Importance of flower size and yellow coloration as influencing factors in the reproductive success of *Pleurothallis sanchoi* (Orchidaceae)

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ABSTRACT

Reproductive success can be linked to pollination success because pollination is needed for plants to reproduce and put out seeds. Factors that affect pollination success include color, nectar, scent, flower shape and size. Past research on *Pleurothallis sanchoi* has indicated that of the three color morphs, yellow, purple, and yellow with purple petals (YPP), yellow is the most attractive color. This study examines the importance of flower size and color in attracting pollinators in *P. sanchoi.* A total of 51 individuals were observed and the number of days required for pollinia removal was noted. The flowers were measured with a caliper for length. In the purple flowers, the amount of yellow area was measured by using a pinhead to estimate the overall yellow area. Color and length were significantly correlated, with yellow flowers being the smallest and purple flowers being the biggest. Pollinia removal was significantly correlated to color and was fastest in YPP flowers and slowest in purple flowers, although yellow flowers showed nearly equivalent rates to YPP flowers. The relation between flower length and days required for pollinia removal was not significant, as well as flower length and amount of yellow in purple flowers. It appears that yellow flowered plants have a slight reproductive advantage. YPP and purple flowers may be maintained in the population if more than one species are the pollinators for *P. sanchoi* and have different pollinator preferences or if these color morphs influence pollinators through scent or nectar.

INTRODUCTION

In animal-pollinated plants, reproductive success depends on the ability to attract pollinators to the flower (Glaetti and Barrett 2008). Reproductive success can be linked to pollination success because pollination is required for plants to reproduce and put out seed sets, thereby guaranteeing that the genes of the plant will be passed on to future generations. Thus, plants put valuable energy and resources into attracting pollinators and ensuring pollination. Plants that rely on animals for reproduction use two important factors to attract pollinators: advertisement and reward (Dressler 1990). Preferential pollinator response to certain colors has been shown in bees, butterflies and hummingbirds (Jones and Reithel 2001), but other attributes can affect pollinator response and may be even more important than color, such as bilateral form (Rodríguez et al. 2004) and odor (Omura and Honda 2005). Other floral characteristics that can influence pollinator response are flower shape and the presence of nectar. While it is assumed that pollinators are the most important selective agents in the evolution of floral traits, herbivores and seed predators also play a significant role in selecting for floral characters such as color, morphology, and the production of rewards (Brody 1997). Variations or combinations of these phenotypic traits can greatly influence, both positively and negatively, the reproductive success of the plant.

Pleurothallis sanchoi is an orchid of the subtribe Pleurothallidinae, the largest subtribe in the orchid family with at least 4000 species. Orchids in this subtribe are characterized by small size, no pseudobulbs and only one leaf on each stem. Pollen grains are clumped into structures called pollinia and are removed from the anther in coherent masses. In *P. sanchoi*, the inflorescence is produced near the base of the leaf. Pleurothallids occur with great diversity in cloud forests as epiphytes, growing mostly in the canopy or wherever there is enough light (Dressler, 1993). Most orchids, even the most primitive, have evolved away from nonspecific, promiscuous pollination systems towards highly specialized, more restricted systems. The great majority of Pleurothallidinae are pollinated by small, nectar-seeking flies with most pleurothallids thought to be pollinated by Drosophila-like flies (Dressler, 1990), although aphids and thrips have been cited as possible pollinators too (Dod. 1986).

Pleurothallis sanchoi exhibits three different color morphologies: yellow, yellow with purple petals, and purple although little is known about how these colors influence reproductive success. Since color has been shown to be highly influential in affecting pollinator response, this study is interested in determining how color affects pollinia removal rates. According to Strozinski (2001), pollinia were removed faster from yellow flowers than from purple flowers or yellow with purple petal flowers in *P. sanchoi*. However, in similar studies using a related species, *Pleurothallis segoviensis*, pollinia removal was the fastest and frequency of pollinator visitation the highest for purple flowers (Goldberg and Nelson, 1999 and Lee, 2000).

The first part of this study seeks to answer this discrepancy by determining how flower color and flower size are linked to reproductive success. *Pleurothallis sanchoi* is an appropriate plant for this type of study because all three distinct colors have clearly distinguishable pollinia, thus allowing pollinia removal rates to be easily determined. Here, I examine how color affects the number of days it takes for pollinia to be removed. I also examine the differences in flower size between each color and its effect on pollinia removal rates. It is hypothesized that yellow flowers will have the fastest pollinia removal rates, in accordance with Strozinski (2001) and because of this, there is reason to believe that yellow color is highly influential in attracting pollinators. Therefore, yellow flowers are predicted to have the smallest flower size and purple flowers the biggest.

The second part of this study addresses the possible explanations for the maintenance of the three distinct color morphs in the population. Since yellow is thought to be the most attractive color, it should be favored under natural selection and stabilization of yellow flowers in *P. sanchoi* should occur (Gegear and Laverty, 2001), yet all three color morphs persist. I test the effect of yellow color in purple flowers on pollinia removal rates to determine if color, specifically the color yellow, is important in attracting pollinators. It is hypothesized that purple flowers with more overall yellow area will have faster pollinia removal rates than purple flowers with less yellow character.

METHODS

Study Site

This study was conducted in Monteverde, Costa Rica between the dates of July 19, 2010 and August 1, 2010. The study site was located at La Jardín de Orquídeas where a total of three *Acnistis* trees supporting populations of *P. sanchoi* were used for this study. These orchids were collected from wild populations and translocated to the *Acnistis* trees. They were in good condition and flowering, but the number of distinct individuals was indeterminable.

Study Organisms

Pleurothallis sanchoi was used for the study and the colors of the plants studied were either yellow, yellow with purple petals (YPP) or purple. YPP refers to flowers with yellow sepals that have purple petals and flower tips. Purple flowers always have some area of yellow on the sepals and thus are referred to as "combo" flowers from this point forward.

Flower size and Pollinia Removal

All open flowers were removed from the plants before the first day of study. Beginning on the first day of study and every morning thereafter, the plants were examined for open flowers. Pollination observations were taken throughout the course of the entire 12-day study starting at approximately 8:30 am and ending at 10:30 am every morning. For every open flower, a 10x hand lens was used to determine if pollinia had been removed. If pollinia were present, a tan, dark brown, green, black or gray colored string was tied around the stem of the leaf that had the open flower. The flower was visited each subsequent day until pollinia removal occurred. A different color was used each day so that the day when the flower opened could be identified later. If no pollinia were found, the flower was removed and placed between two clear, glass slide plates to flatten it. Using a caliper, the length between the tip of the top sepal and the tip of the bottom sepal was measured. The number of days for pollinia removal was also assigned to each flower when it was removed from the plant. A value of one was assigned to flowers that had no string when pollinia removal occurred, a two if the flower had a string and pollinia removal occurred the day after the string was put on, and so on. A total of 51 individuals were observed. It was also noted that in addition to the 51 individuals observed and measured, 13 individuals were preyed upon and thus, length measurements could not be taken. Only the color of the flowers and the days after which pollinia removal occurred were recorded.

Amount of Yellow Character in Combo Flowers

Of the 51 individuals observed and measured for flower size, the amount of yellow area was measured in combo flowers only (n=25). After the combo flowers had been flattened between two slide plates, the top of a flat-head pin was painted with a paint pen and then stamped on top of the glass in the yellow areas of the flower. The pin was repainted after touching the glass each time. Yellow was only measured in the top and bottom sepals of the flower. The area of the pinhead was measured using an ocular eyepiece on a microscope.

RESULTS

Flower Size and Pollinia Removal

Combo flowers were the largest (mean +/- SD = 10.66 ± 0.43, n=27) with YPP flowers being of medium length (mean +/- SD = 10.06 ± 0.63, n=16) and yellow flowers being the smallest (mean +/- SD = 9.39 ± 0.5, n=8; Figure 1). Flower length was significantly correlated with flower color ($X^2 = 22.27$, df = 2, $P < 0.001$, n=51, Kruskal-Wallis test).

In terms of the mean number of days required for pollinia removal, pollinia on YPP flowers were removed the fastest (mean +/- SD = 1.31 ± 0.48, n=16) although yellow flowers showed nearly equivalent results (mean +/- SD = 1.38 ± 0.52, n=8). Combo flowers required the most amount of time (mean +/- SD = 1.85 ± 0.72, n=27; Figure 2). The number of days after which pollinia removal occurred was significantly influenced by color ($X^2 = 7.25$, df = 2, $P < 0.05$, n=51, Kruskal-Wallis test). The frequency of predation on each color was random ($X^2 = 2.70$, df = 2, P > 0.05, n=13; Figure 3).

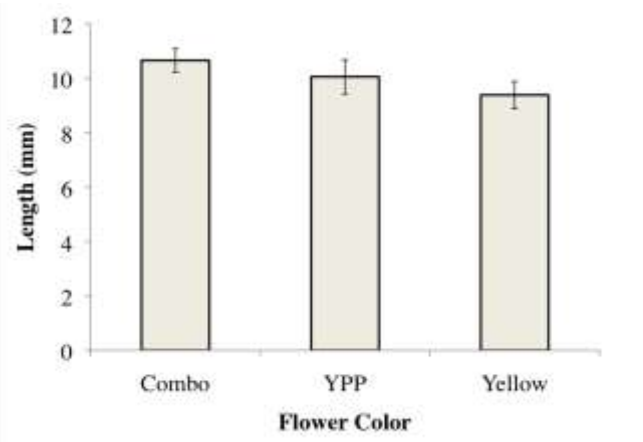

Figure 1. Mean flower length (± 1 SD) of the three color morphs of *P. sanchoi*. Flower length and color were statistically significant. Combo, n=27; YPP, n=16; Yellow, n=8.

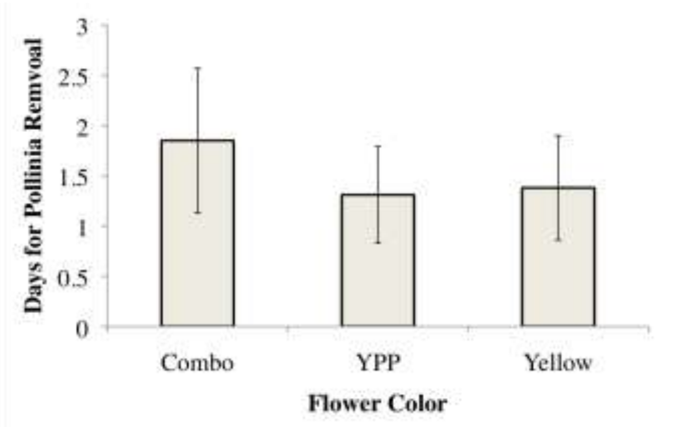

Figure 2. Mean number of days for pollina removal (± 1 SD) of the three color morphs of *P. sanchoi*. The number of days required for pollina removal and color was statistically significant. Combo, n=27; YPP, n=16; Yellow, n=8.

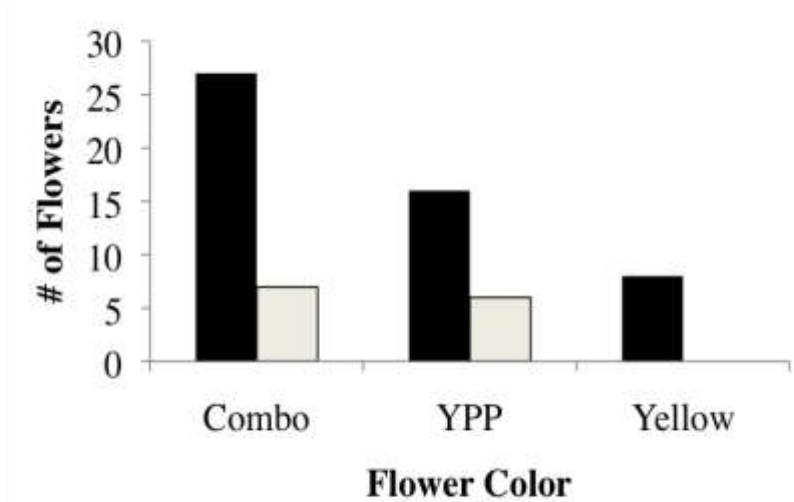

Figure 3. Predation on the color morphs of *P. sanchoi*. Black bars are observed flowers; n=27 for combo flowers, n=16 for YPP flowers, and n=8 for yellow flowers. White bars are flowers eaten; n=7 for combo flowers, n=6 for YPP flowers, and n=0 for yellow flowers. The frequency of predation on each color was random.

There was a nearly significant effect of flower length on days required for pollinia removal in combo flowers ($R^2 = 0.11$, P = .0905, n=27; Figure 4a). There was no significant difference between flower length and days required for pollinia removal in yellow flowers (Spearman Rho = -0.4535, P = 0.26, n=8; Figure 4b) or YPP flowers (Spearman Rho = 0.2486, P = 0.3531, n=16;

Figure 4c). Regression analysis was used for n > 20 (combo flowers) and a Spearman's rank correlation was used for all others.

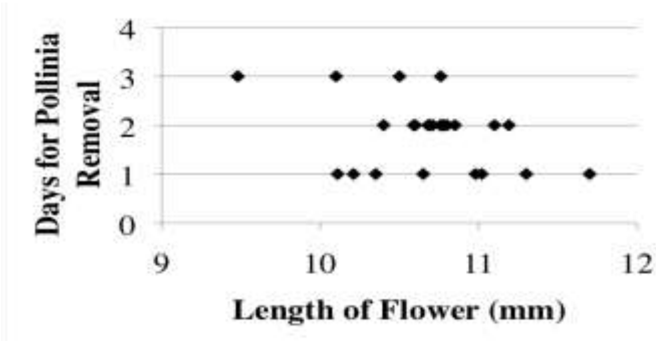

Figure 4a. There was a nearly significant effect of flower length on days required for pollinia removal in combo flowers (n=27).

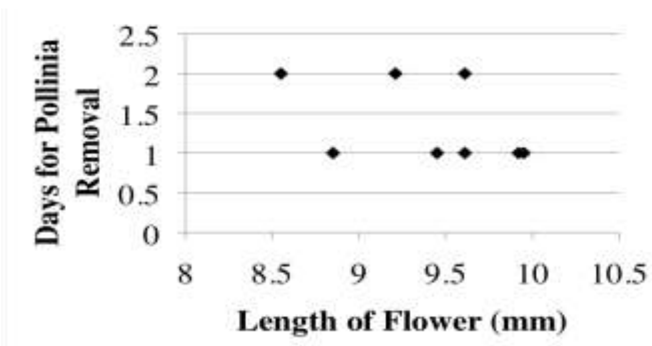

Figure 4b. There was no significant difference between flower length and days required for pollinia removal in yellow flowers (n=8).

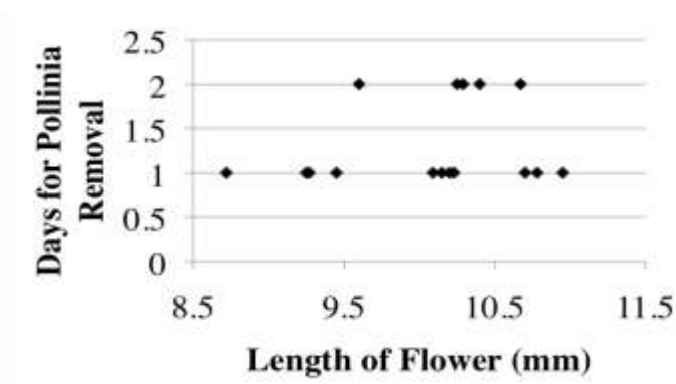

Figure 4c. There was no significant difference between flower length and days required for pollinia removal in YPP flowers (n=16).

Amount of Yellow Character in Combo Flowers

A regression analysis of flower length and area of yellow in combo flowers revealed no correlation and it was nonsignificant (R^2 = 0.0079, P = 0.67, n=25; Figure 5). A multivariate regression analysis of days required for pollinia removal versus area of yellow color and length in combo flowers was also not correlated and nonsignificant (R^2 = 0.12, P = 0.24, n=25).

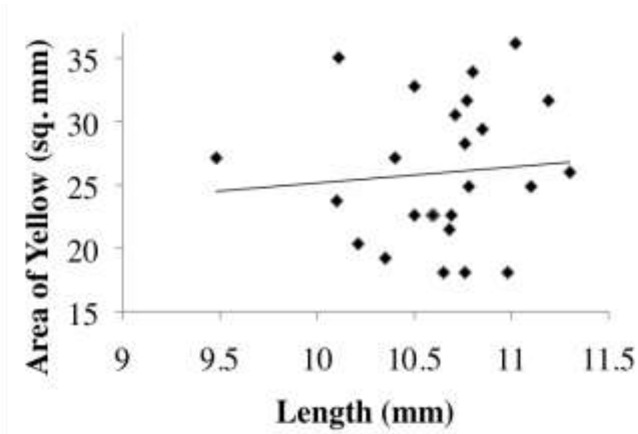

Figure 5. A regression analysis of flower length and area of yellow in combo flowers revealed no correlation and it was nonsignificant.

DISCUSSION

The differences in flower length between color morphs were significant, indicating that color may play an important role in the reproductive fitness of *P. sanchoi*. Consistent with the idea that yellow is the most attractive color for flowers to possess, yellow flowers were the smallest and combo flowers the biggest in length. Yellow flowered plants do not put as many valuable resources into making big flowers because all over yellow flowers have already been shown to easily attract pollinators (Strozinski 2001) and showing off this yellow color in large floral displays is not necessary. YPP flowers, which only have purple petals, are of medium size because they are mostly yellow and only need to be slightly larger than yellow flowers to compete with them reproductively. Yellow color affords yellow and YPP flowers a reproductive advantage over combo flowers, so making large floral displays is a waste of energy. Combo flowers were the biggest because they have to show the greatest amount of yellow area possible in order to attract pollinators, which would be hard to do if the flowers were small. Combo flowers make as large as flowers as possible to make up for the lack of being all yellow. Thus, putting costly resources into making large floral displays to attract pollinators is a consequence of not being all yellow, like YPP and yellow flowers who can make significantly smaller flowers.

This trend is also supported by the findings from Strozinski (2001) who found that combo plants have a greater density of flowers to leaves than yellow plants. This correlation suggests further that yellow is the most desirable color because combo plants consistently put out high densities of flowers per leaf to give the appearance of more overall yellow color in a small area.

While number of flowers per leaf was not part of the study, personal observation of combo plants usually having two flowers per leaf was noted and supports this idea.

Color also had a significant effect on the number of days required for pollinia removal to occur in *P. sanchoi*. There appears to be a pollinator preference for yellow flowers with purple petals, although yellow flowers showed a nearly equivalent mean number of days. Since pollinia were removed from these two color morphs more quickly than from combo flowers, this suggests that being yellow causes increased reproductive success. Faster pollinia removal followed by the subsequent act of setting seeds results in more reproductive changes to produce seeds each season or year. However, it should be noted that pollinia were eventually removed from all flowers, with the longest taking three days. Also, within the combo and YPP flowers, 13 individuals were preyed upon, all of which had pollinia removal rates greater than one day. Yet, the frequency of this predation on YPP and combo flowers was statistically random even though it begs the question of had these YPP flowers had been included, if the rate of pollinia removal for YPP flowers would have been slightly higher than the observed rate. If so, then pollinator preference would clearly be for yellow flowers, the most attractive color, in the sense that pollinia are removed from these flowers faster than from the other two color morphs.

While flower color had an influential effect on the number of days needed for pollinia removal, flower length appeared to have a minimal effect. Yellow and YPP flowers showed no trends to the eye between flower length and days required for pollina removal and this was statistically nonsignificant for both color morphs. This perhaps is explained by the small sample sizes of both yellow and YPP flowers, with n=8 and n=16 respectively. It could also be explained by the fact that yellow flowers are all yellow, YPP flowers are mostly all yellow and pollinators are primarily influenced by yellow color and less heavily by flower size. In combo flowers, there appears to be a trend to the eye that larger flowers had their pollinia removed faster, but there was only a nearly significant effect of length on pollinia removal. It seems probable that combo flowers of *P. sanchoi* would show a relation between flower length and days for pollinia removal because of the role that yellow character plays in attracting pollinators. Bigger flowers, with more yellow exhibited, would attract pollinators faster, causing the number of days after which pollinia removal occurs to be low.

The importance of yellow coloration is further illustrated by the nonsignificant correlation between flower length and area of yellow color in combo flowers. The amount of yellow area in combo flowers was extremely variable across all flower lengths. This evidences how important yellow coloration is because even the smallest combo flowers produce flowers with comparable or greater yellow area than the largest combo flowers and yellow area is just not a result of larger flower size. However, the effect of length and amount of yellow area on pollinia removal rates was nonsignificant. Flower length and amount of yellow area in combo flowers of *P. sanchoi* are important factors in overall pollination success, but they may not be as important as other floral characteristics such as scent or nectar. In *P. sanchoi*, Drosophila-like flies are the hypothesized pollinators and they are often found near decaying fruit or other decomposing vegetation (Dressler, 1990). Flowers that are dark colors, such as brown, purple, and green, are similar in appearance to decomposing material and are more attractive to these flies. Purple and YPP flowers both resemble this characteristic and they may give off some scent of decomposing matter that further attracts pollinators to them, even though they are not yellow (Goldberg and Nelson 1999).

Although subtle, it appears that of the factors studied, color has the greatest effect on reproductive success in *P. sanchoi.* Even though pollinia were removed the fastest from YPP-flowered plants, nearly equal rates were observed in yellow-flowered plants and small sample size was most likely the reason for this. Similarly, yellow-flowered plants were found to have a slight overall advantage in reproductive success in Strozinski (2001) because they tended to be larger and received the attention of pollinators sooner. However, when variations exist in flower color, as in *P. sanchoi*, then the most attractive or frequent color morph should be favored (Tremblay and Ackerman, 2007). Thus, yellow should be the only color variation in *P. sanchoi* if pollinator selection is consistent (Subramaniam and Rausher, 2000).

There are several explanations to why all three color morphs persist in the population. One possible reason is that color may not be genetically determined, but environmentally determined by light levels or substrate condition. Goldberg and Nelson (1999) and Lee (2000) examined if varying light levels affected flower color, but their studies were inconclusive. A second possibility is that YPP flowers and combo flowers make up for the lack of being all yellow in other ways. They may compensate in ways like faster growth and development, seed number, relative viability, longevity and fertility (Niklas, 1997). Another possible reason is that the commonly cited pollinators for the subtribe Pleurothallidinae, Drosophila-like flies, are not really the only exclusive pollinators. Not all pollinator species respond to the same floral characteristics of a given flower in the same way (Schaefer et al. 2004), especially for species that attract an array of pollinator taxa (Waser et al. 1996). If this is the case in *P. sanchoi*, various colors could persist in a the population if the different species of pollinators had different color preferences.

A longer investigation with greater sample sizes for all colors would have lead to more conclusive results and is a possibility for future research. To further understand the pollination system of *P. sanchoi*, future research should include investigations related to why all three color morphs persist in the population and if combo flowers offer some reward, such as nectar, or if they use some other floral characteristic such as scent to attract pollinators.

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LITERATURE CITED

Brody, A.K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology 78(6): 1624-1631.

Dod, D. D. 1986. Áfidos and tripidos polinizan orquídeas en las Pleurothallidinae (Orchidaceae). Moscosoa 4: 200-202

Dressler, R.L. 1993. Field guide to the orchids of Costa Rica and Panama. Cornell University, New York: 7-21, 156.

Dressler, R.L. 1990. The orchids natural history and classification. Harvard University Press, Cambridge: 98, 103, 125.
Gegear, R. J. and T.M. Laverty. 2001. The effect of variation among floral traits on the flower constancy of pollinators. *In* L. Chittka and J.D. Thomson (Eds). Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution, pp. 1-20. Cambridge University Press, Cambridge.
Glaetti, M. and S. Barrett. 2008. Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae). New Phytologist 179(4): 1193-1201.
Goldberg, D. and E. Nelson. 1999. Differences in vegetative and reproductive characteristics of the yellow and purple flower morphs of *Pleurothallis segoviensis* (Orchidaceae). UCEAP Tropical Biology, Fall 143-153.
Jones, K. N. and J.S. Reithel. 2001. Pollinator-mediated selection on a flower color polymorphism in experimental populations of Antirrhinum (Scrophulariaceae). American Journal of Botany 88: 447–454.
Lee, C. 2000. Pollination in *Pleurothallis segoviensis* (Orchidaceae): color and density effects. CIEE Tropical Ecology and Conservation, Spring: 1-8.
Niklas, K.J. 1997. The evolutionary biology of plants. The University of Chicago Press, Chicago: 3-30.
Omura, H. and K. Honda. 2005. Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. Oecologia 142: 588–596.
Rodríguez, I., A. Gumbert, N.H. de Ibarra, J. Kunze, and M. Giurfa. 2004. Symmetry is in the eye of the 'beeholder': innate preference for bilateral symmetry in flower-naive bumblebees. Naturwissenschaften 91: 374–377.
Schaefer, H.M., V. Schaefer, and D.J. Levey. 2004. How plant-animal interactions signal new insights in communication. Trends in Ecology and Evolution 19: 1721-2003.
Strozinski, S. 2001. Effects of plant size and flower color on the reproductive success of *Plerothallis sanchoi* (Orchidaceae). CIEE Tropical Ecology and Conservation, Fall: 109-119.
Subramaniam, B. and M.D. Rausher. 2000. Balancing selection on a floral polymorphism. Evolution 54: 691-695.
Tremblay, R.L. and J.D. Ackerman. 2007. Floral color patterns in a tropical orchid: Are they associated with reproductive success? Plant Species Biology 22(2): 95-105.
Waser, N.M., L. Chittka, M.V. Price, N.M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77: 1043-1060.