

Supernormal Stimulus as a Mimicry Strategy: the case for *Epidendrum radicans* (Orchidaceae)

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ABSTRACT

Epidendrum radicans is a food deceptive Batesian mimic of its sympatric model species *Asclepias curassavica* and *Lantana camara*. Theoretically, food deceptive orchids should be rare with small inflorescences (Johnson et al. 1993; Weins 1978) yet in San Luis, Costa Rica *E. radicans* grows in large monotypic stands with individuals sporting up to 12 open flowers per inflorescence. *E. radicans* might attract pollinators using flower or inflorescence size as a visual supernormal stimulus also implicated in floral mimicry (Scheistl 2004). Pollinia removal of *E. radicans* was measured in plants with i) inflorescence sizes of two and ten growing amongst model species in patches ii) inflorescence sizes of one through eight flowers on plants growing naturally in dense stands without models iii) and unmodified and enlarged flowers. Pollinia removal was proportionally greater for flowers of smaller inflorescence sizes, and greater for unmodified flowers. *E. radicans* does not appear to use visual supernormal stimuli to attract pollinators. *E. radicans* may occur in large monotypic stands as a result of human disturbance. Continued pollinia removal in *E. radicans* may result from constant recruitment of naïve pollinators to the area.

RESUMEN

Epidendrum radicans es una planta que presenta mimetismo Batesiano de las especies modelos *Asclepias curassavica* y *Lantana camara*. Teóricamente, el engaño alimenticio en orquídeas es poco común y con inflorescencias pequeñas (Johnson et al. 1993; Weins 1978), aún en San Luis, Costa Rica *E. radicans* crece en largos tallos monotípicos con individuos que contienen hasta 12 flores por inflorescencia. *E. radicans* puede atraer polinizadores usando el tamaño de flores o inflorescencias como un estímulo supernormal también implicado en mimetismo floral (Scheistl 2004). La remoción de polinia de *E. radicans* fue medido en plantas con i) tamaño de inflorescencias de dos a diez flores entre especies modelo en parches ii) tamaño de inflorescencias de uno a ocho flores en plantas creciendo naturalmente en parches sin modelos iii) y flores alargadas y no modificadas. La remoción de polinia es proporcionalmente mayor para flores con un menor número de flores por inflorescencia y mayor para flores no modificadas. *E. radicans* puede ocurrir en parches largos monotípicos como resultado de disturbios humanos. La remoción continua en *E. radicans* puede resultar por el constante reclutamiento de polinizadores inexpertos en el área.

INTRODUCTION

Animal-mediated pollination relies on floral attractants coupled with a reward (Johnson, S.D. et al. 2003). However, the production of these rewards, which can include nectar, pollen, and oil, is energetically costly (Dafni 1984). Some plants avoid making expensive rewards and, instead, are pollinated by deception (Dafni 1984). In this case, pollination occurs when pollinators are duped by floral attractants mimicking rewarding species (food deception) or flowers mimicking potential mates (sexual deception) (Dafni 1984; Scheistl 2005).

One third of all orchid species are food deceptive (Scheistl 2005). Orchid flowers can fool pollinators by having similar scents, floral shapes or colors of sympatric rewarding plants (Johnson 1994; Weins 1978). Such deception saves energy and increases outcrossing (Jersakova et al. 2006), however food deceptive orchids face the challenge of continually attracting pollinators that may lose interest in or learn to avoid deceptive flowers (Gumbert & Kunze 2001; Smithson and MacNair 1997). Experienced *Apis mellifera* bees foraging on the food deceptive orchid *Orchis boryi* visited the orchid less often than naïve workers (Gumbert & Kunze 2001). It is important, therefore, to ensure the continued pollination of food deceptive orchids by maximizing pollinator attraction while minimizing avoidance learning (Dafni 1984). In short, orchid deceit pollination relies upon the orchid being relatively rare but in close proximity to its model species, and having few flowers per inflorescence (Johnson et al. 1993; O'Connell & Johnston 1998; Scheistl 2005; Weins 1978).

Epidendrum radicans is a food deceptive Batesian mimic of the two sympatric, rewarding model species *Asclepias currasavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae) (Roy & Widmer, 1999; Weins, 1978). All three have overlapping geographical and ecological ranges, are pollinated by butterflies, and have similarly colored orange and yellow flowers on inflorescences (Figure 1: Wiens 1978). However, *E. radicans* is not rare, often growing in large monotypic stands of thousands away from models. Despite this, *E. radicans* in these populations are continually pollinated as indicated by pollinia removal and capsule formation (Bierzzychudek, 1981). The puzzling growth habits of *E. radicans* can possibly be explained by pollinator responses to a supernormal stimulus (Scheistl 2004).

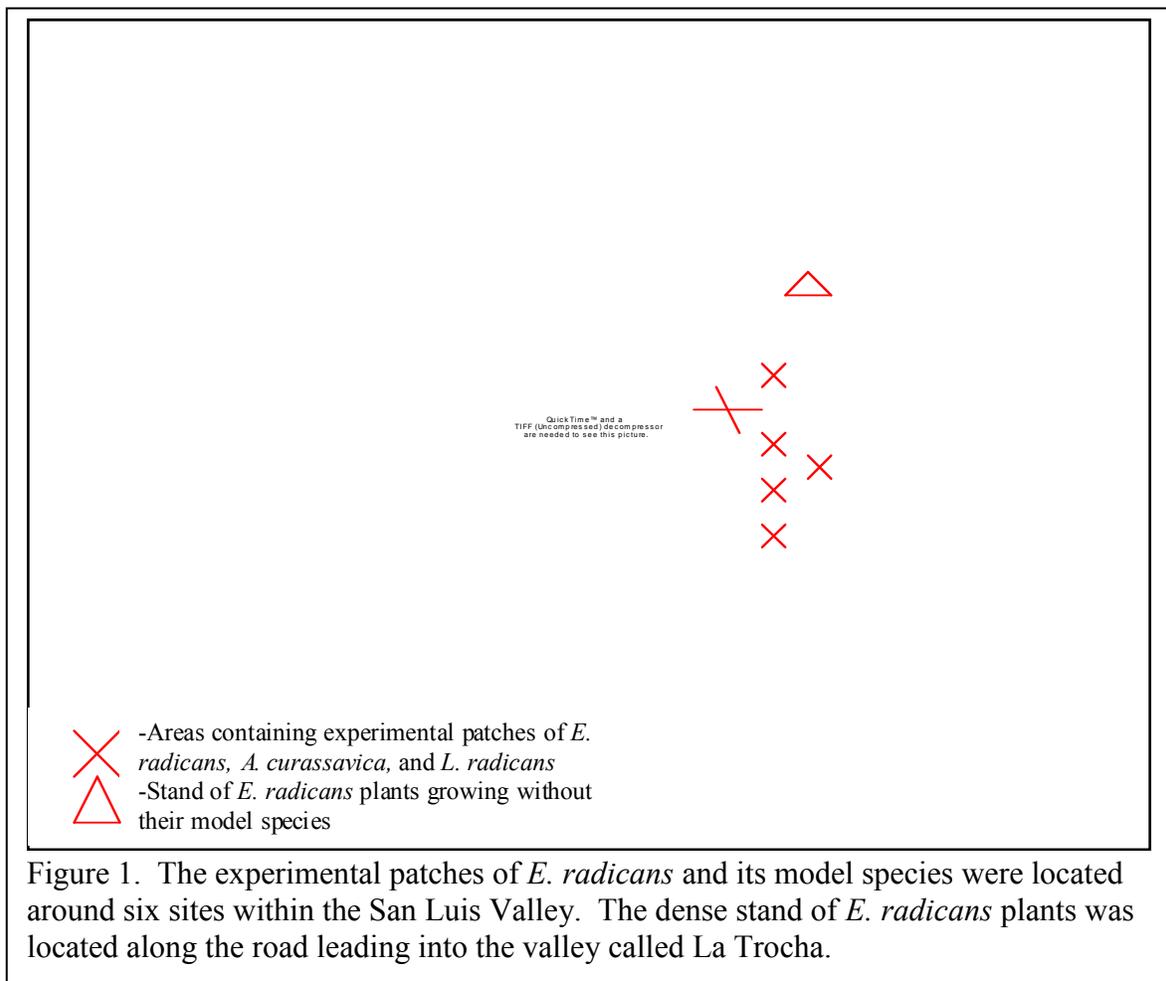
A supernormal stimulus is an exaggerated character that is met with a correspondingly exaggerated response (Christy 1995). Supernormal stimuli in floral mimics exaggerate attractants used by the model (Scheistl 2004). For example, *Chilogottis trapeziformis* is a sexually deceptive orchid that produces up to 100 times more pheromone and has a flower larger than the females of its model wasp species *Neozeleboria cryptoides* (Scheistl 2004). While supernormal stimuli are known in sexually deceptive orchids, it has not been studied in food deceptive orchids. *E. radicans* does not produce scents and its floral coloration is similar to its models. Nonetheless, its floral display could be a visual supernormal stimulus as its individual flowers and inflorescences are both larger than the model species. Large inflorescences correlate with higher pollinia removal and seed pod production in the orchid *Brassavola nodosa* (Schemske 1980), and could act as super attractants that compensate for or even override avoidance learning.

Here, I determine if pollination success of *E. radicans* is impacted by a visual supernormal stimulus, and whether inflorescence size or petal size is the primary contributing factor to the stimulus. To do this I measure pollinia removal of *E. radicans* with i) artificially enlarged flowers ii) inflorescence sizes of ten flowers versus two flowers for plants with models nearby and iii) inflorescence sizes ranging from one flower to eight flowers for plants in large monotypic stands.

Materials and Methods

STUDY SITE

All observations for the experiment were made in the San Luis Valley, Costa Rica (Premontane Moist Forest) from April 4th to April 29th. *E. radicans* were found in stands along the La Trocha hillside, while *A. curassavica* and *L. camara* plants were in disturbed areas throughout the San Luis Valley (Figure 1).



STUDY ORGANISMS

A B C
Figure 2. (A) *E. radicans* is a food deceptive Batesian mimic of its two rewarding model species that have similar floral coloration (B) *A. curassavica* and (C) *L. camara*.

E. radicans is a common orchid found in disturbed areas around the Monteverde region. It flowers year round and produces inflorescences of one to twelve flowers (Bierzychudek 1981). *E. radicans* has similar habitats as its model species *A. curassavica* and *L. camara* but only rarely do they occur in close proximity to one another in San Luis. All three plants prefer areas of human disturbance such as roadsides and pastures (Bierzychudek, 1981). The three species also share common pollinators (Bierzychudek, 1981; Boyden 1980). Butterflies including *Anartia fatima* and *Danaus plexippus* are the most common pollinators. While bees and flies visit the flowers also, pollinia are only known to be transported by butterflies (Bierzychudek, 1981).

EFFECTS OF INFLORESCENCE SIZE ON POLLINIA REMOVAL OF *E. RADICANS* WITH MODELS

Twenty patches of either *L. camara* or *A. curassavica* (defined as all model plants within 3 m² of each other) were used to measure the effects of inflorescence size on pollinia removal of *E. radicans* (Figure 1). Twenty-seven *E. radicans* plants were placed amongst the patches in densities of one to four plants, and pollinia removal measured daily for sixteen days. *E. radicans* were grown with models to maximize pollinia removal according to the magnet species effect described by Johnson et al. 2003. The inflorescence size of all *E. radicans* was either two flowers per inflorescence or ten flowers after the fifth, tenth, and thirteenth days of observation to account for effects of patch placement. Though plants with ten flowers per inflorescence occur naturally, they are rare. Therefore, plants for the inflorescence size of ten group were created by tying together inflorescences of multiple plants. After a plant was pollinated, it was replaced with another plant of the same inflorescence size with intact pollinia.

EFFECTS OF INFLORESCENCE SIZE ON POLLINIA REMOVAL OF *E. RADICANS* WITHOUT MODELS

Eighty *E. radicans* growing in large roadside monotypic stands were used for twelve days to measure the effects of inflorescence size on pollinia removal without models. Here, a range of inflorescence sizes - ten plants for each inflorescence size of one through eight – were observed and followed with replacement. Pollinia removal was measured daily.

EFFECTS OF A SUPERNORMAL FLOWER SIZE ON POLLINIA REMOVAL OF *E. RADICANS*

Ten patches of *Asclepias* and *E. radicans* plants of an inflorescence size of two were created by placing one *E. radicans* plant within each *Asclepias* patch. Pollinia removal of each *E. radicans* was measured daily for ten days. Plants with pollinia removed were replaced the same day. The flowers of *E. radicans* in five patches were modified by enlarging the flowers with paper. A yellow paper square 1 cm x 1 cm was taped to the center of an orange paper square 2.5 cm x 2.5 cm. This square was fitted behind each flower and taped to the top sepal of each flower of the *E. radicans* plant. The *E. radicans* flowers of the other five patches were not modified. Each patch was switched between

the unmodified and modified flower arrangement after five days to account for local effects on pollinia removal of each patch.

RESULTS

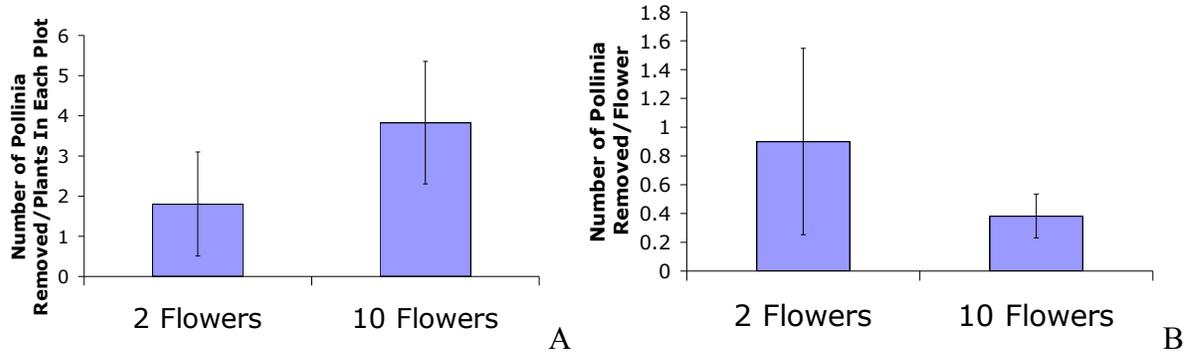


Figure 3. Mean (\pm SD, $n = 20$) of the occurrences of pollinia removal over sixteen days in *E. radicans* plants growing amongst its model species in twenty patches. X-axis bar labels represent inflorescence (A) Number of pollinia removed/plant observed in each patch is the average across twenty patches of the ratio between total occurrences of pollinia removed and the number of *E. radicans* plants in each patch (B) Number of pollinia removed/flower is the average across twenty patches of the ratio between total occurrences of pollinia removal and total flowers in each patch.

The number of pollinia removed/plant was greater for the larger inflorescence size of ten flowers compared to plants with two flowers per inflorescence (Figure 3: Wilcoxon Signed Rank Test, $Z = -3.425$, $P = 0.0006$, $n = 20$). However, as inflorescence size increased, pollinia removal/flower decreased (Figure 3: Wilcoxon Signed Rank Test, $Z = -3.347$, $P = 0.0008$, $n = 20$).

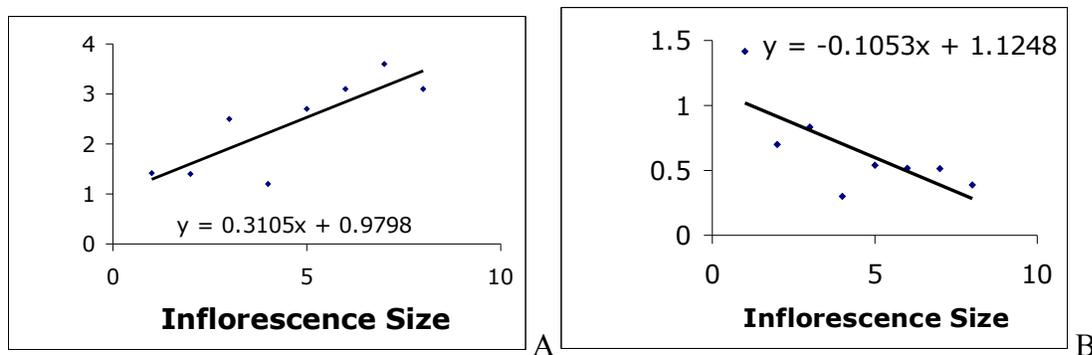


Figure 4. Pollinia removal over twelve days of *E. radicans* plants with inflorescence sizes ranging from one to eight flowers per inflorescence growing in dense roadside stands in San Luis ($n = 10$ and 12 for the inflorescence size of one) (A) Number of pollinia removed is a ratio of the total number of pollinia removed to the total plants for each treatment. (B) Pollinia Removed/Flower is the ratio of total pollinia removed to the flowers offered by all plants of each treatment.

For *E. radicans* growing in dense stands, the number of pollinia removed per plant increases as inflorescence size increases (Figure 4: Spearman Rank Test, *Spearman Rho* = 0.7904, *P* = 0.0195, *n* = 10, 12 for the inflorescence size of one). However, as inflorescence size increases in *E. radicans*, pollinia removal per flower decreases (Figure 4: Spearman Rank Test, *Spearman Rho* = -0.7381, *P* = 0.0366, *n* = 10, 12 for the inflorescence size of one).



Figure 5. Total pollinia removal over ten days in *E. radicans* plants growing alongside *A. curassavica* in ten patches (*n*=5: 5 patches of unmodified flowers and 5 patches of enlarged flowers). Both unmodified and enlarged flower sizes had an inflorescence size of 2. Numbers above the bars represent the occurrences of pollinia removal as a percentage of the total pollinia removed.

For *E. radicans* growing in patches amongst its model species, unmodified control flowers experienced 24% greater total pollinia removal compared to the artificially enlarged treatment flowers, although the difference was not statistically significant ($\chi^2 = 1.190$, *P* = 0.275).

Pollinia removal often occurred in bursts from one pollinator at a time for *E. radicans* in the monotypic stands. Flowers of a small area (3 m²) remained unpollinated for days until all flowers were recorded as pollinated in a single morning (pers. obs.).

DISCUSSION

Food deceptive flowers grow in low densities in close proximity to their model species and have small inflorescence sizes to maximize pollinator attraction while minimizing avoidance learning (Dafni 1984; Scheistl 2005). Weins 1978 studied the pollination success of the food deceptive orchid *Cephalanthera*, which usually grows in low densities compared to its model – the bellflower. Pollination success is higher when *Cephalanthera* grows along side its model compared to growing alone (Weins 1978).

However, *E. radicans* does not exhibit mimic growth patterns possibly because its flower or inflorescence size is a supernormal stimulus (Scheistl 2004).

Artificially large flowers are visited by pollinators less frequently compared to natural flowers. This indicates that larger flower size is not a supernormal stimulus in *E. radicans*. A small flower size possibly influences pollinator attraction because it minimizes avoidance learning. Deceptive plants have increased pollinator visitation when they have flowers closely resembling model species (Gumbert & Kunze 2001; Johnston et al. 2003). Pollinators may not recognize the artificially enlarged flower and preferentially visit the flowers of the model species or the unmodified flowers in nearby patches. The primary pollinator of *E. radicans* - *Danaus plexippus* - has evolved very closely with *A. curassavica* (DeVries 1987; Goode 1999) and may only respond to deceptive flowers of a size similar to *Asclepias*. Also, when artificially large flowers are visited, pollinators may learn to avoid those flowers faster because of their distinctiveness.

The increase in pollinia removal as inflorescence size increases for *E. radicans* in monotypic stands and near its models indicates that more flowers increase the likelihood that any single pollinium is removed from a plant simply because more pollinia are available to be removed. However, as inflorescence size increases, pollinator visits decrease per flower. Therefore, plants with larger inflorescences receiving more visits per plant are not receiving disproportionately high visitations, suggesting that inflorescence size is not a supernormal stimulus utilized by *E. radicans*. Increasing the number of flowers produced increases the likelihood of pollination, however, each flower has a smaller chance of being pollinated. Also, negative conditioning of pollinators visiting plants with large inflorescences may decrease pollinator visitation for plants with large inflorescences. Rather, visitation per flower increases disproportionately as inflorescence size decreases. Therefore, having small inflorescence sizes maximizes pollination and is consistent with plant mimicry growth strategies (Weins et al. 1978). Despite this, sexually reproducing *E. radicans* grow in large stands, often with large inflorescence sizes.

Because pollinators learn to avoid mimics the pollination success of food deceptive flowers relies on naïve pollinators (Boyden 1982; Johnson 2000). Flower production of the food deceptive orchid *Calypso bulbosa* var. *americana* is timed closely with the emergence of its queen bumblebee pollinator in late spring (Boyden 1982). Pollination relies upon the initial deception of the pollinator because after time, the pollinator learns avoidance of the flower (Boyden 1982). While the pollinator avoids the mimic eventually, plant populations are supported by the limited occurrences of pollinations during the late Spring season. *E. radicans* flowers and pollinators are present year round, peaking in the dry season, suggesting that pollinators of *E. radicans* are not naïve, however, recruitment of new naïve pollinators to the area is most likely high. The two main pollinators of *E. radicans* are *Anartia fatima*, the most common butterfly in Costa Rica, and *Danaus plexippus* (Bierzychudek 1981; DeVries 1987). The respective life spans of the reproductively active adult *A. fatima* butterfly is two weeks and two to six weeks for *D. plexippus* (DeVries 1987) meaning there is a high recruitment of young naïve pollinators year round to the area. Also, 80% of Pacific lowland butterfly species in Costa Rica are migratory (Stevenson and Haber 2000). Butterfly populations consisting of first generation offspring migrate to the Pacific slope in April or May as well as during the “veranillo” from June to July (Stevenson and Haber

2000) contributing more naïve pollinators to the area. Therefore, there is constantly recruitment of naïve pollinators to the study site that most likely contribute to the continual pollination of *E. radicans* in stands.

All data suggest that *E. radicans* should utilize typical mimic growth forms that maximize pollinator visitation while minimizing avoidance learning – sparse densities, small flowers, and small inflorescence sizes (Weins 1978). Historically, *E. radicans* followed this pattern and did not grow in dense stands. It is likely that the state of *E. radicans* is not natural, instead reflecting what I call the “disturbance response hypothesis.” After large-scale human disturbance, *E. radicans* most likely quickly colonized areas to form dense stands. Human disturbance also likely increased the prevalence of *Anartia fatima* and *Danaus plexippus*, both are adapted to disturbed habitats. Further as *E. radicans* populations expanded so did “weedy” species, such as the models *A. curassavica* and *L. camara*. However, *E. radicans* likely increased disproportionately compared to its models because of its ability to regenerate from fragments. Continuous chopping of the plants increases *E. radicans* density dramatically and decreases the density of its models, which the dense monotypic stands. Despite this, *E. radicans* populations still prevail because of a human-induced increase in optimal growth habitat and naïve pollinators.

Visual supernormal stimuli are not utilized by *E. radicans* as a strategy to attract pollinators. Rather, their patterns of pollination support typical mimic growth strategies. Because selective pressures acting on the dense *E. radicans* stands most likely differ from those for *E. radicans* growing more sporadically in less disturbed habitats, studies should compare how *E. radicans* fitness has changed. For instance, a primary benefit to being a food deceptive mimic is increased outcrossing - pollinators quickly detect a plant is not rewarding and move to a new area (Jersakova et al. 2006). However the observed pattern of pollinia removal in the stands decreased out crossing because all flowers within a small area of a stand were pollinated by a single pollinator, causing a high degree of selfing amongst plants with inflorescence sizes above one. While *E. radicans*' abundance has changed relative to its models and pollinators because of human disturbance it still maintains dense monotypic populations that sexually reproduce because of a high continuous recruitment of naïve pollinators.

ACKNOWLEDGEMENTS

I thank CIEE Tropical Ecology and Conservation and Alan for their invitation into their study abroad program. I also thank CIEE for providing materials for the project, Anjali Kumar, Pablo Allen, José Calderón, and Yimen Araya, for project input and help with statistical tests. I would also like to thank Alan Masters especially for being my project advisor. Thanks also to the many students of the program for providing help with writing the manuscript and with tips on formatting for Word.

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