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# Effects of plant size and flower color on the reproductive success of Pleurothallis sanchoi (Orchidaceae)

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## ABSTRACT

Factors contributing to the reproductive success *of Pleurothallis sanchoi* were examined in relation to plant size and flower color. This particular orchid has plants of varying sizes as well as three color morphs including yellow, purple, and yellow with purple petals. First, the potential for reproductive success was deduced by measuring, on individuals in natural populations, leaf and flower number and how they are related to flower color. Second, the removal and deposition of pollinia on flowers of different colors was examined. It was found that larger plants generally had more flowers, although there appears to be an upper limit above which increased flower production no longer occurs. Yellow flowers tended to be on larger plants but they did not necessarily make more flowers. Pollinia removal was fastest for yellow flowers while pollina deposition did not differ significantly between the three flower colors. Although yellow individuals tend to be more abundant in this population and appear to have a slight reproductive advantage, the persistence of the two other color morphs suggests that they either compensate for reproductive disadvantages, or that flower color is environmentally determined.

## RESUMEN

Los factores que contribuyen al éxito reproductivo de *Pleurothallis sanchoi* fue examinado en relación con el tamaño de la planta y el color de las flores. Este tipo de orquídea tiene plantas pequeñas y grandes y también hay tres posibilidades para el color de las flores que tiene la planta. Los tres colores son amarillo, violeta y amarillo con pétalos violetas. Se midió el éxito reproductivo potencial de cada planta al contar, en individuos de poblaciones naturales, el número de hojas, flores y se relacionaron con el color de las flores. También se tomó en consideración la presencia y remoción de polinia en flores de colores diferentes. Se encontró que plantas más grandes, en general, tenía más flores, pero parece que hay un límite en el número de flores que una planta puede producir. Flores amarillas se encontraron en plantas más grandes, pero estas plantas no siempre produjeron más flores. La tasa de remoción de polinia fue el más rápido para flores amarillas. La deposición de polinia no fue diferente entre los tres colores de flores. Los individuos con flores amarillas tienden a ser más abundantes en esta población y parecen tener una pequeña ventaja reproductiva, la persistencia de los otros dos colores sugiere que o están compensando esas desventajas reproductivas o que el color de los flores está determinado por el medio ambiente.

## INTRODUCTION

Reproductive success is of crucial importance in the life of a plant because it ensures future representation in a population. Plants therefore invest valuable resources in increasing their chances of reproduction. There are many phenotypic traits that are known to influence plant fitness in regards to reproduction. Plant size has been linked to floral display area and the success of both the male and female flower components (Murren and Ellison 1996). Since pollination success can be a useful measure of reproductive fitness, floral characteristics are often of key importance (Nilsson 1992). Characteristics that have been shown to play a role in pollination include flower shape, the production of nectar or oil, resin glands, scent and color (Barth 1985, Endress 1994). Different variations and combinations of these phenotypic traits can greatly increase or decrease the reproductive success and thus future representation of an individual in a population.

*Pleurothallis sanchoi* is one of over 4,000 species of orchids in the subtribe Pleurothallidinae (Dressler 1993). This subtribe is characterized by mostly miniatures with no pseudobulbs. These factors make them well-suited to habitats such as cloud forests where there is an abundant supply of water. In these areas they can be found in great abundance growing both in the canopy and on the trunks of trees (Dressler 1993). Pollination in this group is generally thought to be by Drosophila-like flies, although aphids and thrips have also been cited as active pollinators (Don 1986 and Dressler 1990). Pollen grains are united into structures called pollinia and removed from the flowers in coherent masses. Personal observations of *P. sanchoi* have revealed differences in plant size as well as a variety of color morphs including individuals with yellow, purple and yellow-purple-and yellow-purple-pedaled flowers, with yellow flowers being the most abundant in the population. Since it is known that such differences can greatly affect reproductive success, this study is interested in determining if a relationship exists between plant size, flower color and reproductive success of this particular species of orchid.

Due to sheer numbers of orchids in this subtribe, many of the orchids in this group are not well-studied, and literature on *P. sanchoi* is particularly scarce. Next to nothing is known about the effects of plant size and flower color on reproductive success. Furthermore, it is not known why or how different color morphs persist in the population. The first part of this study is designed to determine how plant size may be linked with reproductive success. This will be done by measuring factors that influence the potential to set seed such as leaf and flower number. A greater number of leaves may allow a plant to support more flowers. More flowers on a plant means more opportunities for successful seed set. Comparisons of these results between the three color morphs may allow one to determine if a particular color coordinates with an optimal size for plant reproductive success.

The second part of the study seeks to evaluate differences in pollinia removal and deposition between the three color morphs. According to Nilsson (1992), the removal and receipt of pollinia can be used as a measure of male and female reproductive success. This part of the study wishes to address whether or not flower color affects reproductive success measured in this way. That flower color does indeed play a role in at least some flowers is evidenced by the fact that pollination is usually followed by a rapid fading of flower color (Barth 1991). Past studies on a related species with similar color morphs, *Pleurothallis segoviensis,* found that purple flowers were preferred by pollinators in terms of speed of pollinia removal and frequency of pollinator visitation (Goldberg and Nelson 1999 and Lee 2000). There is thus reason to suspect that *P. sanchoi* will demonstrate similar trends. Finally, this study will address possible explanations for the maintenance of three distinct color morphs in the population. It is hoped that in gaining an understanding of how plant size and flower color influence the reproductive success of this orchid, that the mechanisms and reasons behind the maintenance of color variation in this and other species of orchids will become more apparent.

## METHODS

This study was conducted in Monteverde, Costa Rica (10° 18' N, 84° 48' W), between the dates of 23 October 2001 and 16 November 2001. The study site was located in a pasture behind the Estación Biológica in the Lower Montane Wet Forest Holdridge Life Zone. A total of seven trees distributed throughout the pasture and supporting populations of *Pleurothallis sanchoi* were utilized for this study. In addition to these populations, each tree also harbored many other species of epiphytes including individuals from the families Bromeliaceae, Piperaceae and many others in Orchidaceae. Only plants growing at head level or below were observed. Weather conditions during data collection were characterized by gusty winds and blowing mist with few periods of sun.

### Morphological Data

Data for individual plants including number of leaves, number of flowers and flower color were collected during the first ten days of the study. A leaf was included in the count if it was completely open, meaning flat and with no curvature of the edges, regardless of its size. Open flowers and buds of any size were included in the counts of the number of flowers per plant. The color of the flower was recorded as either purple, yellow or yellow with purple petals (YPP). YPP refers to flowers with yellow sepals and purple petals and column tip. A total of 80 individuals were sampled.

### Pollinia Removal and Deposition

Pollination observations were taken during the second ten days of the study. Plant size of sampled organisms was limited to those individuals with five to fifteen leaves in order to account for possible pollinator preferences to smaller or larger plants. On the first day, each selected tree was examined for open flowers. Any open flowers were removed to ensure that open flowers encountered on the following day had bloomed within the last 24 hours. Once a newly open flower was located during the following days, flower color was recorded along with an identification number. It was then examined for intact pollinia using a 10X hand lens. If no pollinia were found, a value of zero was assigned to the flower, indicating that the pollinia had been removed within 24 hours. If pollinia were present, the date was recorded and the flower revisited every subsequent day until pollinia removal occurred. A value of one was assigned if removal was observed the first day following discovery, a value of two for the second day and so on. Similarly, each sampled plant was pruned so that only one flower per plant was open at a given time. This was done in order to minimize possible effects of flower density on pollinator visitation. Evidence of pollinia deposition was also recorded. This included any pollinia attached to the column in an orientation other than the original position in which they are nestled in a small grove at the top. A total of 75 individuals were observed.

### Data Analysis

A regression was performed to determine the effects of leaf size on flower number. Three separate regressions, one for each flower color, were performed to determine how the proportion of flower number to leaf number related to the total leaf number of a plant. A one-way ANOVA was used to determine differences in leaf number per plant for the three color morphs. A second one-way ANOVA was performed to ascertain differences in the number of flowers of an individual for the three groups. A Kruskal-Wallis Test was used to determine differences in pollinia removal time between the colors. Finally, a Chi Squared Test was used to ascertain differences in pollinia deposition among the three color morphs.

## RESULTS

There was a positive correlation between the number of flowers and the number of leaves on each plant ($R^2 = 0.219$, $P < 0.0001$; Figure 1). Regressions of the proportion of flowers to the number of leaves and total leaf number for each color revealed a negative correlation for yellow flowers ($P = 0.0255$) and almost no relationship for purple and yellow purple petaled flowers (Figure 2). Individuals with yellow flowers were significantly larger than those with yellow purple petals and also had a tendency to be larger than individuals with purple petals ($F = 3.237$, $P = 0.181$, two-way ANOVA; Figure 3). There was no significant difference between flower colors in regards to the average number of flowers produced per plant (two-way ANOVA; Figure 4).

There was nearly significant effect of color on pollinia removal (H corrected to ties = 31.416, tied $P = 0.0618$, Kruskal-Wallis Test; Figure 5). Pollinia were removed from yellow flowers faster than from either purple or yellow purple petaled flowers. There was no significant difference in pollinia deposition time among the colors ($X^2= 0.564$, $P = 0.754$; Chi-Squared Test; Table 1).

## DISCUSSION

Several interesting results were found when examining morphological data in relation to the overall reproductive success of *Pleurothallis sanchoi*. The number of flowers was found to be positively correlated with the number of leaves on a plant. This indicates that larger plants have the potential to set more seed than smaller individuals, thus increasing their reproductive success. This is consistent with findings from Murren and Ellison (1996) who found a similar correlation between plant size, floral display area and male and female reproductive success. This increase in numbers of flowers in relation to leaves did not occur indefinitely.

A regression performed on the proportion of yellow flowers to leaves against the number of leaves per plant indicated a negative correlation and suggested that proportionately, flower production decreases with very large individuals. This may be the result of a trade-off between reproduction and growth. Support for this idea comes from the fact that many of the large (many-leaved) plants observed were composed of high numbers of small, developing leaves (personal observation). Additional evidence that trade-offs play a role comes from Nilsson (1992), who asserts that a large fruit production can decrease growth and output during subsequent years so as to make the short-term benefits derived from this increased production inconsequential. If this is the case, it follows that large plants would have proportionately fewer flowers.

Flower color appeared to have a minimal effect on plant size and flower number. The fact that plants with yellow flowers were significantly larger than YPP plants and tended to be larger than purple flowered individuals indicates that they have the potential to set more seed. However, although flower number in general increases with plant size and yellow plants are often large, they are not producing more flowers (Figure 3). This finding is consistent with the previously discussed regression in which it was demonstrated that the number of flowers does not continue to increase with the number of leaves. It appears that there would be an optimal flower size at which the number of leaves and the number of flowers would be maximized. Since yellow-flowered plants tend to have a greater number of medium to large individuals, it is possible that they have a greater proportion of individuals at this optimal reproduction size and are still able to set the most seed.

Regardless of the number of flowers a plant produces, an important determinant of successful seed set is successful pollination. For *P. sanchoi* there appears to be a pollinator preference for yellow flowers in the sense that pollinia are removed from these flowers more quickly than from the other two color morphs. The fact that yellow flowers receive attention earlier may afford them with the opportunity for increased reproductive success. Faster pollination removal may lead to faster pollination and subsequent seed set, resulting in more reproductive changes to produce seeds per season or year. It should be noted, however, that the pollinia of all three color were eventually removed with the longest being exposed for only three days. Finally, although pollinia removal was faster among yellow flowers, pollinia deposition was the same for the three color morphs. This indicates that although yellow flowers tend to be more successful with regards to the male role, the female end of the process is not directly influenced by color.

Although many of the differences are subtle, yellow-flowered plants seem to have a slight overall advantage in reproductive success than both YPP and purple plants. They tend to be larger, receive the attention of pollinators sooner, and are more abundant in the population (personal observation). This begs the question of why the other color morphs persist in the population. There are a number of possible explanations. A simple reason is that they do not have a choice because the color determining factor is environmental as opposed to genetic. Studies examining this possibility by varying light levels to which the plants were exposed were inconclusive (Goldberg and Nelson 1999, Lee 2000). Another possibility is that the other two color morphs compensate in other ways for their slightly inferior reproductive ability in regards to speed of pollinia removal. Factors to examine that could serve as compensatory mechanisms include speed of development, seed number, relative viability, longevity and fertility (Niklas 1997). It is also possible that competitive ability with regards to flower color changes throughout the year and that observing the population during such a small window of time during one year produces skewed perceptions of color dominance. Nilsson (1996) supports this position when he states, “expenditure for maximized sexual reproduction is parceled” over the lifetime of an orchid. A longer study would be needed to examine this possibility.

The remaining two explanations deal with pollinator preferences. The first deals with the fact that the commonly cited pollinators for the subtribe Pleurothallidinae are Drosophila-like flies. Flies in the family Drosophilidae are often found near decaying fruit or other decomposing vegetation. They are thus attracted to blossoms most similar to this appearance including dark colors such as brown, purple, and green. Purple and YPP flowers most closely resemble these traits. It is thus possible that yellow plants need to produce more flowers in order to be noticed by their primary pollinators who normally concentrate their attention on other colors (Goldberg and Nelson 1999). Even if yellow flowers are more prevalent, they will not be more successful if pollinator behavior (preference) undermines this prevalence.

The final proposed explanation takes advantage of the fact that very little is known about the pollinators for this group. Although it is generally believed that most flowers in this subtribe are fly pollinated, both aphids and thrips have been cited as additional pollinators (Dod 1986). If this is the case, it is easy to imagine that various colors could persist in a population if the pollinating insects belonged to different species with differing color preferences. Differences in their relative abundances could cause pollinator limitation that regulates the number of individuals in each color morph.

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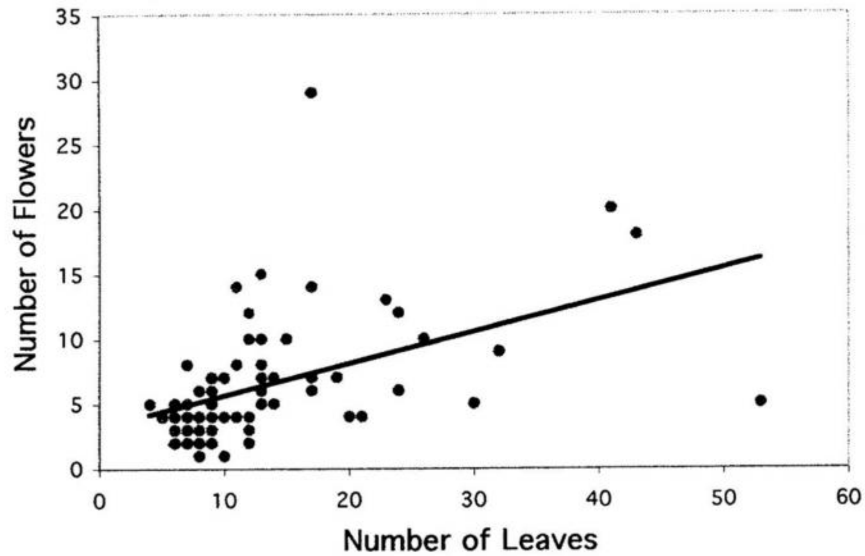


Figure 1. Relationship between the number of flowers and the number of leaves on an individual plant of *Pleurothallis sanchoi* (n = 80).

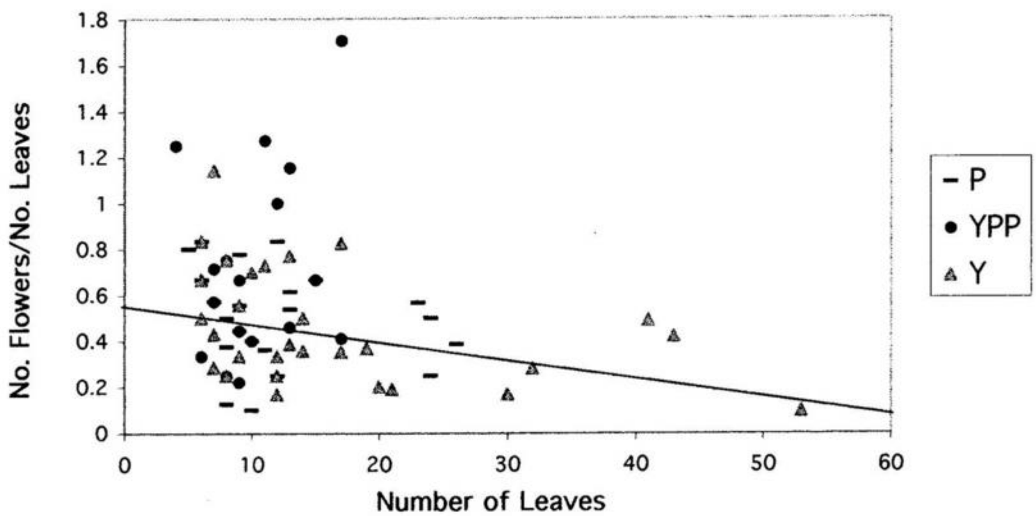


Figure 2. The relationship between the proportion of flowers to leaves and the total number of leaves per individual for purple (P), yellow (Y), and yellow-purple-petaled (YPP) plants. The line indicates a significant P value for the regression for Y flowers.

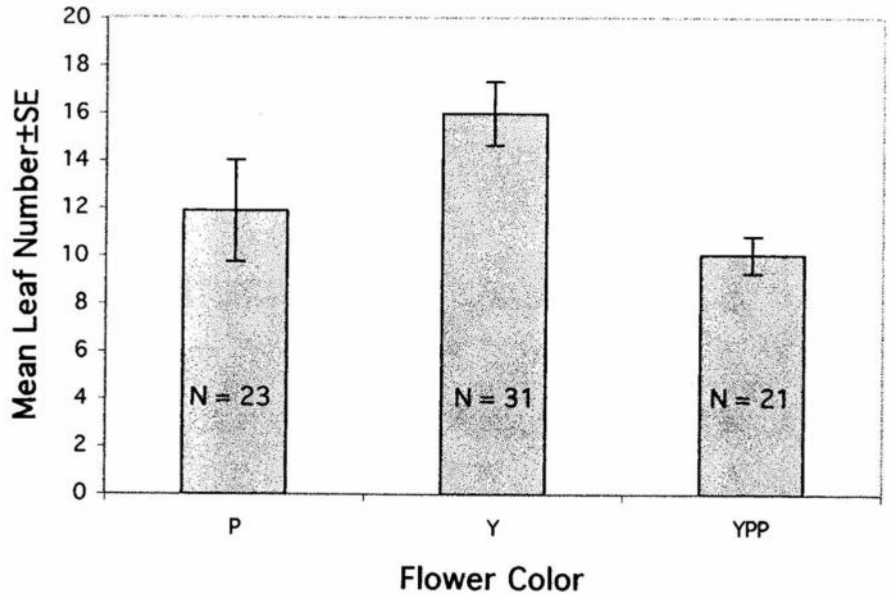


Figure 3 . Differences in mean leaf number among purple (P), yellow (Y) and yellow-purple-petaled (YPP) flowers.

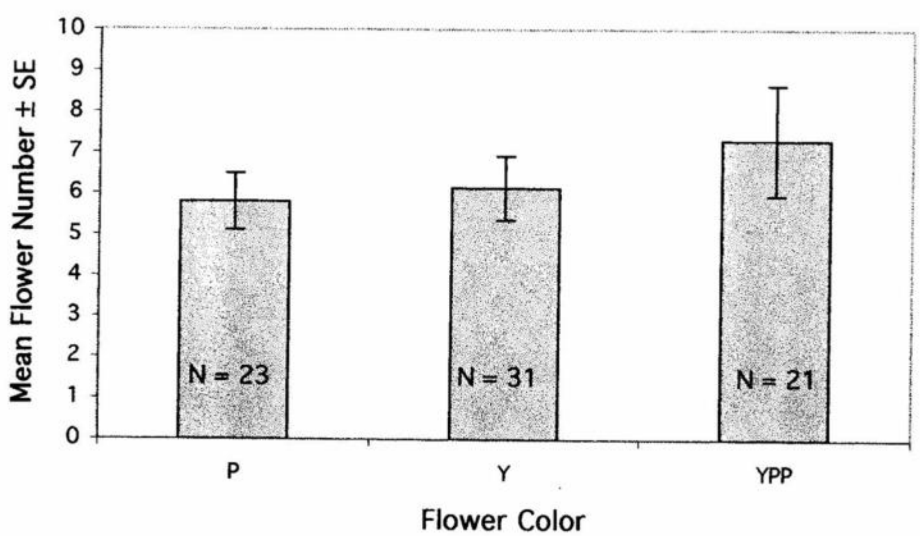


Figure 4. Differences in mean flower number between purple (P), yellow (Y) and yellow-purple-petaled (YPP) flowers.

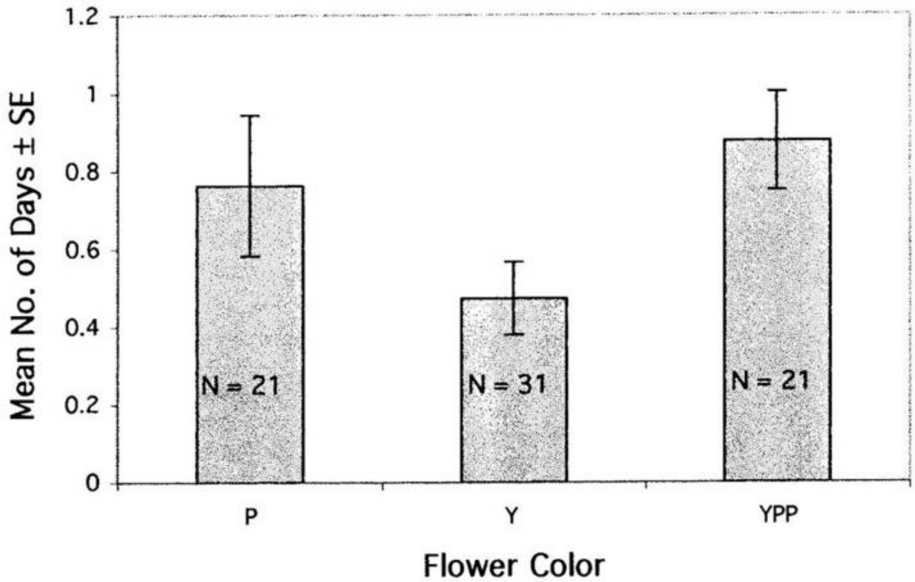


Figure 5. Differences in the mean number of days for pollinia removal between purple (P), yellow (Y), and yellow-purple-petaled (YPP) flowers.

**Table 1**. The number of individuals with and without pollinia deposition for purple (P), yellow (Y), and yellow purple petaled (YPP) individuals.

| Flower Color | Pollinia Deposited | No Pollinia Deposited | Plant Totals |
|---|---|---|---|
| P | 4 | 17 | 21 |
| Y | 8 | 28 | 36 |
| YPP | 6 | 15 | 21 |