November 2005

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ABSTRACT
This study examines the effect of patchy distribution on the production and concentration of nectar of *Habenaria monorrhiza* at Monteverde, Costa Rica. Sixty patches of *H. monorrhiza* were identified; patch size ranged from one to six individuals. One group of patches (N=24) was covered to prevent pollination and were examined for the amount and concentration of nectar. Another group of patches (N=36) were left uncovered and were tested to determine the effect of patch size on visitation rates of pollinators. There was no significant effect of patch size on the amount or concentration of nectar produced ($F = 1.3039$; $p = 0.2568$; df total = 35). However, increasing patch size significantly increased the percent of pollinia removed on a plant ($F = 45.1034$; $p < 0.0001$; df total = 35). Furthermore, many flowering plants depend on the nectar production of their neighbor to attract pollinators, but *H. monorrhiza* was proven in this study not to regulate the amount or concentration of nectar. It does, however, depend on its large patch size to increase visitation by its moth pollinator.

RESUMEN
Este estudio examinó el efecto de la distribución en grupos aislados sobre la producción y la concentración de néctar de *Habenaria monorrhiza* en Monteverde, Costa Rica. Sesenta parches de *H. monorrhiza* fueron identificados; el tamaño de los parches varió entre uno y seis individuos. Un grupo de parches (N=24) fue cubierto para prevenir la polinización y fue examinado para determinar la cantidad y la concentración de néctar. Otro grupo de parches (N = 36) no estuvo cubierto y fue examinado para determinar el efecto del tamaño de grupo sobre la frecuencia de visitas por los polinizadores. No se encontraron diferencias significativas en el efecto del tamaño del parche sobre la tasa de visitas de los polinizadores ($F = 1.3039$; $p = 0.2568$; df total = 35). Sin embargo, el aumento del tamaño del parche incrementó significativamente el porcentaje de polinia removido en una planta ($F = 45.1034$; $p < 0.0001$; df total = 35). Muchas plantas con flores dependen de la producción de néctar de su vecino para atraer al polinizador, pero se demostró que en el caso de *H. monorrhiza* no hay regulación de la cantidad o la concentración de néctar. No obstante, *H. monorrhiza* depende del gran tamaño del parche para aumentar el número de visitas de los polinizadores.

INTRODUCTION
Habenaria monorrhiza is part of the largest genus of orchids in the neotropics with over 500 known species (Gentry 1993). This is a primitive group in which little is known. This lack of knowledge includes, but is not limited to the pollination of *Habenaria*, although the phenotypes of most species in this genus indicate that they are moth-pollinated (Zomlefer 1994; Dressler 1990). Through observation, it is known that *Habenaria monorrhiza* share many of these traits, therefore is also presumed to be moth-pollinated (Walter 1983). The most noticeable such qualities are the large raceme inflorescences that feature 2-15 spirally arranged flowers that bloom synchronously (Hammel et al. 2003). They have a patchy distribution, meaning they grow in clumps, and are found in disturbed, open areas along roadsides and in grassy habitats (Mora and Atwood 1992). These open areas make flowers easy for a moth to find when foraging. The lip of the flower is deeply dissected into 3 linear lobes, which create a good landing

pad for the pollinator (Mora and Atwood 1992). *Habenaria monorrhiza* has a large, specialized spur (up to 25 mm), which contains nectar (Fig. 1a)(Zomlefer 1994; Gentry 1993). The long proboscis of a moth matches this morphology well. Both sectile pollinia are supported on a slender caudicle, which is about 5 mm long (Mora and Atwood 1992). When a moth stops for nectar, the rostellum that surrounds the pollinia (Fig. 1b) gets stuck to the wings or the face of the moth. If the pollinator continues to search for nectar, pollen from the pollinia will be deposited on the stigma of another flower, thus beginning the reproduction process (Mora and Atwood 1992; Zomlefer 1994).

The nectar production of *Habenaria monorrhiza* has also been understudied. However, other wild plant populations have been found to produce flowers with little nectar if a neighbor is already producing large amounts of nectar (Jones and Little 1983). Also, the concentration of nectar for some flower species can be highly variable between individuals depending on their distribution and location (Howe and Westley 1988). It is possible that a similar relationship between spatial distribution and nectar production occurs in *H.* monorrhiza. In theory, larger patches of *H. monorrhiza* will offer more nectar to a pollinator than a single inflorescence. Nectar production, which is presumably energetically expensive, should be avoided if possible, so long as pollinator visitation is not negatively affected. If moths are attracted to larger patches containing more flowers and hence more nectar, it may be possible for *H. monorrhiza* to reduce nectar production without sacrificing pollinator visitation. Therefore, to increase fitness by saving energy, it is predicted that flowers within larger patches of *H. monorrhiza* will produce less nectar at lower sugar concentrations. Additionally, if these larger patches are more desirable to pollinators, then, once said pollinators are present, they will tend to stay within that spatial range, and visit nearby inflorescences (Howe and Westley 1988). Both nectar production and spatial distribution are proposed methods to increase out-crossing, which is the favored pollination mechanism employed by orchids. (Walter 1983).

This study investigates the effect of spatial distribution on nectar production and concentration, as well as pollinator visitation. Plants cannot control how they grow with respect to members of the same or other species. Therefore, when attracting a pollinator, an individual may have to compensate for being in a bad location. Likewise, an individual may be offered the opportunity to take advantage of being in a good location. One solution is to accordingly alter nectar volume and concentration. The prediction is that patches with more plants will produce, on average, a lower amount and concentration of nectar. This is contrary to single plant patches, in which nectar is predicted to be produced in higher amounts and concentrations to attract pollinator visitation. Lastly, a higher percentage of pollinia are predicted to be missing from *H. monorrhiza* flowers that occur on inflorescences in larger patches than on flowers on an inflorescence in a single plant patch. This is predicted because larger patches should attract more pollinators, and, because they contain larger amounts of nectar, moths should tend to remain in them.

METHODS

Study site and determination of patchiness

This study was conducted in Monteverde, Puntarenas Province, Costa Rica from October 22 to November 15, 2005 along the roadside between the Estación Biológica and Hotel Belmar. The first step in this experiment was to define patches within the study site. The distance between each plant and its nearest neighbor was estimated with a tape measure (cm). These measurements were then averaged. “Patches” were defined as a group of two or more plants that were as close or closer to each other than the mean distance (150 cm).

Nectar volume and concentration

To be included, an individual had to have at least three open, unpollinated flowers on its inflorescence. Each inflorescence on a chosen plant was covered using a mesh pollination bag, so that flowers were not visited during the course of the experiment. The height of nectar in the spur of each flower was measured; this entailed removing the three uppermost flowers from each plant and holding a flashlight behind the spur. Subtle lines were exposed on the spur, which marked the height of the nectar within; this was then measured using calipers (mm). Next, each spur was cut open, exposing the nectar. The nectar was transferred to a refractometer to determine sugar concentration. The concentration and volume of nectar were compared between plants in different patch sizes. A regression analysis was used to compare these relationships. The total number of patches of bagged individuals equaled 24 and the total number of individuals equaled 66.

Rate and Visitation

A second set of patches was found using the same criteria above. The only difference was that, to be eligible for this set, the inflorescences only needed to have one open flower, and it could be pollinated. These plants were used to study visitation by pollinators, instead of for nectar observations. Therefore, plants in this set of patches were not bagged. Using a 10X hand lens, the presence or absence of pollinia was noted for each flower on each inflorescence. Patch size was recorded, and the relationship with the percentage of flowers with missing pollinia was analyzed with a regression analysis. If one or two pollinia were absent, the flowers were considered to have been pollinated, because the moth pollinator frequently only removes one pollinium per visit (Singer 2001). A regression graph was used to compare the relationship between the average percent of pollinia missing within a whole patch and patch size; the average percent of pollinia missing for each flower on an individual plant within a patch and patch size was also analyzed. Ninety three total inflorescences, comprising 36 total patches, were observed.

RESULTS

The height of nectar in the spur, the percent concentration of the nectar sampled, and the patch size were regressed. There was no significant relationship found between these parameters ($F = 1.3093$; $p = 0.2568$; df total = 65)(Fig. 2). The total average percent of pollinia removed within a patch and patch size showed a significant correlation ($F = 45.1034$; $p < 0.0001$; df total = 35)(3a.). The relationship between average percent pollinia removed per inflorescence and patch size yielded was also found to be significant ($F= 36.3948$; $p< 0.0001$; df total = 92)(3b.).

DISCUSSION

The results proved the first hypothesis (larger patches would have less nectar amounts and concentrations) to be insignificant (Fig. 2). This means that, since growth patterns are random, single plant patches must find other ways to compensate and attract a pollinator. However, the latter hypothesis, which stated that patch size affects the rate of pollinator visitation, was supported. Percent pollinia removed, when compared to patch size, was significant (Fig. 3). This suggests that pollinator visits increase along with patch size.

Once again, we know that nectar production is presumed to be energetically costly to a plant. For example changing the different types of specialized sugars, depending on the particular pollinator that a plant is trying to attract, this can change seasonally in some species (Howe and Westley 1988). Since there was no trend in the nectar production for different patch sizes, it must be concluded that *H. monorrhiza* does not use nectar regulation in order to save energy. Even though this trend has been seen in other plant species, nectar production for *H. monorrhiza* was so highly variable that it was apparently independent of patch size. One reason that *H. monorrhiza* does not put energy into nectar regulation could be that it can self-pollinate; this means that no pollinator is necessary in order for the plant to reproduce (Tannourji 2000). Most orchids are self-compatible, but their structures tend to favor out-crossing (Dressler 1992). Since this act of selfing is possible, *H. monorrhiza* does not need to depend on nectar regulation to compensate if it does not grow in a patch. Reproduction is still possible without pollination; there have been many cases of solitary orchids that self- pollinate in the absence of other means of reproduction (Dressler 1992). In addition to alternative forms of reproduction, *H. monorrhiza* has other methods of attracting pollinators.

Since missing pollinia imply that a flower has been visited by a pollinator, the observed results suggest that larger patches have more success with pollination (Fig. 3). Not only were a higher percentage of pollinia missing as patch size increased as a whole, but individuals within larger patches also showed a significantly higher rate of visitation. This could be due to the foraging habits of pollinating moths. It has been found that once a pollinator finds a plant, the pollinator is more likely to visit the next closest plant than a further one (Howe and Westley 1988) Thus, a patchy distribution may be an effective way for *H. monorrhiza* to increase visitation by a pollinator to an individual inflorescence. Once the pollinator has been drawn to the patch by nectar cues, all individuals that are lucky enough to be in that patch are likely to be visited.

In conclusion, self-pollination could be a reason that nectar production is not regulated. *Habenaria monorrhiza* does not expend energy to change the amount or concentration of nectar it produces in accordance with its spatial distribution, because it has alternative forms of reproduction in the worst-case scenario (not being pollinated by an external source). Instead, *H. monorrhiza* depends on larger patches to maximize pollinator visitation. Further studies could review the rate of selfing as compared to out-crossing, in accordance with patch size. Furthermore, since the inflorescences of *H. monorrhiza* are so variable, the size of inflorescences and their effect on foraging behavior could be explored.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank Karen Masters for always telling me how well I am doing. Also my beloved Alan Masters for always being so hip and for tons of hugs! Jointly I would like to thank Karen and Alan for providing all those delicious snacks to us rascals. Thanks to Ollie and Marie for answering all my really SMART! questions and for supporting me in all my spinning endeavors. I can't forget Javi, for making my neck purple every time he wrang it. A special thanks to Christian "The Weedwacker" for not cutting down too much of my sample size. Last but not least Estación Biológica and Costa Rica for revealing all its beauty and magic.
P.S. Bottton Feeders stink!!!

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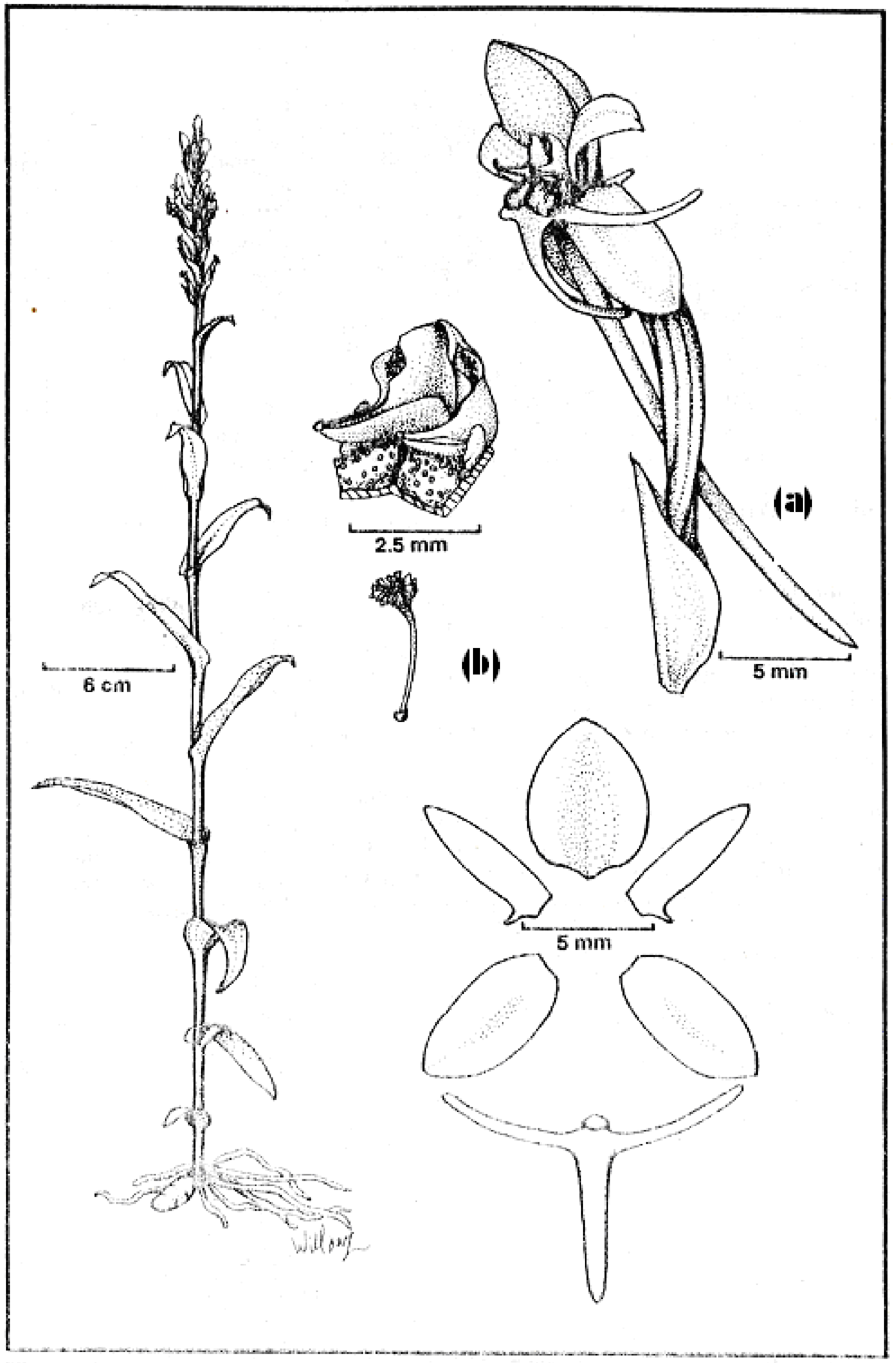
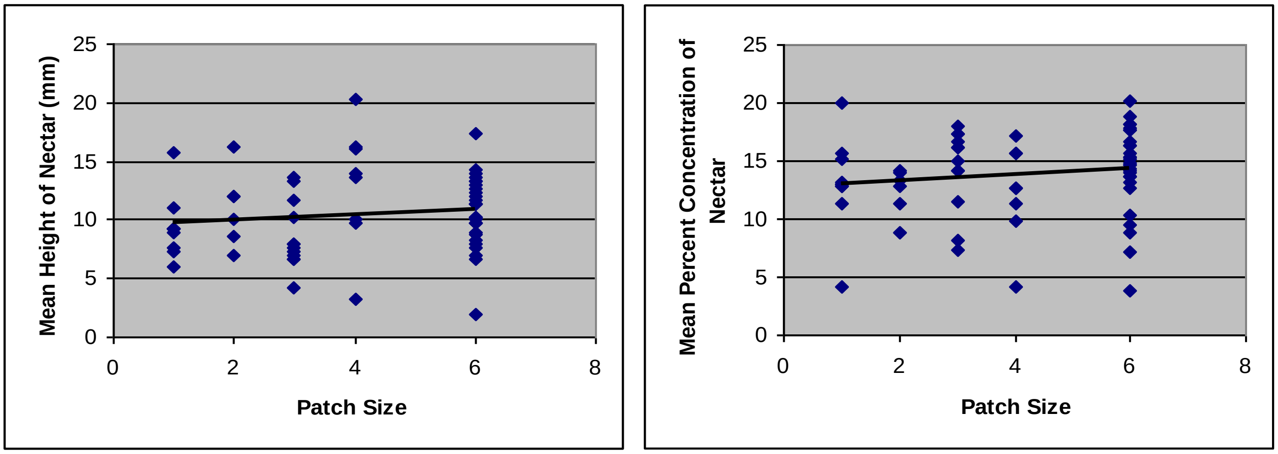
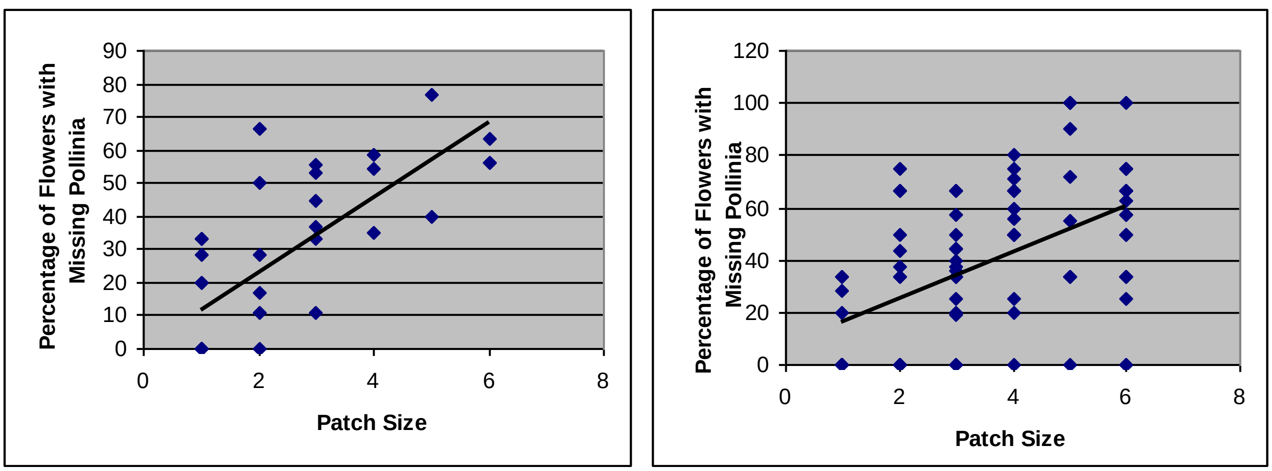


Figure 1. Vegetative and reproductive structures of *Habenaria monorrhiza* (a) specialized spur (25mm) containing nectar of plant (b) pollinia (from Mora and Atwood 1992).



a. b.

Figure 2. See text for details on experiment setup. Patch size equals the number of individual inflorescences that are 150cm or closer from each other (a) Mean height of nectar (mm) between flowers for one plant within a patch vs. the size of patch in which it was present (b) mean percent concentration of nectar between flowers for one plant within a patch vs. the size of patch in which it was present.



a. b.

Figure 3. See text for details on experimental setup. Patch size equals the number of individual inflorescences that are 150cm or closer from each other (a) percent of flowers with missing pollinia per patch, with data from total number of flowers on all plants in patch averaged, vs. the size of patch (b) percent of flowers with missing pollinia per plant, with data from all flowers on a plant averaged vs. the size of patch plant was present in.