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The effects of spatial distribution on the proportion of pollinia removed in Epidendrum radicans (Orchidaceae)

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ABSTRACT

Many plants that do not offer rewards for pollination are involved in a floral mimicry. Mimic flowers copy visual cues from unrelated model species that offer rewards and rely on the chance visitation by confused pollinators. *Epidendrum radicans* is a mimic plant that strongly resembles two plants that provide rewards: *Asclepias curassavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae). This study examines how spatial distributions affect the proportion of pollinia removed (PPR) for the non-rewarding mimic *Epidendrum radicans.* Nearest neighbor distances between infloresencces, total number of flowers, and total number of pollinia removed were recorded at thirty patches of *E. radicans* in Monteverde, Costa Rica. A wide range of patch densities was found along with a negative relationship between proportions of pollinia removed (PPR) and density. Extremely large patches of *E. radicans* did not follow the negative trend between PPR and density. This could be due to multiple visits from naive pollinators who have not realized that no reward is offered or the possibility of multiple visits by pollinators who are reassuring themselves of the non-rewarding characteristic. A patchiness index was also calculated for each patch. However, no conclusions could be made about the relationship of proportion of pollinia removed (PPR) and the index of patchiness. Although *E. radicans* is involved in a floral mimicry, the data did not suggest that the mimic grows in close proximity to its models.

RESUMEN

Muchas plantas que no ofrecen recompensa a sus polinizadores imitan a otras flores que si ofrecen una recompensa. Las plantas que juegan este papel copian ciertas características visuales de otras plantas *y* dependen del los polinizadores se confundan y las visitan casualmente. *Epidendrum radicans* es una planta que imita a: *Asclepias curassavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae), dos que dan recompensa. Este estudio examinó como la distribución del espacio afecta el porcentaje de polinia que esta sacado de esta flor. La distancia del vecino más cerca de las misma especie, el número total de las flores, y el número total de polinia sacado fueron apuntados en treinta parcelas de *E. radicans* en Monteverde, Puntarenas, Costa Rica. Había una gran variedad de las densidades y se encontró una relación negativa entre el porcentaje de polinia sacado (PPR) y la densidad de las plantas. Se puede explicar las visitas múltiples de los polinizadores ingenuos que no se han dado cuenta que no hay una recompensa o la posibilidad de las visitas múltiples por polinizadores que se están asegurando a si mismos de que la flor no tiene recompensa. Las parcelas muy grandes no siguen la tendencia negativa entre el porcentaje de pollinia quitado (PPR) y la densidad. Una índice de desigualdad fue calculada por cada parcela. Sin embargo, no se encontró una diferencia significativa entre el índice de desigualdad " patchiness" y el número de

polinia sacado. Aunque *E. radicans* esta imitado a las otras flores, este estudio encontró que no crece muy cerca a las plantas que se esta imitando.

INTRODUCTION

Visual cues, olfactory cues and the presence of collectable, consumable or usable substance are all characteristics used by plants to attract pollinators (Ackerman 1994). Among the vast number of plants in the world, there are some flowering plants that do not present a pollinator reward and instead use some form of deception to attract pollinators (Ackerman 1994). One form of deception is floral mimicry. This idea suggests flowers without rewards mimic flowers with rewards. However, it is believed that pollinators are likely to visit the model more frequently and spend more time at a model flower than a mimic flower (Haber 1984). Non-rewarding plants attract pollinators by presenting the same cues that a pollinator innately recognizes (Haber 1984) and its flowers are pollinated by the number of "mistake" visits due to imperfect discrimination by the pollinators (Agren 1991).

Floral mimicry characteristics are frequently found among the 20,000 species in the family Orchidaceae (Ackerman 1994). One example of floral mimicry in this family is *Epidendrum radicans* (Orchidaceae). *Epidendrum radicans* is a common roadside weed specie that occurs throughout the Neotropics from 1,000 to 2,000 meters (Deacon 2000) and bears a simple inflorescence with flowers that vary in color from yellow to red (Figure 1). After a flower has been pollinated, the lip (normally a much lighter orange than the sepals and petals) turns darker, becoming less attractive to pollinators (Todzia 1983). It ranges from Mexico to Panama and blooms more or less through-out the year (Todzia 1983). *Epidendrum radicans* grows terrestrially in dense masses on open soils, rocks, and brushy banks. Long white roots are usually found on the bases of the newer leaves. This plant is commonly known for its resemblance with two nectar producing plants: *Asclepias curassavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae) (Todzia 1983). Since *Epidendrum radicans* offers no reward and it obtains visits by resembling other nectar producers, it is considered a Batesian mimic (Bierzychudek 1981). Although *E. radicans, A. curassavica,* and *L. camara* bear the same color flowers and attract the same butterflies, *E. radians* attracts far fewer pollinators than the model it is trying to mimic (Dressier 1993). There have been only a few studies examining the distribution of all of three and how the proximity affects pollinia removal of each other.

In order to compensate for the lack of a reward, *E. radicans* flowers possess other adaptations for infrequent visitation. Single flowers can last up to 10 days and produce as many as 0.5 million seeds. Pollen of *E. radicans* is packaged into single pollinia that attaches to the probiscis of the pollinator. This enables pollinators to visit other species without displacing the pollinia (Bicrzychudek 1981) because different plants attach pollinia to different parts of the pollinator's body.

Another factor that affects the fitness of an *E. radicans* plant is the spatial location between it and other conspecifics. Dense stands of *E. radicans* could provide the opportunity for a pollinator to realize there is no reward and leave before visiting a number of flowers (Bierzychudek 1981). This idea is supported by evidence suggesting that pollinators visiting flowers lacking rewards are less likely to visit an adjacent flower than to leave the patch (Ackerman 1994). Thus, individuals in dense stands of floral mimics may not successfully reproduce.

Similarly, it has been suggested that larger patches of *E. radicans* would negatively affect pollination and the proportion of pollinia removal (Deacon 2000). Nevertheless, there is reason to believe that a large patch of *E. radicans* may in fact increase the probability of reproductive success in certain circumstances. For instance, a larger patch may increase the number of visits by pollinators because as large patches, they are more noticeable and attractive to visitors. Consequently, an increase in number of visits would increase the proportion of pollinia removed. In another circumstance, the sheer increase in patch size could increase the overall number of pollinia removed, even if the proportion of pollinia removed is not affected.

This study will observe the spatial distribution of *E. radicans* flowers and examine the effect of density and patchiness on pollination success. Traditionally, researchers have focused on the female component of reproductive success by measuring fruit size and seed production (Wolfe 1987). However, this study will base pollination success by quantifying pollinia removal because the pollen grains of *E. radicans* are packaged in a pollinaria which can be easily observed and counted (Wolfe 1987).

I expect the proportion of pollinia removed (PPR) will increase as density increases. Furthermore, patches with decreasing nearest neighbor distances or an increase in patchiness will have a positive relationship with the proportion of pollinia removed. In addition, *A. curassavica* and/or L. *camara* will be positively associated with *Epidendrum radicans.*

METHODS

Study site- This study was conducted in Monteverde, Puntarenas Province, Costa Rica from April 9 to May 10, 2001. Natural patches *off E. radicans* were located along the roadsides of Monteverde, on the road to San Luis and to Tilarán, Guanacaste Province.

A patch was defined as a cluster of flowering *E. radicans* that had a minimum distance of 10 m from the outer most inflorescence of the patch to another *E. radicans* inflorescence in every direction. The nearest neighbor distance for each inflorescence was recorded by locating the nearest inflorescence and measuring the distance between the two in cm. The total number of flowers was quantified by counting all flowers on all inflorescences in a patch. The total number of pollinia removed was also recorded. To calculate the area of the patch and make comparisons of different patches possible, the following method was used. An ellipse was drawn around the area filled with inflorescences. The radius was calculated by taking the mean of the length and width of each area filled with inflorescences. Then a larger ellipse was drawn at a distance of 10 m from the perimeter of the area of inflorescences labeled as total area. The area of the entire ellipse was calculated as πr^2, where r= 10 m + the radius of the patch (Figure 2).

The presence of A. *curassavica* and *L. camara* (in flower and not in flower) was also recorded for each patch. Each patch was scanned for flowers of both species by walking back and forth till the entire area of the patch had been covered. If flowers were not present, the structure of possible model plants was carefully examined. The presence of milky white latex was used as the determining characteristic of A. *curassavica* and a square stem was used as the determining characteristic of *L. camara.*

In extremely large patches of *E. radicans* with more than 1,000 flowers, a sub-sample was taken because of the pressure for time and efficiency. A 10 x 10 m sub-patch was chosen for study based on these criteria: it seemed to be representative in terms of the average density of the entire patch and it was easy access. The same observations were recorded using the same methods outlined previously. In these circumstances, the total area was calculated. The total number of flowers and total number of pollinia removed was estimated by the number of times the 10 x 10 m sub-patch could fit into the area of the actual patch.

An index of patchiness was determined by mean nearest neighbor distances and was calculated as $m=l/(4r^2)$, where m= density per unit area and r= mean distance between nearest neighbor (Southwood 1954). A chi-squared test was used to test the association of the presence of *A. curassavica* and *L. camara* with *E. radicans.*

RESULTS

Thirty-three natural patches of *E. radicans* were located throughout Monteverde, Costa Rica. The range of number of flowers was 1 to 258,984. The average number of flowers per patch was 8820 with a standard error of 8628. The calculated areas of each patch ranged 314 m^2 in a patch with a solitary inflorescence to 3896 m^2 in an extremely large patch. The average area was 627.97 m^2 with a standard deviation of 149.825. A frequency distribution of all the patches was created which showed there was a high number of a smaller patch (Figure 3). In contrast, there were only a few extremely large patches with more than 201 flowers.

A simple regression was used to test for correlation between the proportion of pollinia removed (PPR) and the density of each patch (total number of flowers/area). There was no relationship found between all PPR and density values (R^2= 0.004, p-value= .7261, N = 29). Nor was there a relationship between the PPR and density values when there was an exclusion of the PPR values of zero. In contrast, a negative relationship (R^2= 0.252, p-value = 0.0400, N = 16) was found between PPR and the density when two exclusions were made: (1) the exclusion of the PPR values that equaled 0; (2) the exclusion of extremely large density (Figure 4a). We can exclude the zero

PPR values by assuming that these particular patches have yet to be visited by pollinators. The patches with extremely large densities were also excluded because the number of flowers and PPR could have not been accurately reflected because they were calculated by estimation. If this trend was followed, then the PPR values for extremely high densities were unexpectedly high (Figure 4b).

A Spearman Rank test was performed to see if there was a correlation between PPR and the index of patchiness. When the zero values for PPR were included in the test, there were no significant results (N = 20, Rho corrected = -0.108, tied p-value = 0.6279). The same result was calculated when the zero values were excluded from the data set (N = 14, rho corrected = -0.107, tied p-value = 0.6885).

A chi-squared was used to see whether *E. radicans* is positively, negatively, or not associated with *A. curassavica* and/or *L. camara* (in flower and not in flower). *Epidendrum radicans* was found to not have a positive association with flowering *A. curassavica*. Thus, it is non-randomly dispersed without the influence of flowering *A. curassavica* (X^2 = 10.8, d.f = 1). Only one plant of *L. camara* was found in all of the patches of *E. radicans,* which did not allow for a chi-squared for association to be tested.

DISCUSSION

The results from this study support the two different hypotheses on the effect spatial distribution on non-rewarding plants. Conventional theories suggest that non-rewarding plants will not be as successful in larger patches because pollinators will realize there is no reward and move on to a different plant. The second theory in this paper suggested a large patch of *E. radicans* could be successful because it is more noticeable and its size would expedite high pollinia removal. The negative relationship found between the PPR and density (with exclusions) supports the conventional theory of floral mimicry that denser stands of *E. radicans* will decrease visitation because pollinators will realize there is not a reward (Bierzychudek 1981). Thus, lowering the PPR. This theory applies to the smaller patches of *E. radicans* whose visitors were smart enough to learn there is no reward. The data from the extremely large patches of *E. radicans* support the second theory that large patches can be successful because the PPR was surprisingly high and did not follow the trend between PPR and density. In this case, naïve pollinators were relied upon for pollination.

Most theories predict large patches will not be favored by pollinators because they do not offer a reward and it has been demonstrated that smaller, less dense patches have higher pollinia removal success. However, extremely large patches of *E. radicans* still exist. The second theory presented in this paper explains this apparent paradox. A surprisingly high PPR is found which suggests that these large patches of *E. radicans* are not suffering from its non-rewarding characteristic. Figure 4b shows the two large patches which are represented by stars. They had a higher PPR than expected, which suggests they are using different methods to attract pollinators. Large patches are more noticeable and are doing a better job attracting more visitors who are naïve and uneducated of the non-rewarding nature. This increase in number of visitors would increase in the PPR. Another benefit of a large patch is that the overall number of pollinia removed would be much greater than the number of pollinia removed in a small patch. This implies greater reproductive success compared to a small patch, even though the PPR may be lower. Further studies should examine the types of pollinators visiting the different size patches of *E. radicans* to determine if young or naïve visitors are the pollinators of the large patches and smarter older pollinators are visiting smaller patches. The frequency of visits should also be investigated.

Another possibility of high PPR is that smart pollinators may require several visits to distinguish between deceitful flowers and those flowers that have been emptied by previous visitors (Haber 1991). Pollinators are making multiple visits to these large patches because they want to be reassured that a reward is not offered. This also suggests that the second theory is working in the extremely large patches of *E. radicans.*

The Spearman Rank test used see if there was a correlation between PPR and plant spacing (patchiness) could not lead to significant conclusions because a nearest neighbor distance could not be measured for solitary individuals. These individuals were excluded from the sample size, limiting it to 14. According to the conventional theory of floral mimicry, plant spacing would have a negative effect on PPR in the same manner as density. Having inflorescences closer together would limit the number of visits because the smart pollinators would leave before visiting other flowers. The second theory suggests that patchiness could increase pollination success because the size would be more attractive to a naïve pollinator.

While there was no obvious relationship can be made between PPR and plant spacing for *E. radicans,* a study on *Taxus canadensis* by Allison (1990) showed that pollination success was negatively correlated with plant spacing. Allison's study does not consider the non-rewarding nature of *E. radicans,* but Allison's results follow the same trend that a larger sample size would also show a negative relationship between PPR and plant spacing. Future studies investigating the relationship should include larger sample sizes.

Because *E. radicans* relies on the pollinators of its two models, *A. curassavica* and *L. camara*, it would be expected that *E. radicans* would be located near the plants or in the same patch as it models. It was determined that *E. radicans* is not positively associated with *A. curassavica.* This supports Deacon's theory that a non-rewarding mimic should be located within the general region of its models, but not locally in the same patch. Other factors that could have affected the non-positive association of *E. radicans* with *A. curassavica* are different germination requirements or the chance that has not permitted seed dispersal to the same areas. Further studies should look for *A. curassavica* and investigate its association with *E. radicans*.

There was not enough data to make any conclusions about the association between *L. camara*. *Lantana camara* is generally found at a lower altitude so it has been speculated that pollinators of *L. camara* of are visiting *E. radicans* at higher altitudes (Bierzychudek 1981). This suggests that the floral mimicry is working, but the two species are not likely to be found in the same patch.

The results of this study shows support the proposed theory which contradicts the conventional theory of the spatial distribution effects on *Epidendrum radicans.* The new hypothesis proposes that the consequences of extremely large patches of non-rewarding plants such as *E. radicans* may not be a negative as once proposed. These results suggest that the conventional theory should re-evaluate its prediction of pollination success of non-rewarding plants.

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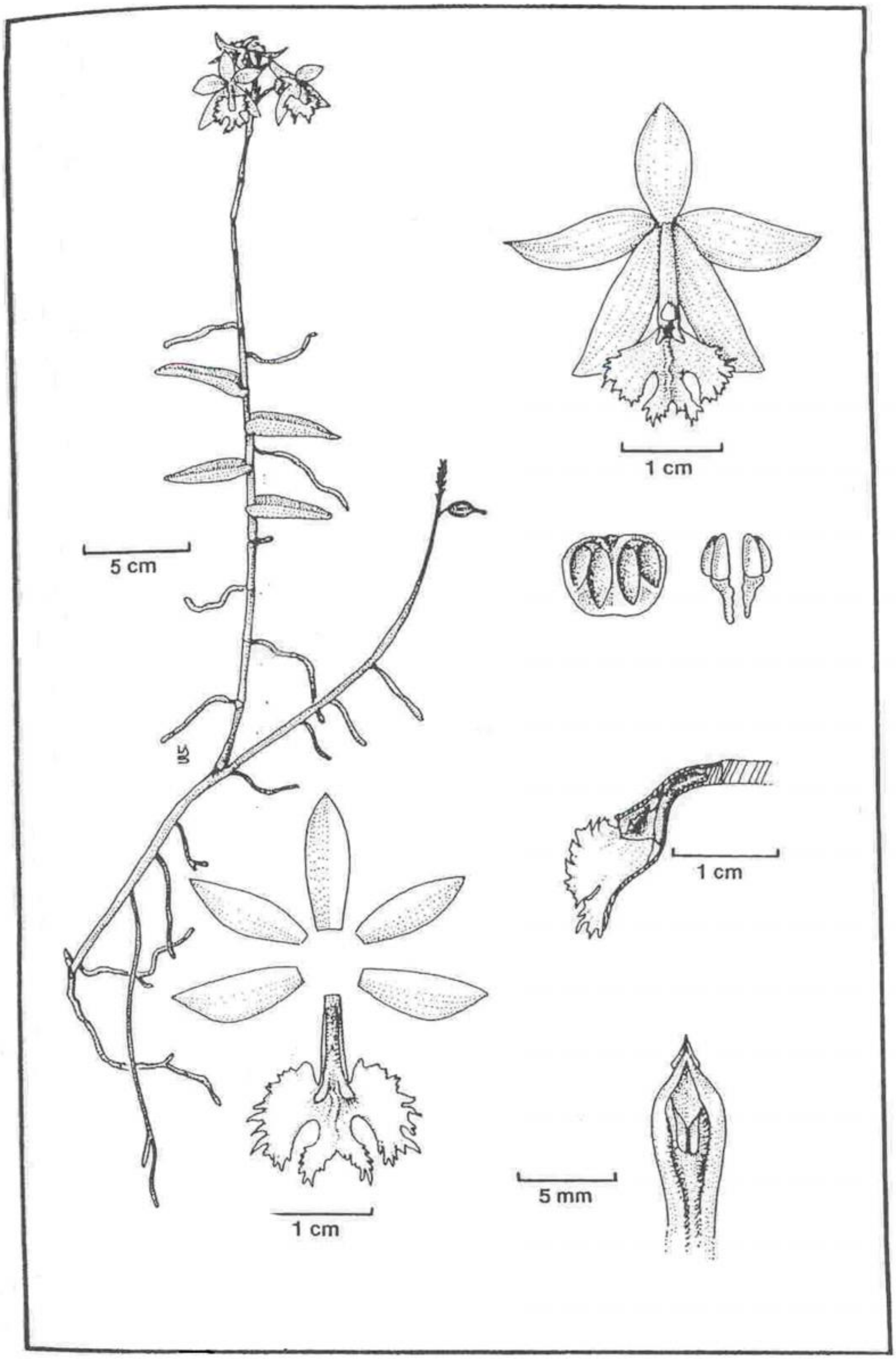

Figure 1. Vegetative and reproductive structures of Epidendrum radicans (Atwood and Mora de Retana 1992)

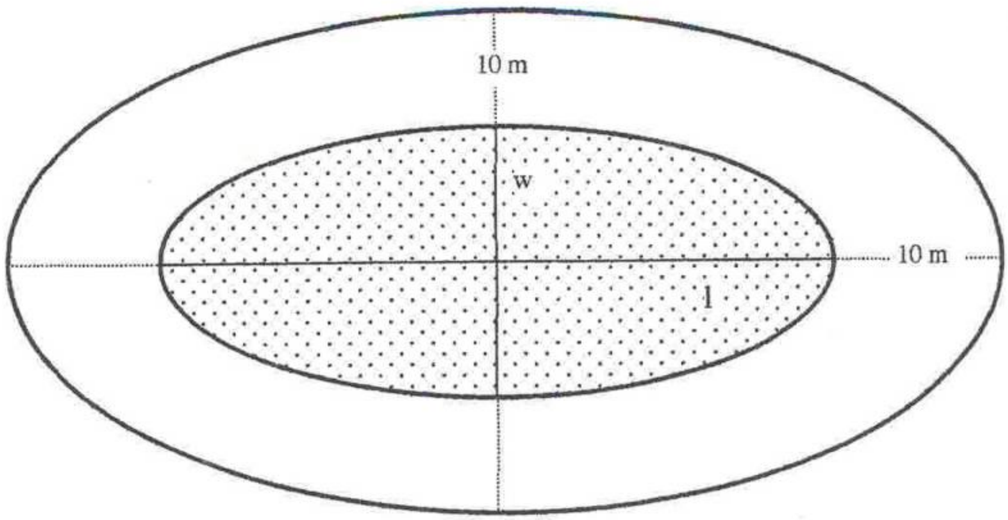

Figure 2. A hypothetical sample patch of *Epidendrum radicans* showing length (l) and width (w). Inflorescences would be found in shaded region (inflorescence area) and the entire patch (total area) would be defined 10 m beyond the perimeter of the inflorescence area.

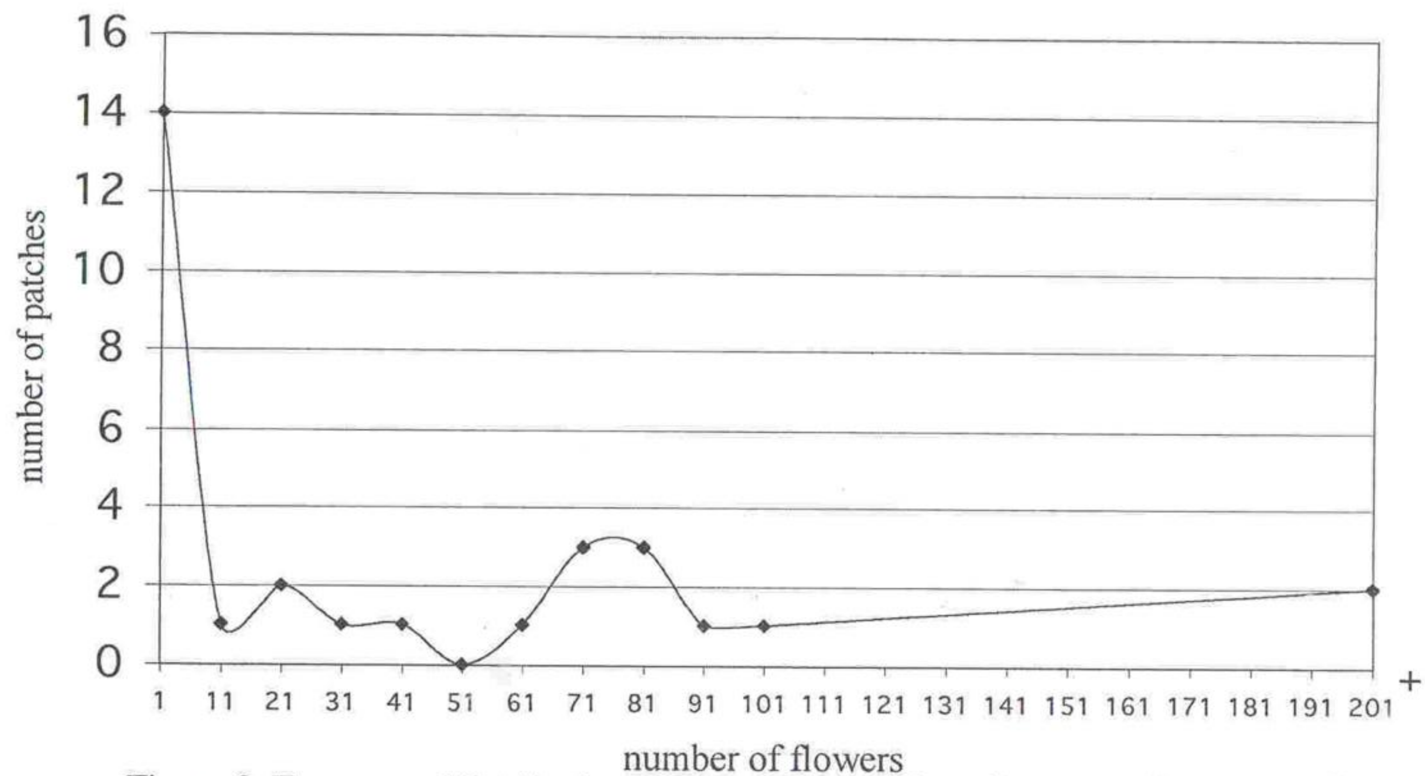

Figure 3. Frequency Distribution of the number of *E. radicans* patches possessing a given number of flowers.

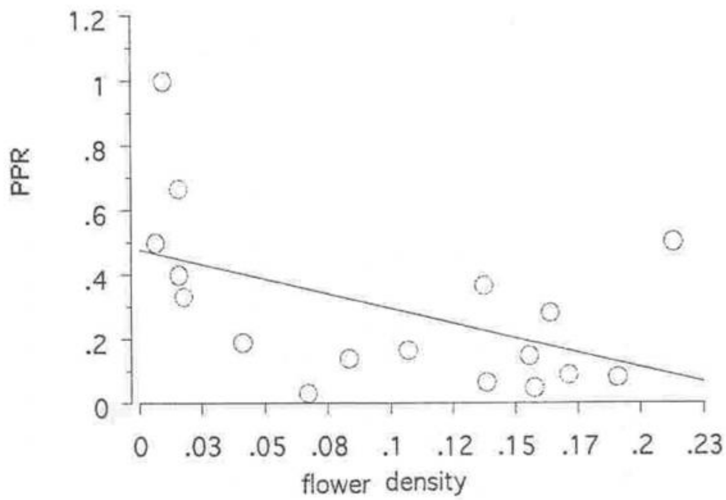

Figure 4a. There is a negative relationship between PPR (proportion pollinia removed) and density (#of flowers/area) excluding zero PPR values and large areas. See text for explanation.

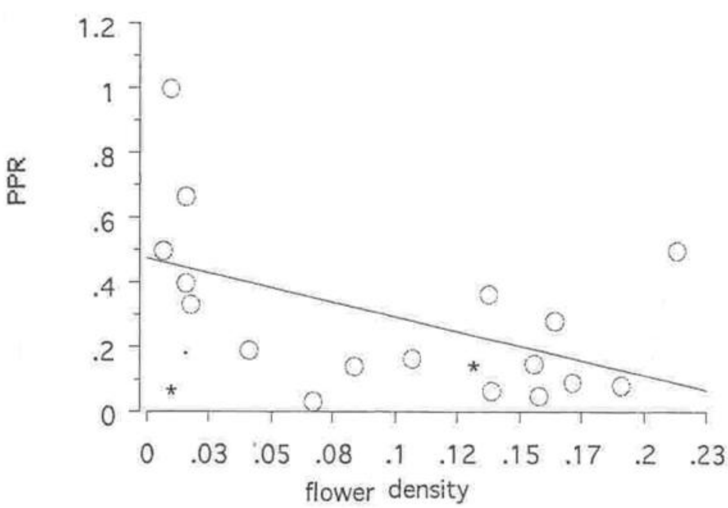

Figure 4b. A negative relationship was found between PPR (proportion pollinia removed) and flower density (# of flowers/area). Because of trend, the PPR values of big patches were higher than expected demonstrated by a * symbol. See text for explanation.