Stomata Densities of Pleurothallids (Orchidaceae) in Different Microhabitats of Monteverde

Chelsea Beebe

Department of EPOBiology, University of Colorado-Boulder

ABSTRACT

The purpose of this study was to test whether there is a difference in the number of open and closed stomata on the northeast and southwest facing sides of trees among seven species of Pleurothallids and one pseudobulb species. Stomata impressions were taken from the leaves using clear finger nail polish and they were then examined under a microscope. The results showed that there was a significant difference between the number of open and closed stomata for every species, always with a higher frequency of closed stomata. Significant affects of species type and position on the tree on stomata density was also found with branch tip orchids having a lower stomata density then non-branch tip species. A possible explanation for the low frequency of open stomata is that they might only open in the rain, when water loss is at a minimum.

RESUMEN

El propósito de este estudio debía probar si hay una diferencia en el número de abierto y cerró stomata en el noreste y el suroeste frente a lados de árboles entre siete especies de Pleurothallids y una especie de pseudobulb. Las impresiones de Stomata se tomaron de las hojas que utilizan el esmalte para las uñas claro de dedo y ellos entonces fueron examinados abajo un microscopio. Los resultados mostraron que había una diferencia significativa entre el número de abierto y cerró stomata para cada especie, siempre con una frecuencia más alta de cerró stomata. Los afectos significativos de tipo de especie y la posición en el árbol en la densidad de stomata se encontró también con orquídeas de punta de rama, teniendo una densidad más baja de stomata entonces no la especie de punta de rama. Una explicación posible para la frecuencia baja de stomata abierto es que ellos pueden solo abierto en la lluvia, cuando la perdida de agua está en un mínimo.

Introduction

The family Orchidaceae is a large and very diverse group among the Angiosperms with between 20,000 and 25,000 species in 725 genera (Dressler 1981). With approximately 1,200 species in Costa Rica, it is the largest group of flowering plants (Hartshorn 1983; Walter 1986). The majority of orchid species are epiphytic (Walter 1986). Epiphytes are more exposed to harsh conditions and thus require specific abiotic and biotic factors for successful growth. One necessary abiotic, factor is a high amount of continuous moisture. With 2500-3500 mm of rainfall annually, as well as a frequent misty periods, the cloud forest of Monteverde, Costa Rica, is a very hospitable habitat for epiphytic orchids (Haber

2000). Orchid diversity is especially high in this type of ecosystem (Dressler 1993). The Monteverde trade winds blow from northeast to southwest most prominently from November through January and more moderately from February to April (Clark et al. 2000). This wind has both positive and negative effects on epiphytic growth, bringing with it either high amounts of mist or drying winds.

In desiccating environments, plants have evolved many adaptations to protect themselves from dehydration. One such adaptation is Crassulacean acid metabolism (CAM) photosynthesis. In this process, plants open their stomata, the structures that regulate gas exchange and water up take, at night instead of during the day (Raven and Johnson 1986). This method of carbon fixation accumulates C_4 compounds at night, allowing the plants to enter the Calvin cycle during the day and thus minimize water lost to harsh daytime environmental factors (Raven and Johnson 1986). Stomata densities are not uniform throughout a species. Different leaves of the same species can have different stomata densities depending on their ages as well as the conditions of the microhabitat into which the leaf was born (Chicurel 2000). Since the loss of water through the stomata is proportional to the stomata density, a leaf born in a drier environment will likely have lower stomata density in order- to limit the loss of water- to such factors as wind, intense UV light, and increased daytime temperatures (Fitter and Hay 1987).

The sub-tribe Pleurothallidinae occurs in cloud forests in great diversity (Dressler 1993). This group is often epiphytic and can be distinguished by their fleshy leaves, thick cuticle, often, sunken stomata, lack of pseudobulbs, and limited storage tissue for water (Walter 1986; Dressler 1993). They are most common in mid to high elevation areas and are thought to be a very vulnerable group to drought and other microhabitat variation (Walter 1986; Frohlich 2002). Due to their lack of pseudobulbs and limited storage tissue, these orchids rely heavily on the rains and misty conditions of the cloud forest for survival. In a study done on mist variation and epiphytic orchid abundance, it was found that Pleurothallids were most abundant where the daily mist frequencies were the highest and most frequent (Frohlich 2002).

This study tested the whether there was a higher frequency of stomata open at night versus the day on the northeast facing side of the tree or the southwest facing side of the tree for seven species of the sub-tribe Pleurothallidinae, and one pseudobulb species. On the postulation that Pleurothallids utilize CAM photosynthesis due to their lack of water storage structures, a higher frequency of open stomata at night was anticipated. Likewise there was a predicted higher frequency of open stomata in the day for the pseudobulb species because they are not limited by water availability, due to their morphological adaptations. Within these results, there was also anticipated a higher number of open stomata on the southwest side of the tree due to their protected location from the northeast trade winds.

Materials and Methods

This study was conducted in a field of secondary growth and open space behind the Estación Biológica in Monteverde, Puntarenas, Costa Rica. The elevation is approximately 1580m above sea level. Eight species of epiphytic orchids, seven from the family Pleurothallidinae including *Pleurothallis sanchoi, Lepanthis pygmeae,*

Speklinia aristata, Pleurothallis tuerckheimii, Lepanthes monteverdensis, Lepanteis sp., and *Masdevallia nidifica* and one species of pseudobulb orchid were chosen for the study. The samples were taken from the northeast and southwest facing sides of the lower two meters of the trees. Twenty leaves from each direction were taken in the day between the hours of 9:00a.m. and 2:00p.m., and at night between the hours of 8:00p.m. and 1 1:00p.m. The six trees from which the samples were taken were chosen based on a mist study previously performed in the same field by Hanna Meisner-Bagdahn in 2003. Her study measured the effects of mist frequency on Pleurothallid abundance and survival. Based on these data, I chose trees from that study that all had more misty days on the north facing side of the tree as compared with the south facing side. These trees are located along the western and northwestern sides of the field.

Impressions of stomata were taken using clear finger nail polish and applying a small amount to the abaxial side of each orchid leaf. After sufficient drying time, the finger nail polish was removed with tweezers and placed in an envelope for transport. Each sample was then placed under a compound microscope at 400x magnification and viewed in three randomly selected locations. At each location the number of open and closed stomata was counted and recorded.

A 2-way ANOVA test was performed on the data to measure the effects *of* species type and position on the densities of stomata. The data from each species from the northeast and southwest and day and night were then summed and analyzed using a 2x4 Contingency test.

Results

After running a 2-way ANOVA (independent variables = species and position; dependent variables = number of stomata), I found a significant effect ($P = 0.0307$) of species on number of stomata as well as a significant interaction effect (Table 1 and Fig. 1). I also ran a Fisher's PLSD post-hoc test and found many species pairs to be significantly different (Table 2). I then ran a 2x4 contingency test on the summed data of open and closed stomata from each species and found a significant difference between the north and the south sides for every species (Table 3 and Fig. 2).

Discussion

The results (Table 1 and Fig. 1) showed that the species of orchid has a significant effect on the stomata density of each leaf. There are also a number of significant differences between pairs of species. There is a significant difference between *P. sanchoi* and M. nidifica and all other Pleurothallids. This can be explained in a number of ways. All the other Pleurothallids here, except for *P. tuerckheimii,* are considered branch tip species of orchids. This means that, in the canopy where orchids are more common, these species are usually found on the very tips of the host branches (K.L Masters, 2003, pers comm.). Here, the orchids are exposed to more wind and sunlight than in other areas where non-branch tip orchids such as *P. sanchoi* and *M. nidifica* generally grow. Branch tip micro-habitats, which are potentially be more desiccating, would be likely to have orchids with lower stomata density than the stomata density of orchids found else where on trees.

There was a significant difference between the total number of stomata on the pseudobulb species and the other Pleurothallids. Pseudobulb orchids can store water for the plant in their enlarged stems. This constant source of water availability protects the plant from desiccation and thus allows it to have more stomata. There was also a higher frequency of open stomata on the southwest side of the host tree. These data suggest that the pseudobulbs species could possibly be more susceptible to the trade winds and thus has a higher frequency of stomata on the more protective southwestern side of the tree.

The comparison of the frequency of open and closed stomata in the night and day and on the northeast and southwest side of the tree yielded a nonrandom pattern. Specifically, more stomata were closed then was expected by chance, for both the night and day and the northeast and southwest aspect. These results were true for both the Pleurothallids and well as the pseudobulb species. This means that the stomata of both groups generally keep their stomata closed regardless of their aspect on the tree or the time of day. This may suggest the importance of water conservation to all orchids, since the high frequency of closed stomata was dramatic. Reasons for the abundant closure of stomata may be explained by a great need to avoid water loss during evapotranspiration. Perhaps they only open their stomata when there is abundant moisture in the air, i.e. when it is raining or heavily misting. Since it is difficult to take good samples in the rain, all night samples were taken in dry weather thus excluding any raining nights. Also, according to Dressler, the CAM system is very flexible, allowing stomata to open in the day in times of moisture abundance, i.e. the wet season in Monteverde. This might have been missed due to infrequent sampling. Further studies need to be preformed to answer this puzzling question.

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Table 1. *ANOVA table showing effects of orchid species and position (northeast and southwest) on number of stomata on orchid leaves.*

	DF	F-Value	P-Value
Species	7	39.162	<.0001
Position	1	2.903	0.0895
Species by position	7	2.244	0.0307
Residual	304		

Table 2. *Pairs of significantly different species in terms of stomata density.*

Species	1	2	3	4	5	6	7	Pseudobulbs
P. sanchoi		*	*	*	*	*	X	*
L. pygmeae	*		*	*	X	X	*	*
S. aristata	*	*		X	X	X	*	*
P. tuerckheimii	*	*	X		X	*	*	*
L. monteverdensis	*	X	X	X		X	*	*
Lepanthes sp.	*	X	X	*	X		*	*
M. nidifica	X	*	*	*	*	*		*
Pseudobulb	*	*	*	*	*	*	*	

[a]* = significantly different pair

[b]X= not significant pair

Table 3. *Comparisons of the difference in the number of open and closed stomata on eight species of orchids.*

Species	X^2	DF	P-Value
P. sanchoi	26.922	3	<.0001
L. pygmeae	10.230	3	<.0001
P. tuerckheimii	18.621	3	<.0001
S. aristata	10.230	3	<.0001
L. monteverdensis	46.853	3	<.0001
Lepanthes sp.	52.992	3	<.0001
M. nidifica	64.643	3	<.0001
Pseudobulb	49.763	3	<.0001

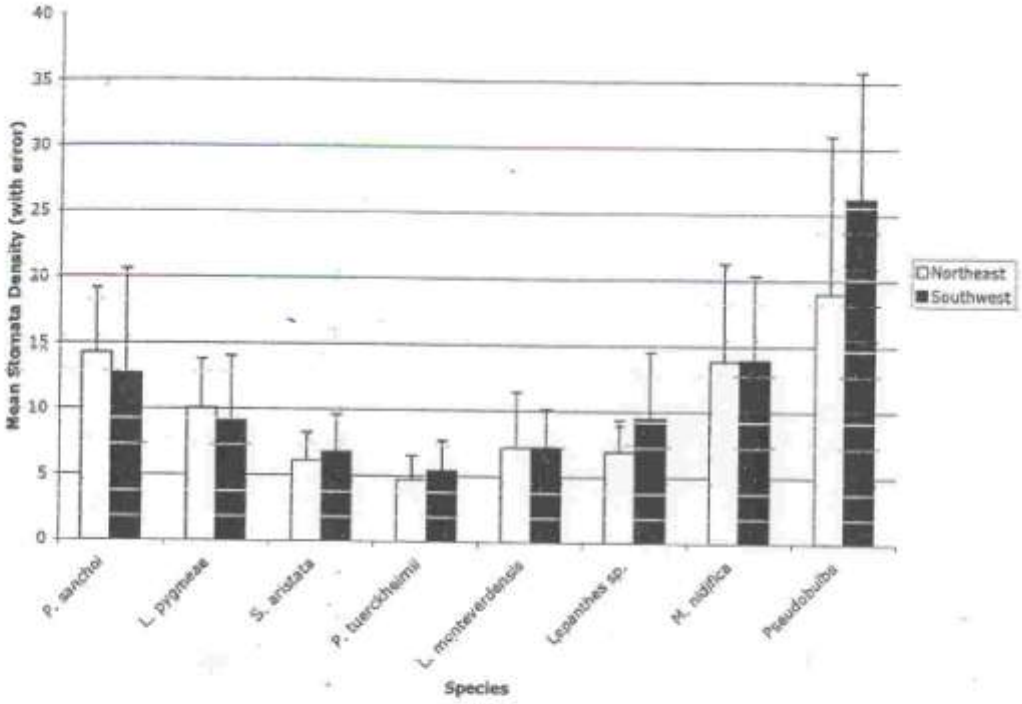

Figure 1. Mean number of stomata on the northeast and southwest side of the tree for eight species of orchids
(*P. sanchoi* = non-branch tip; *L. pygmea* = branch tip; *S. aristata* = branch tip; *P. tuerckheimii* = non branch tip; *L. monteverdensis* = branch tip; *Lepanthes sp.* = branch tip; *M. nidifica* = non branch tip). N = 20 per direction.

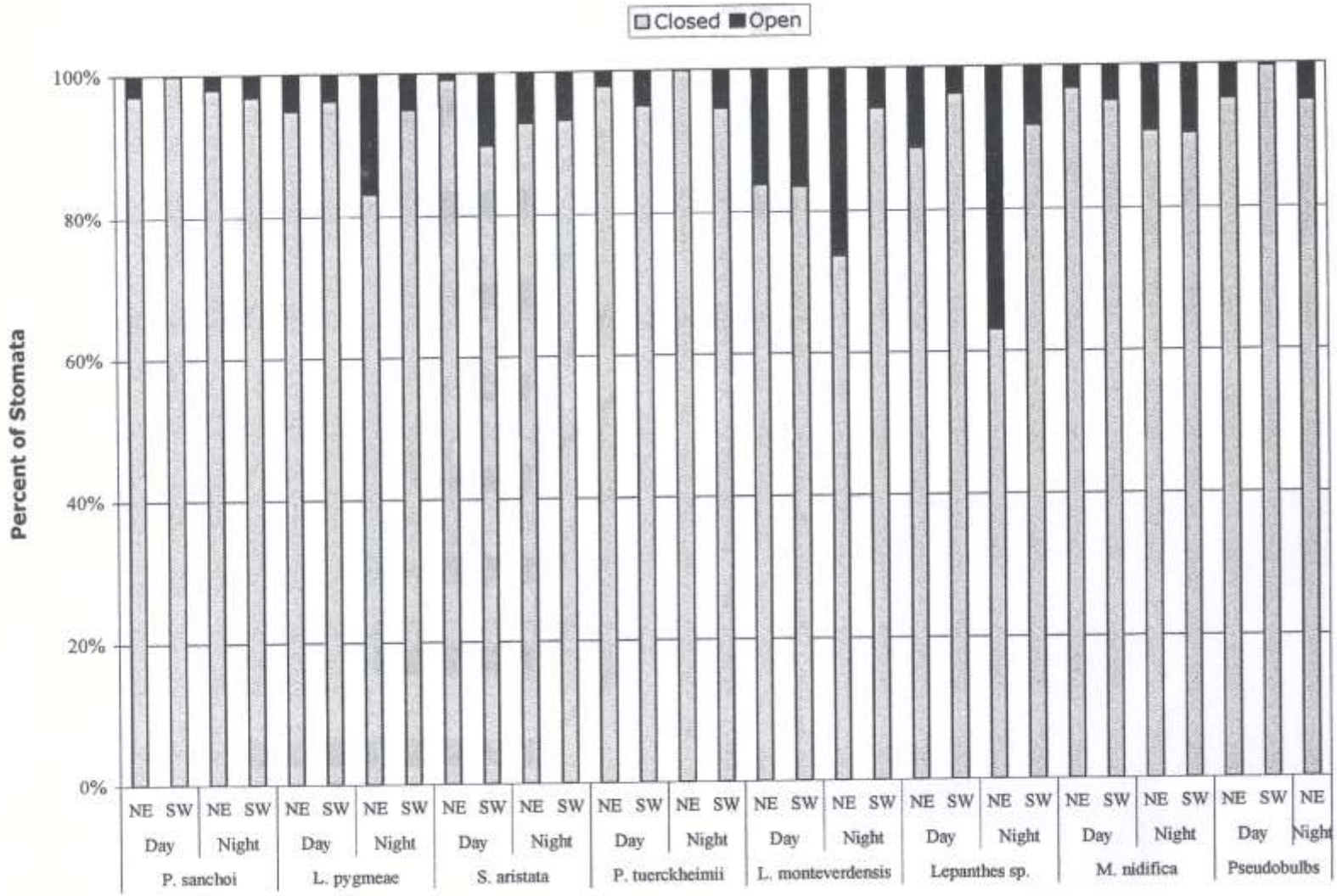

Figure 2. Percentage of open and closed stomata for all orchid species on the NE and SW aspect for the night and day.