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Research Project- Final Draft

Introduction: Orchids have adapted varying forms of mimicry to attract pollinators. Some imitate the scent or appearance of nectar producing flowers, while they don't produce nectar themselves. Others mimic the scent and appearance of female bees or wasps to attract male pollinators and ensure that the male will stay on the flower long enough to collect pollen needed for fertilization. Studies show ants pollinate some orchids. *Epipactis thunbergi* is an orchid that has ants as visitors 40% of the time, compared with only 10-20% of the orchids primary pollinator, the hover fly. These Japanese carpenter ants have been shown to transfer pollen to the stigma of the flower. However, research shows that the ants may damage the pollen grains, decreasing pollination success in comparison to the hover flies (Suigara, 17). Recent research has shown adaptations of some plant species to repel ants that may cause damage or deter pollinators. The researchers conducting this study chose four plant species where ants were observed to observe. They counted the frequency and duration of pollinator visits to the flowers and found that the pollinators' visitation and duration decreased when ant activity was present (Junker 665). After further study, they found that half of the flower species had evolved a method of repelling ants. This is likely a response to the negative impacts of ant activity on pollinator visits. While there appears to be some debate over whether ants are harmful or beneficial to plant pollination, it is clear that their presence has an impact on plant species they inhabit.

There is ample research on the various methods of pollination orchids employ, and a wide variety of methods used by researchers to obtain data on the subject. A study of *Cypripedium parviflourm (*Lady's Slippers) showed that pollinators are capable of learning the

difference between deceptive orchids and the rewarding plants they mimic. They paired budding orchids into a treatment group and a control group and altered the flowers in the treatment group to trap visiting insects and observed their behavior exiting the flower to determine how pollen was transferred. This study shows how effective the orchids adaptations for delaying insects' exit helps ensure transfer of pollen for reproductive success (Case 1-3).

A similar study focused on the floral cues between species that flower at similar times in relation to rewarding vs. nor-rewarding species of flowers. By altering artificial inflorescences and observing their effects on pollinating bumblebees, researchers determined that when orchids that were non-rewarding flowered after the rewarding species they mimic, they were visited four times more than if they flowered before or at the same time (Internicola, 1358). This shows an adaptation of orchids to make sure they have the best chance at pollination by timing their blooms to occur after the species they mimic. Other studies focus more on the structure of the plant itself as an adaptation to maximize pollination by bees (Dressler 182). Some research analyzes the co-evolution of the plant and pollinator by focusing on physical traits, abundance of pollinators and sustained interactions between orchid bees and the flowers they pollinate (Roubik, 321). This study has found links between the evolution of floral traits and the evolution of bees to adapt to these traits.

J.M. Gomez's research on ants as pollinators of the Mediterranean perennial herb *Lobularia maritima*, shows that a specific ant species, *Camponatus micans,* was the primary visitor to blooms during the summer and that almost all ants had pollen present on their bodies as they traveled from bloom to bloom. This shows that some ant species play a large role in pollination. They project further explored the effect of ants on plant reproduction by studying the ants' impact on seed production, germination and overall plant fitness of progeny (Gomez 92).

I hypothesize that pollinators found on orchids in bloom will vary depending on the type of orchid and whether or not the flower mimics the specific pollinator. Orchids that mimic a specific type of pollinator will have a higher number of that pollinator than other orchids.

- **Materials and Methods:** Upon arrival in Belize, I realized that the orchids I had originally chosen to focus my research on were not present in the area of Sibun and most orchids available were not in bloom. I had to adapt my proposed methods to focus on the species of orchids that were available for observation. I found three species in the orange g**roves** of Sibun to focus my research on*: Notylia barkeri, Oncidium sphacelatum,* and *Bulbophyllum pachyrachis (*rat-tail orchid). These orchids were observed found in the citrus grove on the Sibun property, and also on the citrus grove where we did orchid relocation. To observe and note the type and number of pollinators present, I set up a grid in my data notebook and recorded the type of orchids and pollinators present on each tree in the grid. There were fifteen trees with orchids that I observed at Sibun. I noted whether or not each orchid was in bloom. I also noted the presence/ absence of four categories of pollinators; ants, bees/ winged pollinators, spiders or other possible pollinators. I chose to add spiders to my original list of pollinators because I noticed the presence of spiders on several orchids. I recorded data for each tree in the grid, and I further expanded my data using the same method at the other location for four trees with orchids in bloom.

Results: The following table shows the results I recorded upon observing orchids in two locations: Sibun citrus grove (Sibun), and the citrus grove (Grove).

Sample #	***Location***	***#Orchids present***	***Inflorescence***	***Ants***	***Bees/wasps/flies***	***Spiders***	***Other***	***Notes***
1	Sibun	4	None	No	No	Yes	No	
2	Sibun	2	None	Yes	No	Yes	No	
3	Sibun	8	Yes (1)	Yes	Yes	Yes	Yes	Beetle
4	Sibun	9	Yes (2)	No	Yes	Yes	No	
5	Sibun	4	None	Yes	No	No	No	
6	Sibun	3	None	Yes	No	Yes	No	
7	Sibun	5	None	No	No	Yes	No	
8	Sibun	6	None	Yes	No	No	No	
9	Sibun	3	None	No	No	Yes	No	
10	Sibun	4	Yes (1)	No	Yes	No	Yes	Beetle
11	Sibun	2	None	Yes	No	Yes	No	
12	Sibun	6	None	Yes	No	No	No	
13	Sibun	5	None	No	No	Yes	No	
14	Sibun	3	None	Yes	No	No	No	
15	Sibun	7	Yes (1)	No	Yes	No	No	
16	Grove	10	Yes (3)	Yes	Yes	No	No	
17	Grove	6	Yes (1)	Yes	No	No	No	
18	Grove	5	Yes (1)	Yes	No	No	No	

19	Grove	8	Yes (2)	Yes	No	No	No	
20	Grove	11	Yes (3)	No	No	Yes	No	
21	Grove	6	Yes (2)	Yes	Yes	No	No	
22	Grove	4	Yes (1)	Yes	No	No	No	
23	Grove	8	Yes (2)	No	No	Yes	No	
24	Grove	3	Yes (1)	Yes	No	Yes	No	

I found that the original hypothesis could not be tested with enough data because the orchids I found in bloom were not the type whose blooms mimic pollinators. However, I added spiders to my original list of possible pollinators of orchids because I noticed that spiders or their webs were present on most of the orchids observed. Flying pollinators such as bees, wasps and flies were always found on orchids that were in bloom and not on orchids that were not in bloom. Ants were found on more than half of the orchids observed, but their presence seemed unrelated to the presence of inflorescence. The presence of other pollinators was rare and seems unrelated to the orchids themselves.

Conclusion: After arriving in Belize and discovering that I could not adequately test my original hypothesis due to lack of available samples, I modified my hypothesis to fit the data I could collect in my time in Belize. The new hypothesis is, “Orchids use many different species of insects as possible pollinators. The presence of pollinators such as ants and spiders plays a role in orchid pollination.” Upon collection of the data shown in the previous table, I observed that ants were present on 15 out of 25 samples. Only 8 of those samples had inflorescence. Therefore, I conclude that ants are likely pollinators of orchids since they are present on more than half of the

samples observed, but they likely are not influenced by the presence or absence of blooms, and do not seem to take part in deception by orchids to lure pollinators. Bees, wasps and flies were only present on 6 samples, and all of those samples contained inflorescence. Based on this data, I conclude that winged pollinators are only attracted to orchids in bloom, which shows that they are lured in either by the presence of nectar or by the bloom itself. I observed that the blossoms on *Notylia barkeri* emitted a strong scent resembling vanilla, while the blossoms of *Oncidium sphacelatum* are shaped to resemble possible pollinators. Both of these factors are likely to contribute to attracting winged pollinators to the inflorescence, thus resulting in the spread of pollen to other orchids in bloom. Spiders and/ or their webs were observed on 12 of 25 samples, making them a likely accidental pollinator of the orchid species studied. I saw no correlation between the presence of spiders and the presence of inflorescence. Based on my observations, I hypothesize that spiders are likely to use orchids as locations for constructing their webs because of the presence of insects such as ants, bees, wasps and flies that serve as prey to spider species. Spiders also do not seem to be influenced by common methods of deception used by orchids to lure pollinators, but are indirectly attracted to these deceptive tactics because they are attracted to the pollinators lured by the orchids. The presence of other possible pollinators was minor, only on 2 samples, and seems to be unrelated to the orchids. I would like to do further research on ants and spiders as pollinators of orchids. I would choose two or three species of orchids to study, and note the presence/ absence of ants in correlation to the presence and type of inflorescence. I hypothesize that ants are also pollinators of many orchid species. I would like to conduct further research to determine which orchids ants pollinate regularly and what characteristics those orchids have that may attract ants to them. I also hypothesize that spiders are accidental pollinators of orchids because of their presence on the blooms to catch prey insects

that are attracted to orchid inflorescence. I would like to conduct further research on this hypothesis by observing spiders on orchids and determining if they collect and transfer pollen through their behavior and presence on orchids.

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