quadrant interactions (Figure 5b).

In testing my second prediction, I used an unpaired t-test to determine differences in stomata density, toughness and thickness between full sun and full shaded habitats. Stomata density showed a significant difference between habitats ($p < 0.0001$, $df = 308$, $t = 11.4$). Mean stomata density for orchids in shade was 8.6 and for sun 6.5. Toughness between habitats showed a significant difference ($p < 0.0001$, $df = 100$, $t = -9.3$). Mean toughness for shaded individuals was measured at 550.5 grams, while individuals in complete sun measured at 732.3 grams. Thickness in different habitats differed significantly also ($p < 0.0001$, $df = 100$, $t = -20.5$). Mean thickness in full shaded individuals was 0.9mm, while individuals in complete sun had a mean thickness of 1.9 mm.

DISCUSSION

Epiphytes characteristically live in areas with sharp gradients of photon flux density, water availability, and nutrient availability (Rundel and Gibson 1996). These gradients are caused by microhabitat differences in abiotic factors and resource availability. This can affect plant size and morphological characteristics. Light availability has been directly correlated with leaf size and shape, abundance, and other morphological traits (Murren and Ellison 1996). Microhabitat effects can be seen in epiphytes growing in varying locations on a tree. Johansson (1975) has identified five distinct zones within

trees, which epiphytic orchids are typically found, and each zone can be characterized by a suite of abiotic factors. Because of this, special distribution of orchids on trees should differ across different areas and quadrants of trees. This was not supported from my results since my chi-squared test of habitat preference was not significant. *Pleurothallis sanchoi* showed no preference for tree zones or quadrants in the two trees studied. This suggests that *P. sanchoi* can survive along a gradient of abiotic conditions, and is relatively insensitive to small scale variations in physical conditions. The adaptations tested might be the mechanism that enables *P. sanchoi* to live in different tree zones and quadrants.

Wheeler (1998) stated that there is a difference in stomata densities among plants as you move from shade to sun. Morse (1999) showed that *Psychotria monteverdensis* had significantly greater stomatal density in the secondary forest as opposed to the edge habitat. Epiphytes must continue to adjust with adaptations such as stomata density to dryer and wetter conditions over the course of their lives (Fletcher et al. 1994). Fletcher et al. (1994) states that it is not because these changes are significant, it is the differences that can arise between individuals of the same species occupying slightly different microclimate habitats. Therefore, morphological traits that conserve water should vary across zones and quadrants in a tree. My results supported this in certain ways and did not in ways. *Pleurothallis sanchoi* showed a difference in stomata density in zones and quadrants in tree one. Individuals in zone one and three showed lower stomata density than zone two. This suggests that zones one and three experiences harsher conditions, i.e., more sun and wind, and *P. sanchoi* tends to lower stomata density to avoid high levels of transpiration that would lead to desiccation. In the north to west quadrant, stomata density was lower compared to all other quadrants. This is attributed to more wind and sunlight from the north to west, creating a harsher environment. Toughness on tree one did not show differences among zones or quadrants, which would indicate either a lack of abiotic conditional gradient on the tree, or that other non-abiotic factors, such as herbivory may affect toughness. On tree one I found thickness to differ significantly between zones. Zone one individuals had thicker leaves than did zone three, showing higher water storage in zone one. This indicates harsher conditions in zone one compared to zone three. It would seem logical that in a forest, with lower zones protected from wind, harsher conditions would tend to be higher up in the canopy. Although in a pasture, the wind and sun can easily reach the bole, and in fact might do so much more depending on the position of surrounding trees and forest edge. These factors could lead to zone one experiencing harsher conditions.

These comparisons of stomata density, toughness and thickness in tree one suggests that zone one experiences the harshest conditions followed by zone three, then

zone two. This is shown by zone one individuals having the lowest stomata density and the highest leaf thickness, followed by zone three, and then zone two. Leaf toughness, however, does not vary by microhabitat and thus suggests little about abiotic variation.

In the second *D. americana*, stomata did not vary between zones or quadrants, but there was a zone*quadrant interaction in zone three. Quadrants one and two showed lower stomata density than quadrants three and four. This adaptation differences indicates an allocation to conserve water in response to harsher conditions such as wind and sun coming from the Northeast and Northwest, which are quadrants one and two respectively. Individuals in quadrant four of every zone showed tougher leaves than any other quadrants. This, contrary to the deductions from the stomata density differences, indicates harsh conditions from south to east. This could be the result of differences in abiotic conditions as individuals developed on the tree. Environmental conditions might have changes, getting worse after orchids have already allocated resources and determined their morphology. Plants might not be able to add more stomata after maturation of the leaf, while they could always add material to make their leaf tougher. Thickness of leaves on tree two showed no variation in response to microhabitat turnover.

The differences in patterns of morphological adaptations between the two *D. americana* can be explained by differences in tree microhabitat within the pasture. The trees may each experience unique conditions in the pasture including wind variance between canopy and bole, differences in light penetration to the bole and among the trees limbs. *Daphnopsis americana* losses its leaves yearly from April to May due to caterpillar herbivores (Haber et al. 1996), and this loss of canopy might affect light penetration into the trees differently dependent on their position near other trees and forest edge. These differences change the abiotic gradient among trees enough to change the adaptations needed to survive.

If exposure to sun increases dessication, then *P. sanchoi* should show water conservation strategies. The results of testing my second prediction supported this, and I found that the individuals in harsher, complete sun habitats allocated more resources to water conservation. Individuals demonstrated this by having more succulent leaves, lower stomata counts, and greater leaf toughness in habitats exposed to greater levels of sun and wind. These physiological adaptations may allow the plant to survive in harsher, drier microhabitats. The increased leaf thickness in individuals in fully exposed habitats demonstrates a morphological strategy to conserve water. The decreased stomata density suggests a strategy to fight excessive transpiration due to the increased sun and wind on fully exposed parts of trees. The increased leaf toughness in full sun shows an adaptation to provide rigidity to the leaf so it can support itself against the wind.

Global warming is affecting ecosystems all over the world, and plants are experiencing warmer, dryer conditions. My results demonstrate that orchids can employ adaptations for water conservation that would help fight desiccation, but phenotypic plasticity is not infinite. There will be limits to conditions under which orchids can germinate, grow and reproduce. Orchid demographics can be affected greatly by changing weather conditions. By determining the threshold conditions under which an orchid can be successful, we can predict what changes will occur and what precautions need to be taken to prevent population crashes.

It would be interesting in the future to look at physiological processes that lead to morphological trait differences such as tougher leaves or denser stomata for understanding desiccation and its causes, and to attain a wider biological perspective. Also it would be important to understand physiological mechanisms such as CAM photosynthesis, which is used for water conservation.

ACKNOWLEDGEMENTS

Karen Masters deserves much praise and recognition for her plethora of orchid knowledge, her great advice and guidance, and her never-ending supply of excitement and enthusiasm. Thanks to Gabriel Barboza and his orchid garden in helping me identify orchids and for his great generosity and willingness to help. Thanks to the Andrew and Tim for their support and patience. Thank you Arnoldo Beche for allowing me to study in your beautiful pasture. My study was made much more pleasure with the warmth and friendship, food and support of Nena and Eloy Jimenez. Finally I would like to thank the Estación Biológica Monteverde and its staff for the use of facilities and their efforts in education.

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Table 3: Two-way ANOVA testing the effects of stomata density between zones, quadrants, and zone*quadrant interactions in (a) tree one, and (b) tree two.

(a)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	2	82.451	41.225	9.848	<.0001
Quadrant	3	65.861	21.954	5.244	0.0015
Zone*quadrant	6	14.907	2.485	0.594	0.7355
Residual	312	1306.074	4.186		

(b)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	2	19.056	9.528	1.812	0.1651
Quadrant	3	22.256	7.419	1.411	0.2396
Zone*quadrant	6	228.623	38.104	7.245	<.0001
Residual	312	1640.815	5.259		

Table 4: Two-way ANOVA testing the effects of toughness between zone, quadrant and zone*quadrant interactions in (a) tree one, and (b) tree two.

(a)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	2	49375.694	24687.847	2.303	0.1087
Quadrant	3	14004.167	4668.056	0.435	0.7284
Zone*quadrant	6	85560.417	14260.069	1.33	0.2579
Residual	60	643191.667	10719.861		

(b)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	3	1013.194	506.597	0.058	0.9435
Quadrant	2	130795.833	43598.611	5.01	0.0036
Zone*quadrant	6	41364.583	6894.097	0.792	0.5797
Residual	60	522175	8702.917		

Table 5: Two-way ANOVA testing the effects of thickness between zones, quadrants and zone*quadrant interactions in (a) tree one, and (b) tree two.

(a)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	2	0.841	0.421	3.764	0.0288
Quadrant	3	0.696	0.232	2.076	0.1129
Zone*quadrant	6	0.545	0.091	0.813	0.5641
Residual	60	6.706	0.112		

(b)	DF	Sum of Squares	Mean Square	F-Value	P-Value
Zone	2	0.181	0.09	1.293	0.2819
Quadrant	3	0.369	0.123	1.758	0.1648
Zone*quadrant	6	0.432	0.072	1.031	0.4143
Residual	60	4.195	0.07		

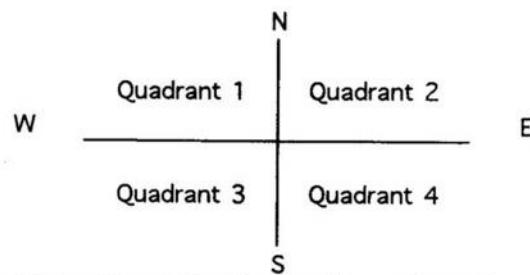


Figure 1: Quadrant designations on the two *Daphnopsis americana* trees.

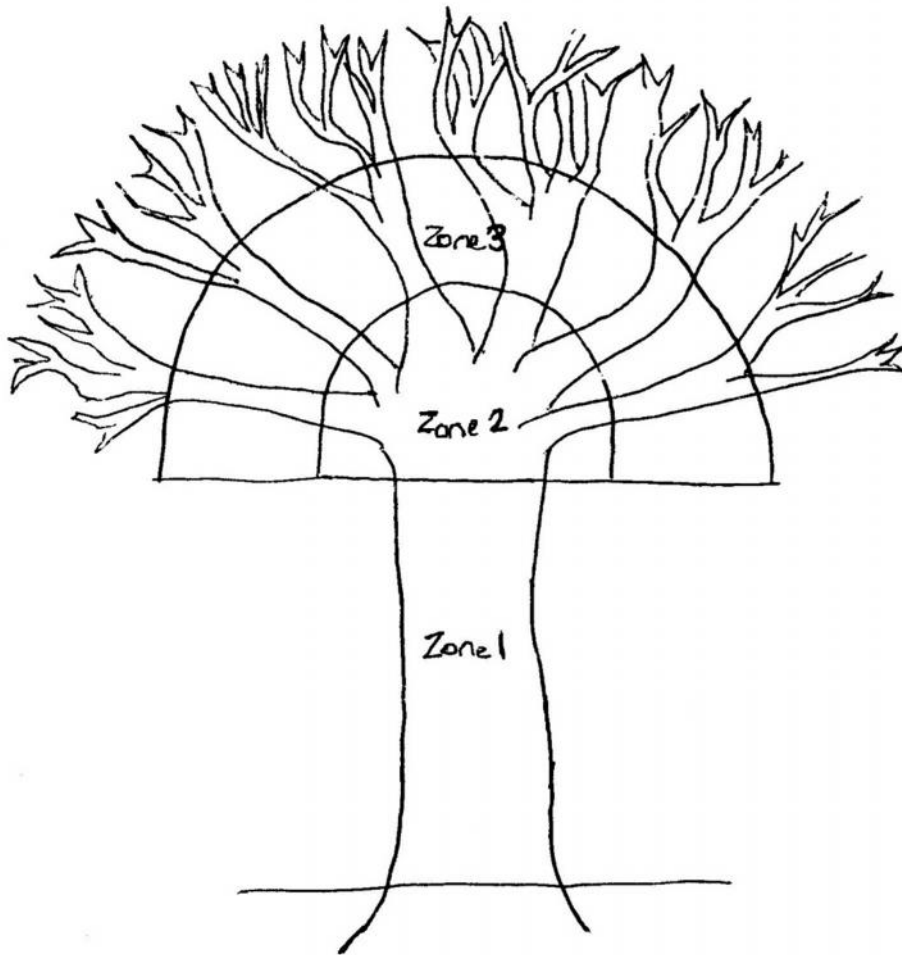


Figure 2: Tree zone designations. Zone 1: the bole up to the major branching; Zone 2: the basal part of the large branches and out a foot from branching; Zone 3: first third of canopy from the first major branching.