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Stomata Density of Orchids and Cloud Forest Humidity

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ABSTRACT

This experiment compares stomata density of the epiphytic *Pleurothallis aristata* and *Maxillaria sp.* orchids under experimental conditions of dry and humid environments. *Pleurothallis aristata* is in the sub-tribe Pleurothallidinae and lacks pseudobulbs, while *Maxillaria sp.* has pseudobulbs. The study seeks to determine what differences in stomata density exist between the two species, and if there is a difference in mean percent stomata open in humid and dry environments. The study takes stomata impressions from the leaves of twenty individuals of each species using clear nail polish. The results show a significant difference in stomata density between the *Pleurothallis aristata* and the *Maxillaria sp.* (Rank Sum Test: $t=55$, $n_1=10$, $n_2=10$, $p<0.05$). Additionally, both species have a higher percentage of open stomata in humid environments than in dry environments (Wilcoxon sign rank test). An explanation for these results is that *Maxillaria sp.* has a pseudobulb for water storage, has a larger leaf surface area, and therefore has higher stomata density. The study showed both species closed their stomata in drier conditions in order to reduce water loss and desiccation. The results of this experiment help demonstrate how different orchid species function in humid and dry environments, and their ability to succeed in the event of global climate change and shifting of biomes.

INTRODUCTION

Orchidaceae is one of the largest and most diverse angiosperm families in the world, including approximately 20,000 to 35,000 described species (Dressler 1981). In cloud forests and other wet forests, many epiphytic orchids live in the canopy, where species experience desiccating conditions of high winds and direct sunlight (Dressler 1993). Thick, fleshy leaves and pseudobulbs (large, bulbous formations on the stem) are both adaptations of epiphytic orchids used to store water and reduce drying (Dressler 1993). There are other morphological adaptations of epiphytic orchids such as succulence and the ability to fix carbon with Carassulacean acid metabolism, similar to members in the succulent family Crassulaceae (Dressler 1981). Orchid species that share this trait have tiny pores in their leaf surfaces (called stomata) that open at night to take advantage of the time where atmospheric humidity is highest

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(Dressler 1981). Thick, succulent leaves are characteristic of these species and they will often open stomata during the day if atmospheric humidity is high, or keep all of their stomata closed if dry conditions occur (Dressler 1981).

Stomata are found in the epidermis and cuticle layer of leaves, which provide the primary route for water vapor transfer when they are open (Hopkins 1995). Water loss and uptake are mediated by the guard cells, which can activate stomata closure if they sense water stress (Taiz and Zeiger 1991). The process of stomata closure is one of the very important protective processes to prevent severe water stress (Fitter and Hay 1987). This defense helps prevent tissue desiccation before leaves reach low water levels (Fitter and Hay 1987).

In this study, I experimented with two controlled environments: one in a wet environment and the other in a dry environment (Figure 1). The experiments were performed with individuals of two orchid species, *Pleurothallis aristata* and *Maxillaria sp*.(Figure 2), chosen because they differ greatly in overall size. *Maxillaria sp*. is much larger; *Pleurothallis aristata* is a miniature orchid. Additionally, *Maxillaria sp*.has pseudobulbs while *Pleurothallis aristata* does not. Having smaller leaves means that *Pleurothallis aristata* is more limited in the amount of water it can hold. Thus, Pleurothallids (referring to the miniature orchid sub-tribe, Pleurothallidinae) will face a more serious threat of desiccation than the *Maxillaria sp*. under identical environmental conditions. Lacking pseudobulbs means that Pleurothallids cannot depend upon their stems for water storage. Therefore, they only have their leaves for water storage and must adapt physiologically or micro-morphologically in order to prevent desiccation (K. Masters, personal communication, August 11, 2011). Thus, it is likely that Pleurothallids should evolve to have a lower stomata density and show a greater responsiveness to dry conditions by closing their stomata. Conversely, it was expected that *Maxillaria sp*. would have greater stomata density because they have larger leaves and the presence of a pseudobulb justifies the increased rate of gas exchange. Furthermore, it was predicted that in dry conditions both orchid species would have a smaller percentage of open stomata in order to prevent water loss.

Figure 1. Set up of environmental conditions. (A) Dry conditions aquaria and workspace. (B) Outdoor humid environment.

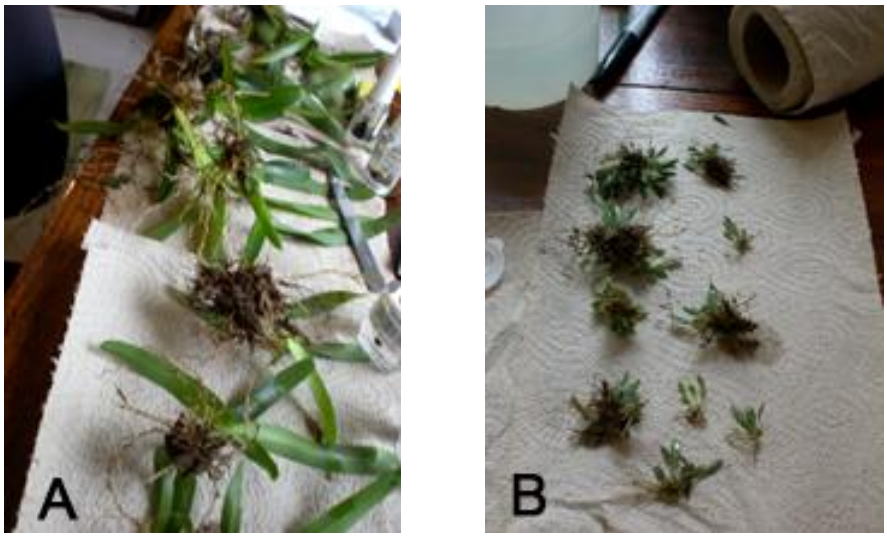

Figure 2. Orchid specimens. (A) *Maxillaria sp* and (B) *Pleurothallis aristata*.

MATERIALS AND METHODS

Twenty *Pleurothallis aristata* individuals and twenty *Maxillaria sp*. were collected from primary or secondary growth cloud forest in Monteverde, Costa Rica. They were retrieved from locations on the property of Karen and Alan Masters as well as the biological station forest. They were removed from their epiphytic habitats by cutting out the entire root system from the host tree so as to keep the individual as intact as possible. Selection criteria for each individual required that each have at least four healthy leaves and be of medium age and size.

Once the individuals were collected, they were paired up with another individual of the same species that was most similar in size and number of leaves, and then each pair was divided into two groups: one destined for the dry, one destined for the wet experimental condition. For each specimen, the weight (g), length (cm) and surface area (cm^2) of the longest leaf, width of the stem (mm), and total number of leaves were determined. The surface area was calculated using ImageJ software, which scaled scanned images of the traced leaves.

The two experimental conditions consisted of aquaria placed in either (1) an indoor, dry environment with a controlled de-humidifier, which kept relative humidity at approximately 50%, or (2) an outdoor, wet environment with a humidifier, which kept relative humidity at approximately 95%. Ten of each species were placed in a bed of moss in each environment and given three days to adjust to the humidity and temperatures. The individuals in the dry conditions were sprayed with 100-250mL of water in the morning and at night each day, and the individuals in the wet conditions had a humidifier inside the aquarium that would turn on if the humidity dropped below 92%.

After waiting three days for the orchids to adapt to the environments, the experiment was initiated on the first set of stomata peels. This consisted of painting a small stroke of clear nail polish on the largest leaf of each specimen and allowing it to dry. Once dry, the polish was

removed in a single peel with clear sticky tape, and attached onto a glass slide. When the nail polish is painted onto the leaf, it obtains an imprint of the stomata as it dries. Each slide was placed into a compound microscope and three views were taken for the peel of each leaf. In each view, the total numbers of stomata were counted, as well as the number of stomata open. From these three views, I calculated the average stomata density for each leaf, as well as the average percent of stomata open. The two sets of orchid specimens were then transferred to the opposite environment and given another three days to adjust to the new environmental condition. After the three days, the same stomata peel test was performed on each individual plant. The specimens remained in their current environments after these tests.

A final measurement was taken two days after the second set of stomata peels. Leaf cross-sections of the longest leaf of each individual plant were taken and were viewed in the cross-sections in a dissecting scope equipped with a micrometer in the eyepiece to measure the thickness of each leaf.

RESULTS

STATISTICAL ANALYSES

Since this experiment had a relatively small sample size, non-parametric statistics were used. These include the Wilcoxon sign rank test (used for comparing related samples' means), Spearman's rank correlation test of relationship (to measure the correlation of dependence between two variables), and the rank sum test of differences between means (also for comparison of two samples' means).

RELATIVE HUMIDITY AND MEAN OPEN STOMATA

In order to determine if the average percentage of stomata increased as individuals were moved from a dry environment to a humid environment a Wilcoxon sign rank test was performed four times, for each change of conditions of both *Pleurothallis aristata* and *Maxillaria sp*. From this test, results show that for *Pleurothallis aristata* and *Maxillaria sp*. the move from the dry to the wet conditions resulted in a significant increase in percent of stomata open (65.1% increase for *P. aristata*, 42.4% increase for *Maxilaria sp*.). Likewise, the results show that for both species the move from the wet to the dry conditions resulted in a significant decrease in the percent of stomata open.

The experiment was designed to determine if there was a significant correlation between leaf surface area and the mean percent of stomata open. Spearman's rank correlation test (n = 10, critical value = 0.648) was performed for each species in the conditions they started in and the condition they were in after the environments were switched. For both *Maxillaria sp*. and *Pleurothallis aristata* there was a significant difference in the number of stomata open from dry to wet conditions. As each species was moved from the dry to the wet environment, the mean percentage of open stomata increased significantly (Figure 3; Figure 5). Likewise, as each

species was moved from the wet to the dry environment, the mean percentage of open stomata decreased significantly (Figure 4; Figure 6).

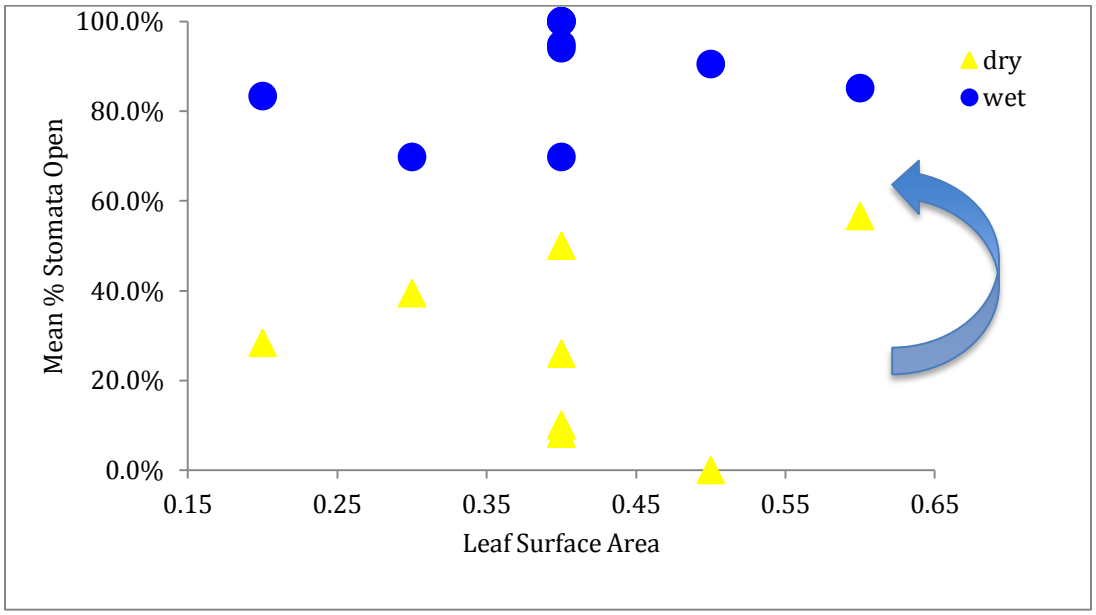

Figure 3. Leaf Surface Area and Mean Percentage of Open Stomata of *Pleurothallis aristata* from Dry to Wet Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Pleurothallis aristata* from dry to wet environments. The arrow demonstrates which environment the species was moved to. As *Pleurothallis aristata* was moved from a dry to a wet environment, the percentage of open stomata increased.

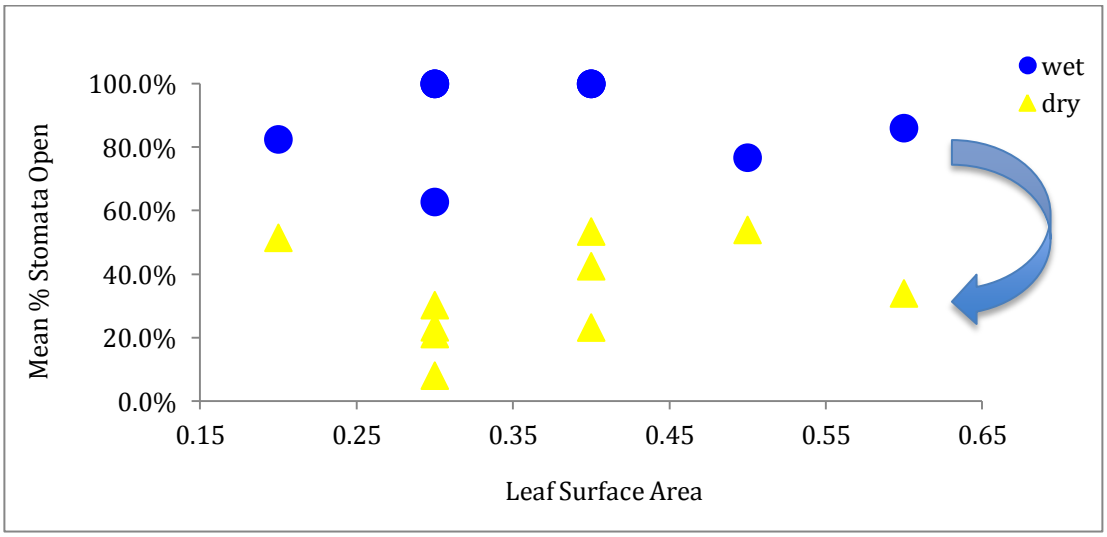

Figure 4. Leaf Surface Area and Mean Percentage of Open Stomata of *Pleurothallis aristata* from Wet to Dry Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Pleurothallis aristata* from the wet to dry environments. The arrow demonstrates the move from the wet to the dry environment. As *Pleurothallis aristata* moved from wet to dry, the percentage of open stomata decreased.

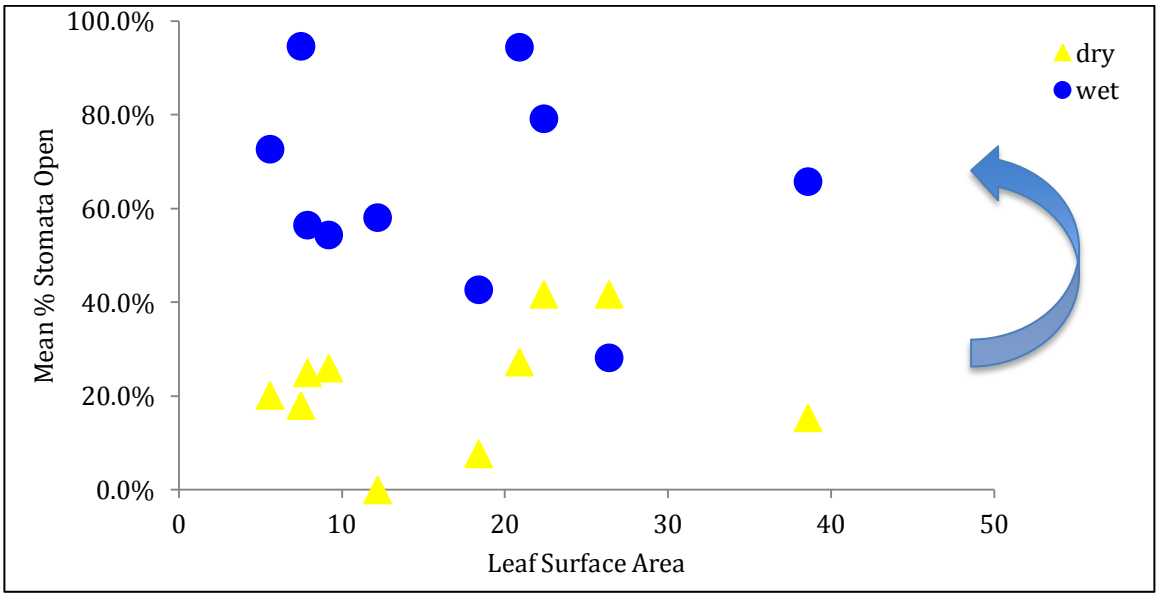

Figure 5. Leaf Surface Area and Mean Percentage of Open Stomata of *Maxillaria sp.*from Dry to Wet Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Maxillaria sp.* from the dry to wet environments. The arrow demonstrates the move from the dry to the wet environment. As *Maxillaria sp.* was moved to the wet environment, the percentage of open stomata increased.

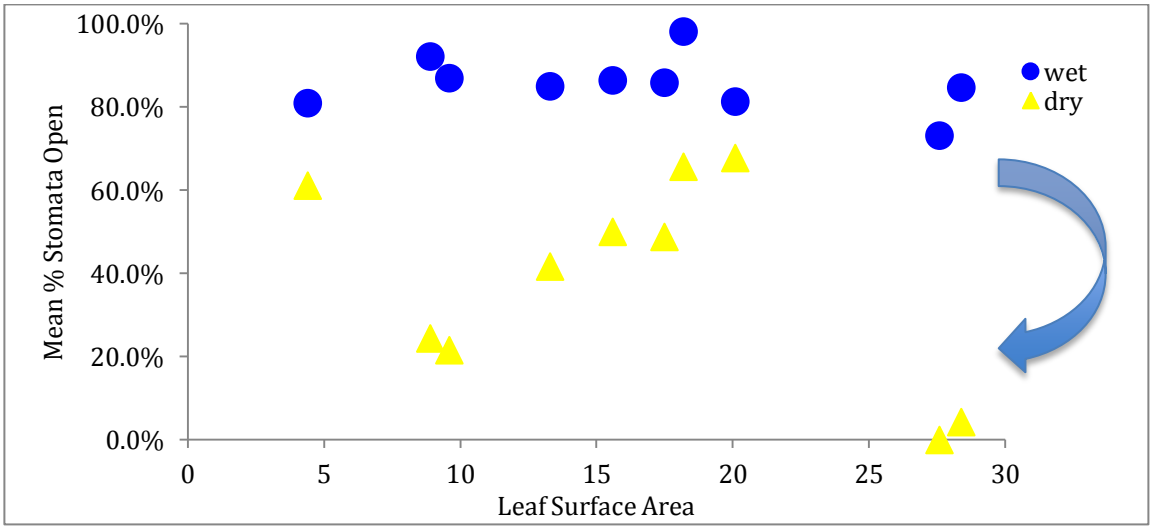

Figure 6. Leaf Surface Area and Mean Percentage of Open Stomata of *Maxillaria sp.*from Wet to Dry Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Maxillaria sp.* from the wet to dry environments. The arrow demonstrates the move from the wet to dry environment. As *Maxillaria sp.* was moved to the dry environment, the percentage of open stomata decreased.

MEAN STOMATA DENSITY

In order to determine if there was a difference in stomata density between *Pleurothallis aristata* and *Maxillaria sp.,* a Rank Sum Test was performed of differences between means (t =

55, n_1 = 10, n_2 = 10, $p<0.05$). The results show a significant difference in stomata density between the two species (Figure 7). *Maxillaria sp*. has approximately four times more stomata on average than does *Pleurothallis aristata*.

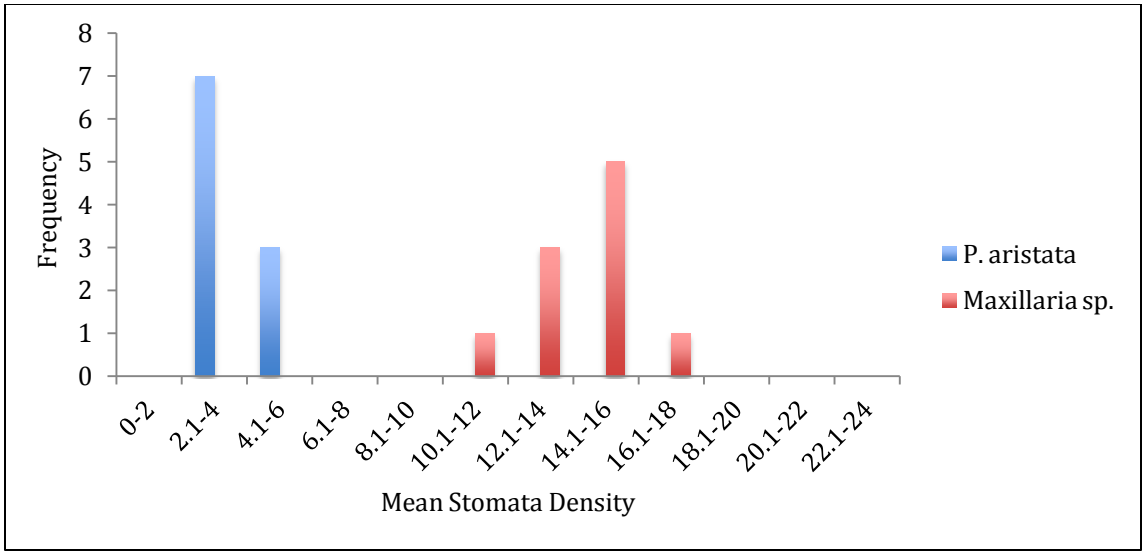

Figure 7. Mean Stomata Density Frequencies.
This figure compares the mean stomata density frequencies of *Pleurothallis aristata* (μ=3.8, σ=0.5) and *Maxillaria sp.* (μ=14.4, σ=1.9). There is a significant difference in stomata density between the two species (Rank sum test, n_1=10, n_2=10, t=55, $p<0.05$).

LEAF SURFACE AREA

To determine if there was a significant difference in leaf surface area between the two species the same Rank Sum Test was used (t=55, n_1=10, n_2=10, $p<0.05$). The results show a significant difference in leaf surface between the two orchid species (Figure 8). *Maxillaria sp*. (x=16.4, sd=7.8) has a leaf surface area that is 41 times larger than the surface area of the *Pleurothallis aristata* (x=0.4, sd=0.1) and it has four times the stomata density.

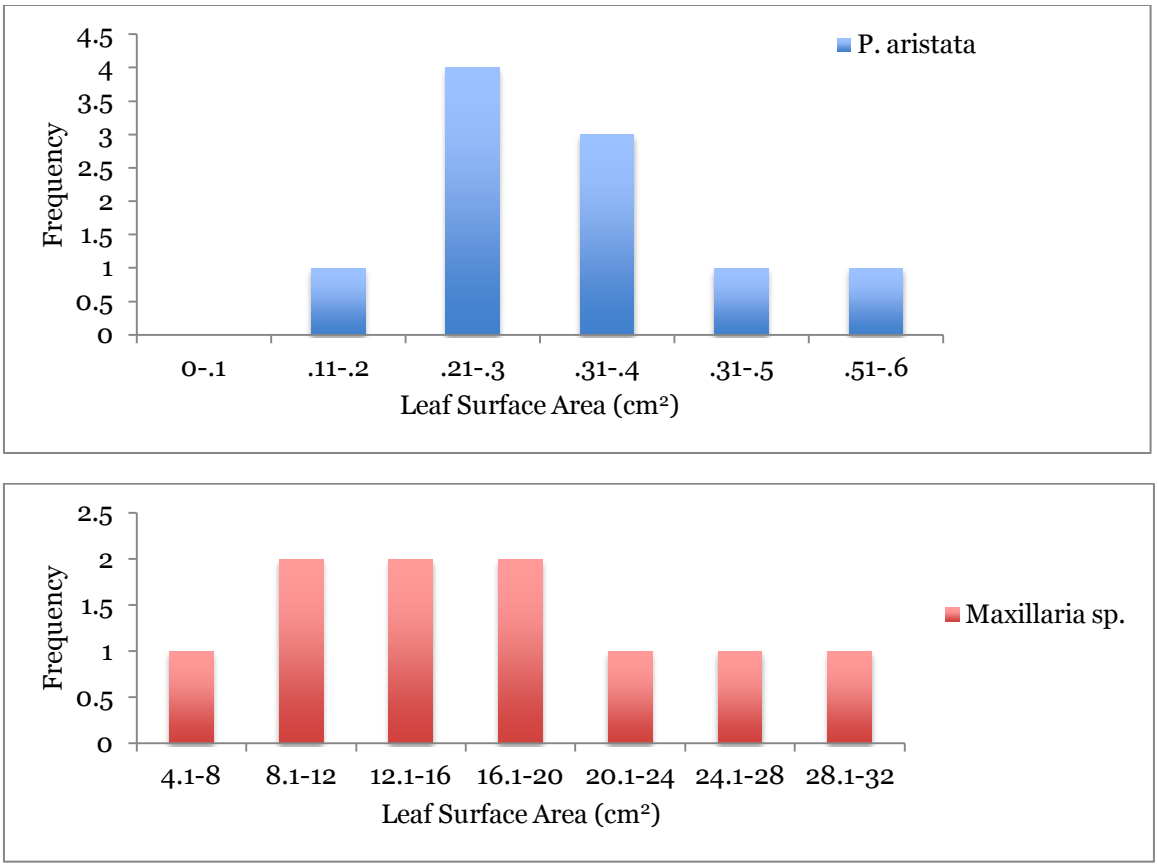

Figure 8. Leaf Surface Area Comparison.

These figures compare the leaf surface areas of Maxillaria sp. and Pleurothallis aristata. The x-axis displays intervals of leaf surface area. There is a significant difference in leaf surface area of the two species.

LEAF THICKNESS

One other factor taken into consideration was the difference in leaf thickness between the two species. A Rank Sum Test was used to derive the differences ($t=68$, critical value=78, $n_1=10$, $n_2=10$, $p<0.05$). There was a significant difference in leaf thickness of the two species; *Pleurothallis aristata* had leaves twice as thick as the *Maxillaria sp*. leaves (Figure 9).

DISCUSSION

The results show that *Pleurothallis aristata* and *Maxillaria sp*. differ significantly in leaf thickness, leaf surface area, and mean stomata density. These differences can be explained in a number of ways. First, Pleurothallids are orchids that lack pseudobulbs, so they must be more conservative with their water and use structures other than their stems for water storage or use other adaptations to mitigate water loss in drier environments. Having a smaller leaf surface area and mean stomata density reduces the amount of water loss through the leaves. Since *Maxillaria sp*. have pseudobulbs, it was expected that they would be less conservative in gas exchange, and therefore would have higher stomata densities. Furthermore, the Pleurothallid

species had leaves that were twice as thick as the *Maxillaria sp*., despite their small size. It was observed that the Pleurothallid leaves were relatively waxy and succulent-like, which would justify their thicker nature if they were storing water in the leaves. The dissecting scope used to measure the leaf cross sections was used to identify presence of different tissue types. In the *Maxillaria sp*. the cross section consisted mostly of photosynthetic mesophyll. In contrast, the Pleurothallid species had a thin layer of photosynthetic cells and tissue and a large quantity of spongy and vascular tissues. This could be an adaptation of *Pleurothallis aristata* to cope with water stressed environments.

The results also show that when moving from a dry environment to a humid environment, the percentage of open stomata significantly increases in both *Pleurothallis aristata* (65.1% increase) and *Maxillaria sp*. (42.4% increase). Similarly, when moving from a wet environment to a dry environment, the percentage of open stomata significantly decreases in both species. This result is reasonable because when species of moist climates are exposed to drier environmental conditions, they reasonably respond by attempting to hold onto water to survive. Therefore, it is expected that most of their stomata would be closed in dry conditions to prevent excess water loss and desiccation.

The results of this experiment indicate that stomata density differs between the *Pleurothallis aristata,* a miniature orchid species and *Maxillaria sp*.pseudobulb-containing orchid species. The behavior of each species in wet conditions demonstrates how these two species function in the cloud forest in Monteverde, Costa Rica, where it is humid for a great portion of the year. Because most of the stomata close up in dry conditions, gas exchange is taking place at a much slower rate. This relates to issues such as climate change and the resulting biome shifts, as some species may be more likely to succeed in periods of prolonged drought or less humidity. For example, in discussing the orchids in this experiment we would expect many Pleurothallid species to be stressed in the onset of global climate change, as they have minimal water storage. Other plant species with similar water storage adaptations as Pleurothallids would also be stressed, and their populations could diminish. It is crucial to look at smaller species and their environmental adaptations when considering global climate change, as their response to change is an indicator to the success of the overall environment or biome.

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