The Effect of Altitude on Stomatal Density of a Greenleaf Manzanita (*Arctostaphylos patula*)

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During photosynthesis, plants utilize carbon dioxide and release oxygen. The rate of this gas exchange depends on the size and density of stomata on the underside of the leaf. The density of leaf stomata can vary depending on plant species, location, and other environmental factors including altitude and CO$_2$ concentration. We predict that the stomatal density on the leaves of a Greenleaf Manzanita (*Arctostaphylos patula*) would be greater when grown in higher altitude. In this experiment, sixty leaves of the plant were collected at both high altitude (2127 m) and low altitude (113 m, N=120). Each leaf was super-glued to a microscope slide in order to create an impression, which allowed the stomata to be easily observed and counted using a compound microscope. A one tailed t-test showed that the average number of stomata per unit area was significantly greater in leaves collected at high altitude (30.03 stomata/mm$^2$ ± 0.57 SEM) than those collected at low altitude (19.13 stomata/mm$^2$ ± 0.72 SEM, $p = 7.46 \times 10^{-19}$). We believe that this difference is a compensation for lower partial pressure of carbon dioxide at high altitude.

**Introduction**

Stomates are small pores located on the underside of leaves and function as the source of gas exchange, mainly involving carbon dioxide. The photosynthetic rate of plants is dependent on the stomatal density within their leaves. Atmospheric partial pressure of the gases involved in photosynthesis has been shown to have an effect on plant functional types (PFT) with specific plant species (Wang et al 2014). Since there is a lower partial pressure of carbon dioxide in higher altitude, plants would need a greater number of stomata to compensate. In this study, the hypothesis tested was that a Greenleaf Manzanita (*Arctostaphylos patula*) would have a higher stomatal density in a higher altitude than in a lower altitude. In order to test this hypothesis, a sample of leaves was collected in both high and low altitudes. Counting and comparing the number of stomates at each altitude will show if there is a significant difference.

**Methods and Materials**

Sixty leaves of Greenleaf Manzanita were collected in Big Bear, California at an altitude of approximately 2127 m (6,978 ft). The temperature was 13.9 °C at 3:45pm on an October afternoon. In addition, sixty leaves of the same plant were collected at a lower altitude of 113 m (369 ft) from a nursery in Laguna Niguel, California. The temperature at lower altitude was 22.8 °C and time of collection was 2:50pm. Thereafter, the underside of the leaves was pressed into a single drop of Krazy brand super-glue on microscope slides. Once dried, the leaves were gently peeled off, leaving an imprint on the slide. These slides were then taken to Saddleback College in Mission Viejo, CA, where compound
microscopes were used. The number of stomates in a single field of view was counted at a total magnification of 400x.

Results
The average stomatal density differed in high and low altitudes (Figure 1). The sample of leaves taken from the higher altitude (N=60) displayed a greater stomatal density (30.03 ± 0.57 stomata/mm², ± SEM) than the sample of leaves collected at a low altitude (19.13 ± 0.72 stomata/mm²). Leaves taken at higher altitude had significantly greater stomatal density than leaves taken at lower altitude as shown in Figure 1 (p = 7.46x10⁻¹⁹, one-tailed t-test).

Discussion
In this experiment, the hypothesis tested was that Greenleaf Manzanita would have a higher stomatal density if grown in a higher altitude. After data collection and analysis, the average number of stomata per unit area was significantly greater in leaves collected at high altitude than those collected at low altitude.

The difference in stomatal density in a higher altitude in comparison to lower altitude could be attributed to various factors. One possible explanation is that the Greenleaf Manzanita with greater stomatal density

![Figure 1](image-url)
density had grown in an environment where the air is less dense, containing a low concentration of CO\textsubscript{2}. Therefore, the plant would need a greater amount of stomates to establish greater O\textsubscript{2} production and efficient gas exchange (Woodward 2002). The Greenleaf Manzanita grown in the lower altitude contained a lesser density of stomates because there is a greater concentration of CO\textsubscript{2}, in which the plant does not need a greater amount of stomates to establish gas exchange and O\textsubscript{2} production.

An alternative explanation suggests that the differences in stomatal density at various elevations are related to drought effects and light exposure as well as carbon dioxide uptake (Kouwenberg et al 2007). In a study presented by the Mineralogical Society of America, researchers examined the climatic variables that contributed to changes in leaf stomatal frequency. Their results also demonstrated a clear increase in stomatal density at higher altitudes. Light intensity generally increases with altitude and can therefore have an influence on the increase of stomatal frequency. Nevertheless, the study concluded that atmospheric carbon dioxide was the most significant factor in terms of stomatal variation at different elevations (Kouwenberg et al 2007).

The effects of altitude on stomatal density vary between different plant functional types (Fucai et al 2014). Although there are other studies that have reported a strong pattern of increased stomatal density at high altitude, there are still some cases that contradict or show the reverse trend. Depending on the plant species, there may be variations in stomatal density that contradict our results. Environmental factors such as light, temperature, water availability and the species of plant could also have a significant effect on stomatal density.

This study supports our hypothesis. In addition, this study investigated one species occurring at low and high altitude. There may be other compensatory mechanisms for a lower partial pressure of CO\textsubscript{2} seen in other species of plants.

The success of finding a significant difference may have been due to having a large sample size. This allowed more precise and accurate findings from the data. An area of improvement in this study would be to measure the concentrations of CO\textsubscript{2} in the atmosphere at high altitude and compare it to the CO\textsubscript{2} concentration in low altitude. To further this study, the same species plant in a high and low altitude can be translocated to the opposite altitude to see if the plant will grow with an increase or decrease its stomatal density. Since the stomata in leaves are the primary structure used for gas exchange in vascular plants, stomatal size and density play a major role in the function of leaves and photosynthesis. It is important to examine other factors that may contribute to the altitude-related differences.

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Literature Cited