Evidence for floral mimicry in *Epidendrum radicans* (Orchidaceae) with *Asclepias curassavica* (Apocynaceae) and *Lantana camara* (Verbenaceae).

Sarah Dupre

Department of Secondary Education: Biology and General Science, University of Rhode Island

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ABSTRACT

Floral mimicry may occur between plant species with geographic overlap, similar flowering times and a shared set of pollinators. To demonstrate mimicry in such species, visitors must enhance fitness of individual flowering plants. This study focuses on potential floral mimicry for a Neotropical roadside weed, *Epidendrum radicans* (Orchidaceae), which produces no nectar but resembles nectar-producing, sympatric and phenologically similar plant species, *Asclepias curassavica* (Apocynaceae) and *Lantana camara* (Verbenaceae). Twenty pollinia of *E. radicans* were placed in four different groups. One containing the orchid alone, the second consisting of the orchid and *A. curassavica*, the third consisting of the orchid and *L. camara* and the fourth group containing all three species. Proximity to *L. camara* increased butterfly visitation in *E. radicans* nearly four-fold and more than doubled visitation when near *A. curassavica* (Friedman Test, Chi-squared = 22.23, df = 3, p = 0.0001; MCN Test, q = 5.962, df = ∞, p < 0.05). Likewise, pollinia removal increased as a result of proximity to both *A. curassavica* and *L. camara* (Friedman Test, Chi-squared = 24.825, df = 3, p = 0.0001; MCN Test, q = 6.261, df = ∞, p < 0.05). Simply doubling *E. radicans* flowers in monospecific groups to the same total flower number as mixed patches had no effect on visitation nor pollinia removal. Hence, the experiments presented here support the case for floral mimicry in *E. radicans*, though its impact on wild populations remains in doubt.

RESUMEN

La mímica floral puede ocurrir entre las especies de plantas con traslapo geográfico, épocas florecientes similares y un sistema compartido de polinizadores. Para demostrar la mímica en tales especies, los visitantes deben realzar la adaptabilidad de plantas florecientes individuales. Este estudio se centra en la mímica floral potencial para una mala hierba neotropical del borde de la carretera, *Epidendrum radicans* (Orchidaceae), que no produce ningún néctar pero que se asemeja a otras especies de plantas que producen nectar, son simpátricas y fenológicamente similares, *Asclepias curassavica* (Apocynaceae) y *Lantana camara* (Verbenaceae). Los polinios de veinte *E. radicans* se colocaron en cuatro grupos diferentes. Uno que contenía solamente la orquídea, el segundo que consistía en la orquídea y *A. curassavica*, el tercero que consistía en la orquídea y *L. camara* y el cuarto grupo que contenía las tres especies. La proximidad a *L. camara* aumentó las visitas de las mariposas a *E. radicans* casi cuatro veces y, cuando *A. curassavica* estaba cerca, las visitas a *E. radicans* se duplicaron. (Prueba de Friedman, Chi-cuadrada = 22.23, df = 3, p = 0.0001; prueba de MCN, q = 5.962, df =3, p = 0.0001; Prueba de MCN, q = 5.962, df = ∞, p < 0.05). De la misma manera, la remoción del polinio aumento debido a la proximidad de *A. curassavica* y *L. camara* (Prueba de Friedman, Chi-cuadrada = 24.825, df = 3, p = 0.0001; prueba de MCN, q = 6.261, df = ∞, p < 0.05). Cuando se dobló el número de flores de *E. radicans* en los grupos monoespecíficos al mismo número total de flores como los parches mixtos, no tuvo efecto en las visitas o en la remoción de polinios. Los experimentos reportados aquí dan evidencia en favor de la mímica floral en *E. radicans*, aunque su impacto en las poblaciones silvestres sigue en duda.

INTRODUCTION

Floral mimicry is said to occur when two or more plant species converge on a common flower morphology. This might occur in two ways: Müllerian mimics converge to attract similar pollinators to their rewarding flowers, while Batesian mimics offer no reward and deceive the pollinator by resembling a rewarding plant species (Roy & Widmer 1999). For floral mimicry to occur, supposed mimics must overlap geographically and phenologically, share individual pollinators and the resemblance must enhance fitness (Bierzychudek 1981; Roy & Widmer 1999). Though proposed for many sets of overlapping species, no case of floral mimicry has ever been verified (Roy & Widmer 1999).

Conditions for floral mimicry exist for three species of plants throughout Neotropical America: *Epidendrum radicans* (Orchidaceae), *Asclepias curassavica* (Apocynaceae) and *Lantana camara* (Verbenaceae). These species share common floral characteristics, have broad overlapping geographical distributions and share a potential set of general butterfly pollinators (Bierzychudek 1981). They are all roadside weeds that grow singularly and together at an elevation between 1,000 and 2,000 meters. Both *L. camara* (Todzia 1983) and *A. curassavica* (Galil & Zeroni 1965; Wyatt 1980) are reported to produce large amounts of nectar, making them potential Müllerian mimics. *E. radicans*, on the other hand, produces no nectar (Bierzychudek 1981; William Haber, personal communication 2004), making it a candidate for Batesian mimicry.

Several studies conducted in the past have failed to support the idea of floral mimicry in *E. radicans*, *A. curassavica* and *L. camara*, possibly because they dealt with too many variables that potentially impacted visitation and pollination success beyond floral resemblance. For example, Bierzychudek (1981), studied naturally occurring stands whose "densities varied considerably." Studies of *E. radicans* show that density is an important determinant of visitation and pollinia removal (Deacon 2000; Wolfe 1987; Woo 2001). This study returns to test the impact of floral mimicry on visitation and pollinia removal in *E. radicans*, taking care to control for patch size and floral density.

MATERIALS AND METHODS

The study site was located in Cañitas, Monteverde, Costa Rica, an elevation of 1,265 meters, which is Tropical Lower Montane Moist Forest (Hartshorn 1983). The different groupings of plants were located on an inclined embankment with a river running along the base. The slope did not have any trees growing on it, however it did have forests starting at the bottom and continuing away from the slope. The only two flowering plants that grew on different parts of the embankment were *A. curassavica* and *L. camara*. The study site received direct sunlight from 8:30 a.m. until approximately 3:30 p.m.

Approximately one hundred *E. radicans* were collected along the roadsides in San Luis, Costa Rica. Plants were then taken to the study site in Cañitas and four groups of these three species were made. Every group contained a patch of *E. radicans* that had a count of twenty flowers and hence, twenty pollinia, since each flower has one pollinium per inflorescence. If the flower's pollinia had been removed by its pollinator or was not a younger, yellow and orange flower, it was removed from the plant.

E. radicans always contained twenty flowers while the other two species had approximately twenty to forty flowers, always looking similar in size and floral appearance. The first group included twenty flowers of only *E. radicans*. The second group had a patch of orchids with a patch of *A. curassavica*. The third group had a patch of orchids with a patch of *L. camara*. The final group included all three of the species located in close proximity of each other. Each group was at least seven meters away from the next closest group.

There were twelve one hour trial periods in which butterfly visitations were observed and recorded for each of the species in every group. After an hour of observation had passed, the pollinia that had been taken by its pollinators were counted, removed from the orchid and then replaced with the necessary new flowers to replace the removed ones. When the study was done on consecutive days, pollina removals were recorded from the time the site was left the previous day until the arrival to the site the following morning.

Towards the end of the study, it was noted that *E. radicans* growing alone might have a disadvantage. Although *E. radicans* did have the same number of pollinia as the other orchid patches, it was still lacking an additional patch of coloration and the presence of a nectar-producing neighbor (*A. curassavica* and/or *L. camara*) that the other groups had. For six days, an additional patch containing only *E. radicans* with forty flowers and thus, forty pollinia were put on the embankment along with the other four groups. The additional group was observed and recorded the same way previous trials had been done.

RESULTS

There was a clear trend, displaying that butterflies were visiting *E. radicans* much more frequently when they were located with both *A. curassavica* and *L. camara*. There was a slight increase of butterfly visitation when *E. radicans* was with *L. camara* rather than when it was with *A. curassavica*. These observations are evident in Figure 1, where there was a significant difference in visitation rates (Friedman Test, Chi-square = 22.23, df = 3, $p = 0.0001$). When the group of *A. curassavica*, *L. camara*, and *E. radicans* were compared to *E. radicans* alone, there was also a significant difference in butterfly visitation rates, but there was not a significant difference between any other groups (MCN Test, $q = 5.926$, $df = \infty$, $p < 0.05$). In addition, there was a significant difference in pollinia removal overall for *E. radicans* when it was with the other two species (Friedman Test, Chi-squared = 24.825, df = 3, $p = 0.0001$) and also when the three species were together were compared to when the orchid was alone (MCN Test, $q = 6.261$, $df = \infty$, $p < 0.05$). This suggests that when *E. radicans* is with the other nectar-producing plants, its visitation rates and pollinia removal increases.

For a period of five days, there were two different groups of *E. radicans*. One group contained twenty flowers while the other group contained forty flowers. It is illustrated in Figure 2 that whether there were twice as many flowers and thus, twice as many pollinia in a group of orchids, there was not a significant difference in visitation rates or in pollinia removal (Wilcoxon Signed Rank Test, (visitation) $n = 5$, $z = -0.137$, $p = 0.891$ and (pollinia removal) $n = 5$, $z = -1.342$, $p = 0.18$). Therefore, even a doubling of flower number did not impact visitation or pollinia removal.

When the study was conducted on consecutive days, pollinia were counted and recorded from the time the site was left on the previous day until the next morning of arrival. It is apparent in Figure 3 that there was not a significant difference in the overall pollinia removal (Freidman Test, Chi-squared = 5.475, df = 3, p = 0.132) (MCN Test, q = 3.291, df = ∞, p > 0.05). Evidently, butterflies did not remove a substantial number of pollinia between the hours of leaving the site the previous day (~1:00 p.m.) and the arrival time of the following day (~8:30 a.m.).

Visitation rates were compared between *A. curassavica* and *L. camara*. In Figure 4, there was a significant difference in the amount of butterfly visits for the two species (Wilcoxon Signed Rank Test, n = 12, z = -3.065, p = 0.002). *L. camara* received many more visits than *A. curassavica* in every single trial that was performed.

In Figure 5, there was not a significant difference for the average number of butterfly visitations to *L. camara* when it was with *E. radicans* and then when it was with *E. radicans* and *A. curassavica* (Wilcoxon Signed Rank Test, n = 12, z = -0.551, p = 0.582). Additionally, there was not a significant difference for the average number of butterfly visitations to *A. curassavica* when it was with *E. radicans* and then with *E. radicans* and *L. camara* (Wilcoxon Signed Rank Test, n = 12, z = -0.707, p = 0.48).

Additional Observations

The most common butterflies observed on the study site were *Anartia fatima*, *Papilio polyxines*, *Heliconius clysonymus*, *Siproeta epaphus epaphus*, and *Lycorea cleobaeae* atergatis. During the observation periods, it was noted that these butterflies would stay on *A. curassavica* and *L. camara* for longer periods of time rather than when they were on *E. radicans*. Also, throughout the duration of this study, a unique association was observed between a wasp and *E. radicans*. The wasp would travel up and down the orchid's stem and when a butterfly would land on the flower, the wasp would climb to the top of the plant and nudge the butterfly off of the flower with its head. It was noted that the wasp seldom visited the other two plant species and that it would fly around the orchid in a territorial way, flying after any butterflies that would try to land on the flowers. This would only occur occasionally, since the wasps were frequently swatted away by the observer during the study periods.

DISCUSSION

Unlike other studies that fail to support the concept of floral mimicry for *E. radicans* with *A. curassavica* and *L. camara*, data here support that *E. radicans* increases its fitness as a result of proximity to the other two species. Because *E. radicans* produces no nectar, this is the first field test of Batesian mimicry in a plant. Gigord et al. (2002) show similar patterns for a non-nectar producing orchid in Europe, but only under restricted greenhouse conditions where flower selection by naïve bees were heavily frequency-dependent.

When *E. radicans* is growing in close proximity with *A. curassavica* and *L. camara*, it is more frequently visited by butterflies and therefore having higher pollinia removal than when it is growing by itself. The presence of the other nectar-producing plants is thought to be a reason for higher rates of visitation and pollinia removal. The

orchid has been noted to possibly mimic the other two plant species in order to attract its pollinators since it does not provide a reward itself. If it imitates the other nectar-producing flowers, pollinators may mistake the orchid's flowers for those that provide a reward. These ideas support the belief that *E. radicans* is indeed a floral mimicry complex, unlike the study done by Paulette Bierzychudek (1981) that gave insufficient evidence of such an idea. Because of this floral mimicry, the orchids are able to be pollinated more often than if they did not look so similar to *A. curassavica* and *L. camara*.

When there was forty flowers in the orchid patch verses the patch that contained twenty flowers, there was not a significant difference of butterfly visitation and pollinia removal. This may have resulted due to the absence of other nectar-producing plants. It is suggested that even though there was twice as many flowers in the patch that had forty flowers, there still was not an increase of visitation and pollinia removal because the pollinators may have noticed the absence of *A. curassavica* and *L. camara* and chose not to visit and thus, remove pollinia from the orchids. Pollinators visiting flowers lacking rewards, such as nectar, are less likely to visit an adjacent flower and more likely to leave the patch (Ackerman 1994). This proposes the idea that nectar-producing plants are visited first and then the pollinators move onto another flower until it reaches an orchid at which time it will pollinate the flower. On the other hand, in a patch consisting of solely *E. radicans*, the pollinator is less likely to visit flower after flower perhaps because the pollinator realizes the lack of nectar.

On days when the study was consecutive, a pollinia count was recorded the following day. There was not a significant difference in pollinia removal, which is logical since butterflies are most active between 10:00 a.m. and 2:00 p.m. (Drummond 1976) and thus, pollination decreases. The study was conducted at 9:00 a.m. and usually finished by 1:00 p.m. This only left the hours between 1:00 p.m. to 8:30 a.m. the following day to allow pollinia removal. Since butterflies tend to be less active as it gets later in the day and also in the early hours of the morning, it is reasonable that the pollinia removed during those hours would be inconsequential.

Additionally, there was a significant difference in the visitation rates between *A. curassavica* and *L. camara*. *L. camara* was visited more frequently every single trial period. On the study site, *A. curassavica* plants were taller and had larger flowers than the *L. camara* plants. The *L. camara's* flowers were more numerous, causing more of an attractive appearance for pollinators. If the butterflies were more attracted to *L. camara's* flowers, they would have visited and pollinated them more frequently than *A. curassavica*. One other explanation for such high rates of visitation and pollinia removal could be that *L. camara* has a greater nectar percentage than *A. curassavica* (Haber, personal communication 2004).

Most importantly, there was not a significant difference of butterfly visitations to *L. camara* when it was with *E. radicans* or then when it was with *E. radicans* and *A. curassavica*. Also, no significant difference was found in butterfly visitations to *A. curassavica* when it was with *E. radicans* or when it was with both. This is crucial because it demonstrates *A. curassavica* and *L. camara* are Müllerian mimics. If there were a significant difference between *A. curassavica* and *L. camara* butterfly visitations, this would mean that the two species do not mimic each other and thus, do not enhance their fitness via Müllerian mimicry. Simultaneously, the data support the idea that *E.*

radicans displays Batesian mimicry because *E. radicans* has higher visitations in the presence of either model.

The data taken throughout this study could have been more accurate if the individual flowers of *A. curassavica* and *L. camara* were counted and made equivalent to the number of *E. radicans* flowers. If this was done, butterfly visitation and pollinia removal counts would not have been affected by the size or coloration differences between the different species. Another factor that may have influenced the data collected was that another study was being conducted at the same time and place as this study. Butterflies were being collected and not released back into their habitat, which reduced the amount of butterflies that foraged on the field site. For future investigations relating to this study, it is suggested that there be more trials conducted. In addition, more individual groups should be used, including additional groups of *A. curassavica* and *L. camara* by themselves. It would also be interesting to measure how much nectar is taken from each of these species and compare the success of pollination of all four groups.

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LITERATURE CITED

Ackerman, J.D., J.A. Rodriguez, and E.J. Meléndez.1994. A meager nectar offering by an epiphytic orchid is better than nothing. *Biotropica* 26 (1): 44-49.

Bierzychudek, P. 1981. *Asclepias*, *Lantana* and *Epidendrum*: A floral mimicry complex? *Biotropica* 13: 54-58.

Deacon, N. 2000. Pollinia removal and visitation in *Epidendrum radicans* (Orchidaceae) and *Asclepias curassavica* (Asclepiadaceae). CIEE Program. Fall 2000.

Drummond, B.A. III. 1976. Comparative ecology and mimetic relationships of Ithomiine butterflies in Eastern Ecuador. PhD Dissertation. University of Florida.

Galil, J. and M. Zeroni. 1965. Nectar system of *A. curassavica*. Bot. Gaz. 130: 1-4.

Gigord, L. et al. 2002. The potential for floral mimicry in rewardless orchids: an experimental study. Proc. R. Soc. Lond. B 269, 1389-1395.

Haber, W. 1984. Pollination by deceit in a mass-flowering tropical tree *Plumeria rubra* (Apocynaceae). *Biotropica* 16 (4): 269-275

Haber, W. 2004. Personal communication.

Hartshorn, G.S. 1983. Holdridge Life Zone. *In* D.H. Janzen. Costa Rican Natural History. University of Chicago Press.

Roy, B.A. & Widmer, A. 1999. Floral mimicry: a fascinating yet poorly understood phenomenon. *Trends Plant Sci.* 4, 325-330.

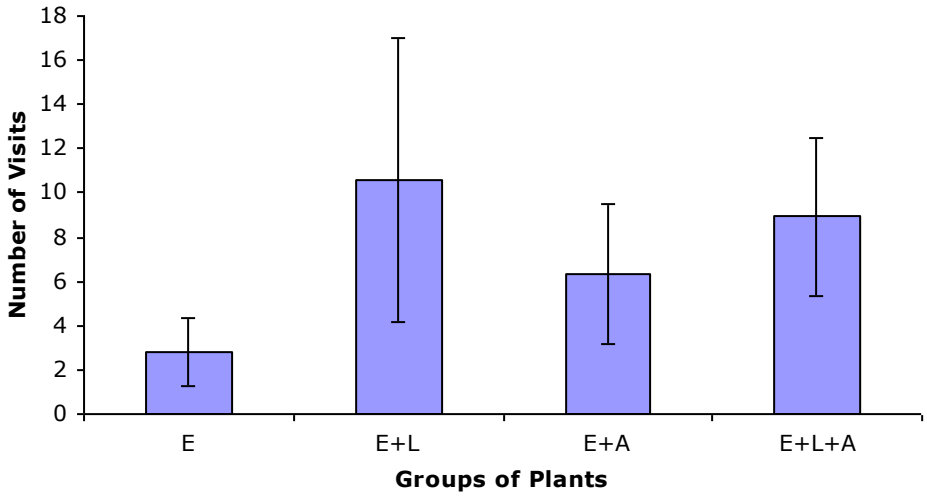
Todzia, C.A. 1983. *Epidendrum radicans* (Bandera Espanola, Gallito). *In* D.H. Janzen. Costa Rican Natural History. University of Chicago Press.

Wolfe, L.M. 1987. Inflorescence size and pollinaria removal in *Asclepias curassavica* and *E. radicans*. *Biotropica*:19(1): 86-89.

Woo, J. The effects of spatial distribution on the proportion of pollinia removed in *Epidendrum radicans* (Orchidaceae). CIEE Program. Spring 2001.

Wyatt, R. 1980a. The impact of nectar-robbing ants on the pollination system of *Asclepias curassavica*. Bull. Torrey Bot. Cl. 107: 24-28.

A



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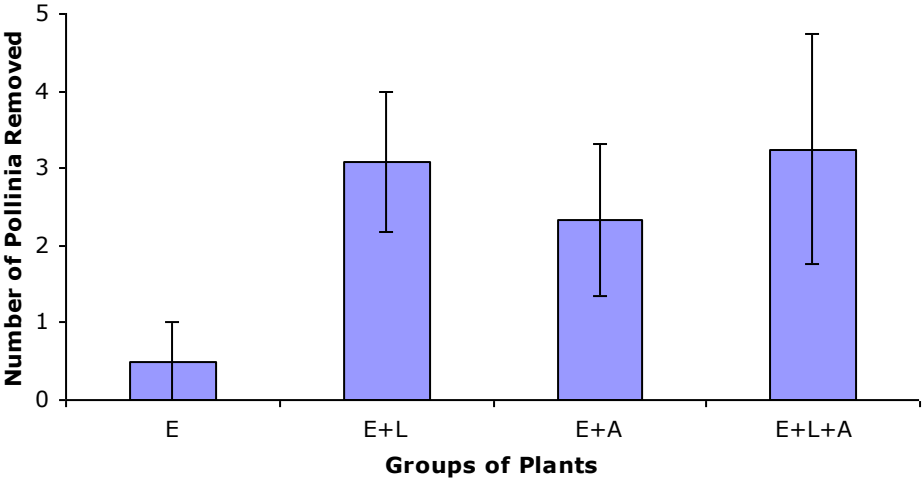

FIGURE 1. (A) Average butterfly visitation rates for *E. radicans* in the four different plant groups (Friedman Test, Chi-square = 22.23, df = 3, p = 0.0001) (MCN Test, q = 5.926, df = ∞, p < 0.05). (B) Average pollinia removal rates for *E. radicans* (Friedman Test, Chi-squared = 24.825, df = 3, p = 0.0001) (MCN Test, q = 6.261, df = ∞, p < 0.05)

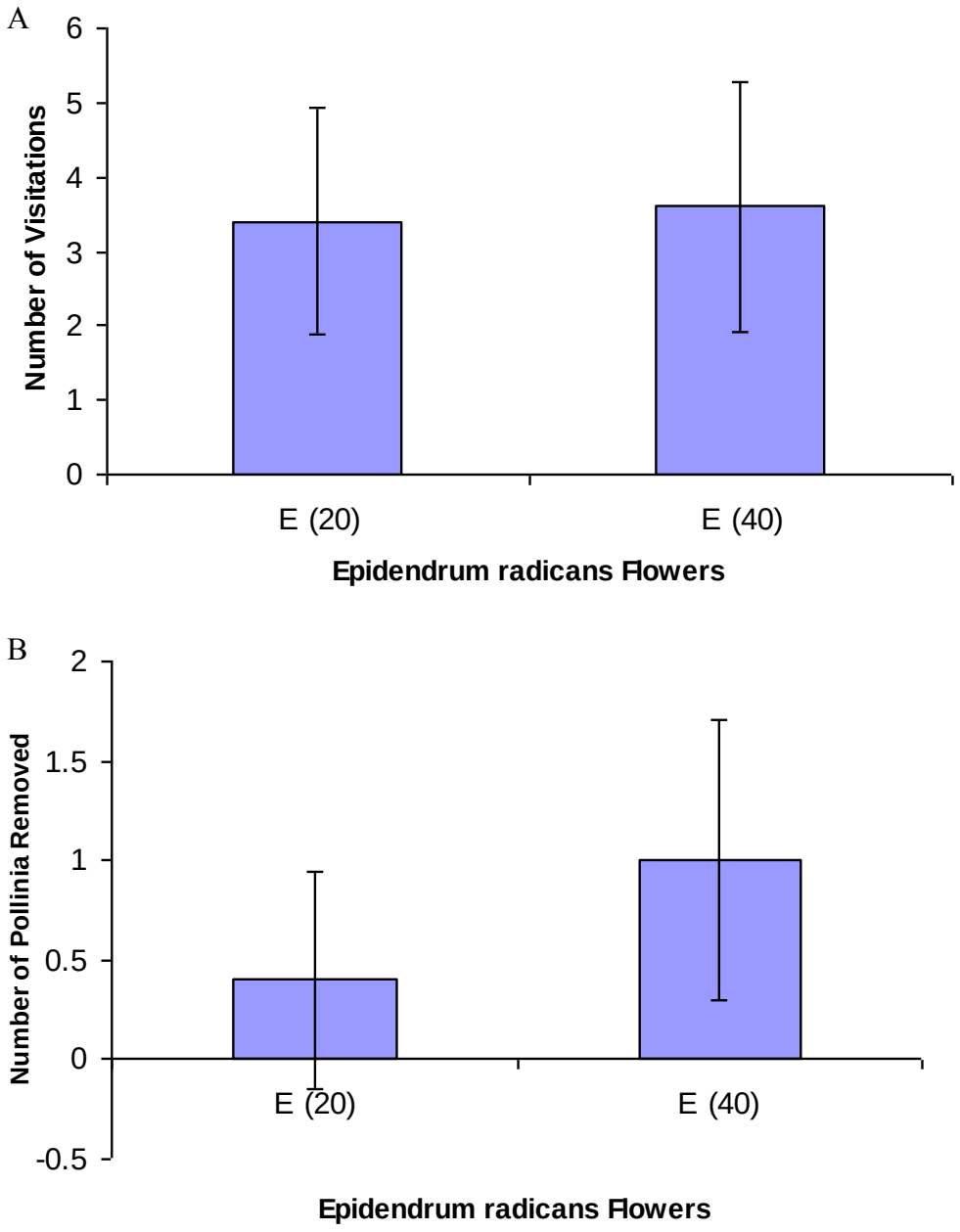

FIGURE 2. (A) Average visitation rates for both *E. radicans* patches (Wilcoxon Signed Rank Test, n = 5, z = -0.137, p = 0.891). (B) Average pollinia removal rates for both patches (Wilcoxon Signed Rank Test, n = 5, z = -1.342, p = 0.18).

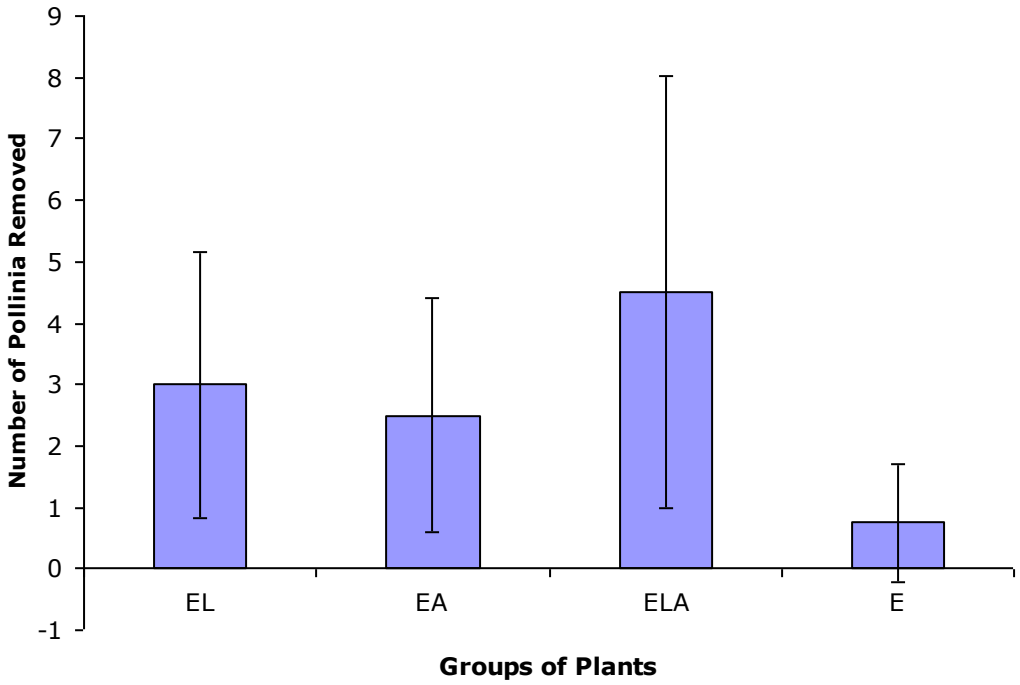

FIGURE 3. Morning pollinia removal counts for *E. radicans* (Friedman Test, Chi-squared = 5.475, df = 3, p = 0.132) (MCN Test, q = 3.291, df = ∞, p > 0.05).

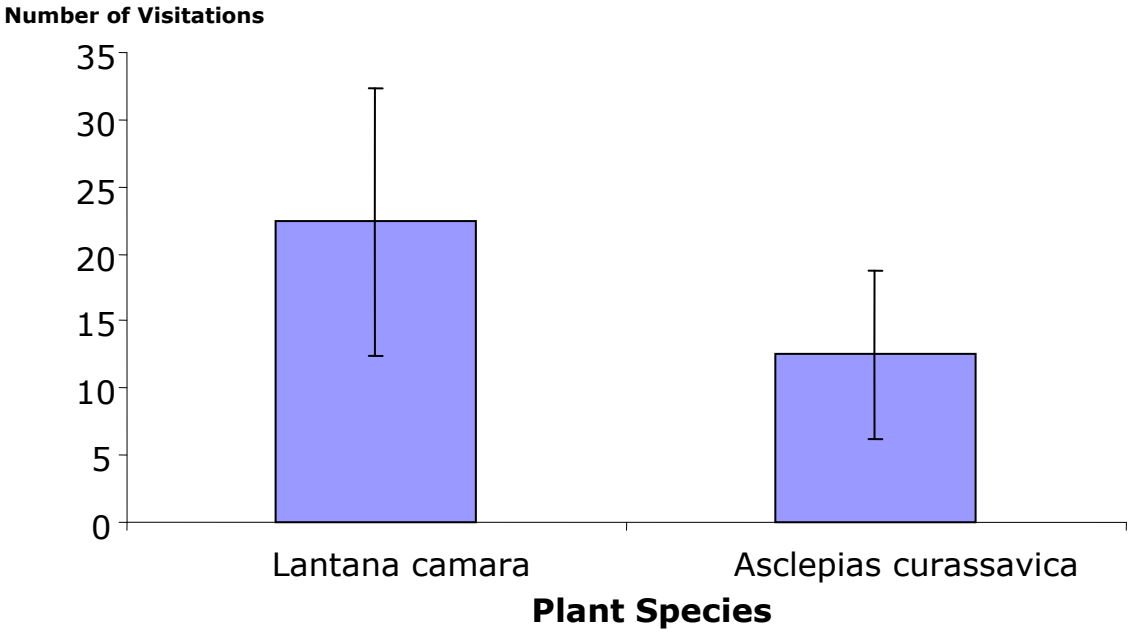

FIGURE 4. Average butterfly visitation rates for the two different plant species (Wilcoxon Signed Rank Test, n = 12, z = -3.065, p = 0.002).

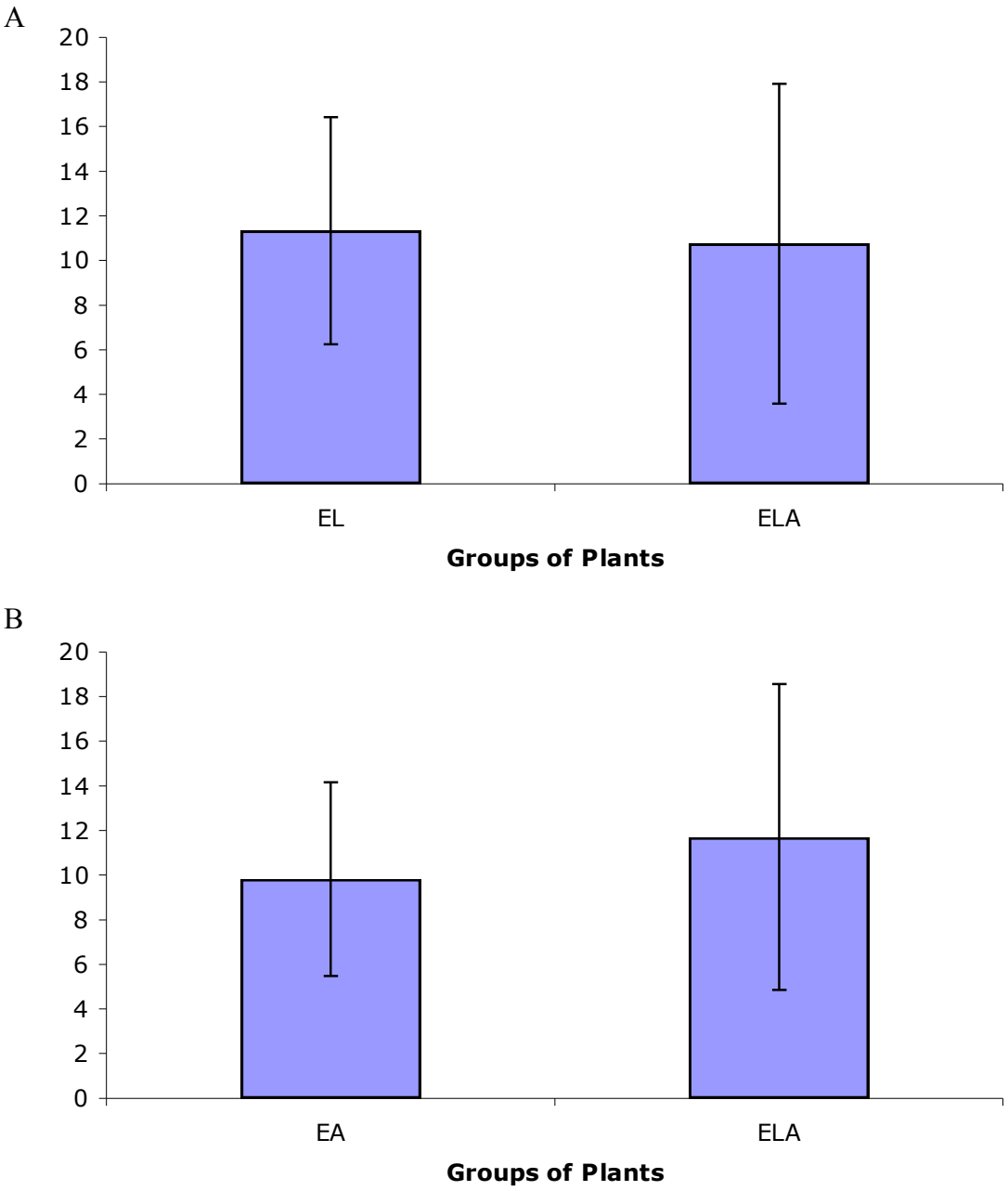

FIGURE 5. (A) Average number of visits to *L. camara* when it was with *E. radicans* and with *A. curassavica* and *E. radicans* (Wilcoxon Signed Rank Test, n = 12, z = -0.551, p = 0.582). (B) Average number of visits to *A. curassavica* when it was with *E. radicans* and with *L. camara* and *E. radicans* (Wilcoxon Signed Rank Test, n = 12, z = -0.707, p = 0.48).