Reproductive Ecology of Euglossine Bee-pollinated Orchid *Catasetum Integerrimum* Hook (Ochidaceae)

Angélica María Hernández-Ramírez (✉ angehernandez@uv.mx)

Universidad Veracruzana

Research Article

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Abstract

Catasetum is a dimorphic and dioecious genus of orchids with a pollinaria release mechanism triggered by pollinator visitation. The reproductive ecology of *Catasetum integerrimum* Hook (Orchidaceae) was characterized in Central Veracruz, Mexico. For that, it was determined whether floral senescence and fruit production are resulted of pollination efficiency in the species, and if so, to what extent present reproductive event constraint the investment in subsequent reproductive event. In the sampled population, efficient pollinaria removal/deposition triggered floral senescence. *Eulaema polychroma* bee visited earlier and with lower frequency the staminate than pistillate flowers. Alleviating pollen limitation increased fruit production but it caused a reduction in flower and fruit production in the subsequent reproductive event. *C. integerrimum* seemed to exhibit reproductive cost-saving mechanisms linked to the pollinator identity and behavior, whereas pollinator efficiency appeared to be mediated by dioecism and dimorphism in floral traits. This study highlights the importance to understand the adaptive significance of dimorphic floral traits in animal-pollinated plants, stimulating new research avenues on the role of pollinators in maintaining safe reproduction threshold of plants.

Introduction

Neotropical region harbors one orchid genus that display a fascinating adaptation for forcibly attaching the pollinaria onto visiting insects' body by triggering a catapult mechanism. Although catapult mechanism has evolved repeatedly in different angiosperm families, those typically are limited to few knowledge species per family (Papilionoideae, Alemán et al. 2014; and Ericaceae, Switzer et al. 2018). By contrast, *Catasetum* Rich. (Orchidaceae) comprises about 160 species distributed in the neotropical region with a potential differential attachment of pollinaria to pollinators' body by catapult mechanisms (Romero and Nelson 1986; Milet-Pinheiro et al. 2015).

Darwin termed "sensitiveness" at this pollination mechanism in *Catasetum* and he argued that this phenomenon offered a strong evidence for advantages of cross-fertilization and natural selection (Darwin 1862). Regardless of the remarkable pollination mechanism, *Catasetum* is of particular interest within orchids, due to is one of the few genera of the family in which species are functionally dioecious with a pronounced sexual dimorphism in floral traits (i.e. each individual is able to produce pistillate flowers or staminate flowers at different times; Romero and Nelson 1986; Zimmerman 1991; Murren 2003; Milet-Pinheiro et al. 2015).

Aside from functionally dioecious trait, the flowers of the genus produce no nectar as reward, instead, the reward comprises floral fragrance compounds that euglossine bees, especially *Euglossa* and *Eulaema* collect and store (Janzen 1981; Romero and Nelson 1986; Whitten et al. 1986; Del Mazo Cancino and Damon 2007; Nunes et al. 2017). In this regard, floral scent in orchid species is of key importance for attracting pollinators and promoting floral visitation constancy through its high specificity (Romero and Nelson 1986). The orchids belonging to *Catasetum*, *Ophrys* and *Stanhopea* genus have been subject of floral fragrance research since 1960s (reviewed by Raguso 2008). Those studies show that scent

chemical composition is species-specific, promoting specialized pollination through using pre-existing biases in the sensory systems of pollinators (Romero and Nelson 1986; Del Mazo Cancino and Damon 2007; Raguso 2008).

From an evolutionary perspective, floral trait specialization in angiosperms may have evolved for increasing reproductive success through the attraction of a low number of suitable pollinators to enhance pollen transfer (Romero and Nelson 1986; Tremblay 1992; Johnson et al. 2005). In other words, floral trait specialization may have evolved in order to maximize an organism's fitness under certain circumstances (Tremblay 1992; Johnson et al. 2005). Hence, highly specialized pollination systems may be less prone to pollen limitation in presence of suitable pollinators (Romero and Nelson 1986; Tremblay 1992). Despite specialized pollination in *Catasetum*, several studies have documented a low fruit set under natural pollination regime (Janzen 1981; Milet-Pinheiro et al. 2015; Brandt et al. 2020).

In this research, the reproductive ecology of *Catasetum integerrimum* Hook (Orchidaceae) was characterized in Central Veracruz, Mexico. Specifically, I aimed to determine whether floral senescence and fruit production are resulted of pollination efficiency in the species, and if so, to what extent the present reproductive event constraint the investment in the subsequent reproductive event. The efficiency of pollination process was defined as the suitability of a vector to pick up and deposit male gametes on conspecific stigmas. The specific questions addressed were: 1) Do pollinaria removal/deposition efficiency modify floral longevity? 2) Do floral visitors are efficient pollinators in the species? and 3) Does be a maximum threshold of pollination effectiveness in setting fruits that constraint the investment in reproductive structures in the subsequent reproductive event? In this study, it was hypothesized that *Catasetum integerrimum* would exhibit floral traits and reproductive cost-saving mechanisms associated with its main pollinators' identity and behavior.

Catasetum integerrimum is a good model system for investigating the linkage between dimorphism in floral traits and pollinator performance, because of its floral scent is integrated by 15 chemical compounds in pistillate flowers and 29 chemical compounds in staminate flowers (Del Mazo Cancino and Damon 2007). Furthermore, specialized pollination system between *C. integerrimum* flowers and bees has reported previously in the literature. Whitten et al. (1986) observed that *Eulaema cingulata* and *Eulaema polychroma* were the pollinators of the species in Veracruz, México, whereas Del Mazo Cancino and Damon (2007) observed that *Eulaema cingulate*, *Eulaema meriana*, and *Exaerate frontalis* were the pollinators of the species in Chiapas, México. Thus, *C. integerrimum* exemplifies those taxa with bee specialized pollination system that exhibits sexual dimorphism in its floral traits, hence, similar tendencies in other species with dimorphic floral traits and visited by the same pollinator guild would be expected. Furthermore, studying pollinator performance in moderate to substantial specialized pollination systems may provide insights for understanding the adaptive value of floral dimorphism in angiosperms.

Materials And Methods

Field work was carried out during the raining season of 2016–2019. The study area was located in a commercial polyculture of shaded coffee plantation located in Coatepec, Veracruz México (19°28"N, 96°55"W, 1224 m a.s.l.). The epiphytic plants of *C. integerrimum* were located on trunks and branches 2–10 m above ground of trees of *Inga jinicuili* and *Citrus spp.*

Catasetum integerrimum Hook (Orchidaceae) is an epiphytic orchid distributed from Mexico to Central America. Small and scattered populations of the species have been reported in Mexico (Whitten et al. 1986; Del Mazo Cancino and Damon 2007). The species has spindle-shaped pseudobulbs and linear-lanceolate leaves. Inflorescences bear female or male flowers, which are dimorphic in morphology. The flowers produce droplets of fragrance as attracting and/or reward to pollinators. Those fragrances contain near to 15 chemicals compounds in the female flowers and 29 chemical compounds in the staminate flowers (Del Mazo Cancino and Damon 2007). *Eulaema cingulata*, *E. polychroma*, *E. meriana*, and *Exaerate frontalis* have observed foraging the flowers of the species (Whitten et al. 1986; Del Mazo Cancino and Damon 2007).

Effects of pollinaria removal/deposition efficiency on floral longevity Floral senescence triggered by pollinaria removal and/or disposition is a commonly observed trait in long-lived flowers of tropical orchids species (Vale et al. 2011; Huda and Wilcock 2012; Vega and Marques 2014). To test this prediction, I recorded the flower longevity of ten pistillate and ten staminate individuals of *C. integerrimum* in 2016. I randomly selected and tagged with plastic rings three recently opened flowers per individual. Of these tagged-flowers, the first flower was bagged with mosquito netting to avoid pollinaria removal/deposition (intact-bagged). The second flower was leave open to natural pollinaria removal/deposition (intact-unbagged). The third flower was hand-pollinated or pollinaria were hand-removal (hand-manipulated). I carefully performed pollinaria removal or deposition by using tweezers. Then, I bagged the flowers with a mosquito netting to avoid potential effects of floral visitation by pollinators. I surveyed floral longevity daily from the anthesis (flower opening) to tagged-flower withering.

I used a nested ANOVA for testing differences in floral longevity. In the model, sexual form (pistillate or staminate) was treated as a fixed factor and treatment (intact-bagged, intact-unbagged, and hand-manipulated) was nested within sexual form (Zar 1999). The response variable was the floral longevity measured in days. The variable was square root transformed to accomplish the parametric assumptions of the analysis (Zar 1999).

Floral display size and pollinator efficiency In fragrance-producing orchids, euglossine bees exhibit a highly specialized pollination efficiency to pick up and deposit male gametes on conspecific stigmas (Tremblay 1992). To test this prediction, I carried out field observations on twenty flowering individuals (ten of each sexual form) in order to describe the identity and behavior of floral visitors in the species in 2017. I counted the number of inflorescences, as well as the number of pistillate and staminate flowers per plant (Milet-Pinheiro et al. 2015). Plants remained bagged with a mosquito netting previous to floral visitation record. Each flower was tagged with a color plastic ring in order to record successive visits to a same flower by floral visitors.

The floral visitor record was conducted from 0800 to 1600 hrs for each focal plant (Nunes et al. 2017). I recorded the time of day, the species of floral visitor, the number of flowers visited, and behavior of floral visitors for each focal plant (Milet-Pinheiro et al. 2015). I recorded the beginning of observations as time zero and the consecutive foraging events on *Catasetum* flowers as minutes from the start time. I used a nonparametric Survival Analysis linked to the log-rank (Mantel Cox) statistic to analyze the probability of pistillate and staminate flowers being visited (Muenchow 1986). I used Mann-Whitney tests for testing differences in the number of inflorescences or number of flowers produced between pistillate and staminate plants (Zar 1999).

Pollination effectiveness in fruit production and resource allocation on reproductive traits Insufficient receipt of male gametes limits fruit production (pollen-limitation). Parallely, present reproduction event may constraint the investment in future reproduction event, due to reproductive value of individuals is optimized across its entire lifespan (Akerman and Montalvo 1990; Meléndez-Ackerman et al. 2000). I carried out an experimental design to test the previously predictions on fourteen individuals during the 2016–2019 seasons. First, I recorded the number of inflorescences, flowers and fruit production in the fourteen tagged individuals during 2016–2017 flowering seasons (Meléndez-Ackerman et al. 2000). Second, in seven randomly selected plants among the fourteen tagged plants, I performed manual pollinators in two consecutive years (2018–2019) according to Meléndez-Ackerman et al. (2000). Those plants were treated as effectively pollinated plants (N = 7 plants). The remainder plants were treated as ineffectively pollinated plants (N = 7 individuals).

In effectively pollinated plants, I performed hand-pollinations in 80% of flowers produced by the plant (hand-manipulated), then they were tagged and bagged with mosquito netting to avoid pollinarium removal by floral visitors. The remaining flowers (20%) were leave to be open pollinated (Akerman and Montalvo 1990). The flower was pollinated by a donor of pollinaria randomly selected in the sampled population. Hand-pollinating were performed between 09:00 and 10:00 hrs. The pollinaria were collected from a 1-day staminate flower and placed directly on the stigmas of 1-day pistillate flowers.

In ineffectively pollinated plants, I touched the stigma with a fine brush in order to simulate a floral visitation without performing an effective pollination (hand-manipulated) in 80% of flowers produced by the plant. Then, flowers were tagged and bagged with mosquito netting to avoid pollinaria deposition by floral visitors. The remaining flowers (20%) were leave to be open pollinated (Akerman and Montalvo 1990). I recorded the number of inflorescences, flowers and fruits produced per plant. I calculated the fruit set per plant at the end of fruiting season (i.e., proportion of flowers that produced fruits; Akerman and Montalvo 1990; Meléndez-Ackerman et al. 2000).

I used repeated-measures ANOVAs for testing differences in reproductive traits produced by the plants. In the model, type of plant (effectively pollinated or ineffectively pollinated) was the main factor, whereas the number of inflorescences or flowers produced by year were the repeated measure (Zar 1999). Similarly, I used a nested repeated-measures ANOVA for testing differences on fruit production (fruit set; proportion of flowers that produced fruits). In the model, type of plant (effectively pollinated or

ineffectively pollinated) was the main factor, and treatment (open pollinated or hand-manipulated) was nested in the main factor. Fruit set produced by year was the repeated measure.

Response variables were transformed in order to achieve the parametric assumptions of the analyses. Counts (number of inflorescences or flowers) were square root transformed and fruit set was arcsine-square root transformed.

All statistical analyses were run using generalized linear modeling with StatView and SuperAnova (Abacus Concepts 1996).

Results

Effects of pollinaria removal/deposition efficiency on floral longevity ^¾ Pistillate flowers exhibited a longer floral longevity than staminate flowers (Means $\pm$ S.E. of intact-bagged flowers, 32.60 ± 0.63 , $N = 10$ pistillate flowers and 8.9 ± 0.79 , $N = 10$ staminate flowers; $F = 25.14$, $p < 0.0001$). Pollinaria removal/deposition modified the floral longevity of flowers ($F = 60.12$, $p < 0.0001$). Specifically, the floral longevity of manual-pollinated flowers was reduced by approximately 31% with regard to intact pistillate flowers (Means $\pm$ S.E., 10.6 ± 0.42 ; $N = 10$ manual-pollinated flowers). Similarly, manual pollinaria-removal reduced the flower lifespan by approximately 22% with regard to intact staminate flowers (Means $\pm$ S.E., 2.2 ± 0.24 ; $N = 10$ manual pollinaria-removal flowers). The floral longevity of open pollinated flowers was variable and similar to intact-bagged flowers (Means $\pm$ S.E., 27.7 ± 3.01 , $N = 10$ pistillate flowers and 9.7 ± 0.33 , $N = 10$ staminate flowers).

Floral display size and pollinator efficiency ^¾ The *C. integerrimum* flowers opened in an acropetalous sequence from the base to the top, reaching its maximum floral display size after 1-2 days. *C. integerrimum* individuals produced either pistillate or staminate flowers arranged in one or two inflorescences produced by plant. There were not differences in the number of inflorescences produced between staminate and pistillate plants (Means $\pm$ S.E., 1.30 ± 0.15 , $N = 10$ pistillate individuals and 1.40 ± 0.16 , $N = 10$ staminate individuals, $U' = 45$, $p = 0.70$). Nonetheless, staminate individuals produced a higher number of flowers than pistillate individuals (Means $\pm$ S.E., 6.30 ± 1.12 , $N = 10$ pistillate individuals and 16.90 ± 0.23 , $N = 10$ staminate individuals, $U' = 45$, $p = 0.70$).

C. integerrimum flowers were visited by *Eulaema polychroma* (Fries) (Euglossini, Apidae). Males of *E. polychrome* approached, alighted at the labellum (occasionally at the column), and entered into the flowers, scratching the interior surface of flower with their anterior tarsi. In staminate flowers, the scratching behavior caused that bees contacted the rostellum lateral extensions, triggering the ejection of pollinaria and attaching them on the thorax of the bee. After forcible pollinaria attachment, the male bees flew away from the plant. In pistillate flowers, the scratching behavior did not cause a catapult-out effect, so that bees flew to the next unoccupied and unvisited flower on the same plant (from the base to the top). Bees hovered around staminate or pistillate flowers, but they only approached and entered into unvisited flowers during the study period.

A total of 106 floral visitation events were observed in *C. integerrimum*. Of these, 86% were observed in pistillate individuals and 14% in staminate individuals. The waiting time for a given pistillate or staminate flower to be visited by bees was statistically different (Chi = 9.83, $p = 0.002$). Staminate flowers were visited earlier by bees than pistillate flowers in the study population (means $\pm$ S.E.; 189.75 ± 13.20 , $N = 91$, pistillate flowers and 103.13 ± 15.36 , $N = 15$, staminate flowers; Fig. 1). All floral visitation events caused that pollinaria were forcefully attached to the body of bees, promoting an early departure of bees from staminate plants. In pistillate plants, a relatively low number of bees were observed carrying pollinaria. Nonetheless, all pollinaria-carrying bees lodged the pollen mass on the stigma of pistillate flowers. As a result, pistillate flowers that were naturally pollinated initiated and achieved capsule production at the end of fruiting season (near to 15% of tagged flowers). The remainder pistillate flowers wilted two days after being visited by a bee and not being pollinated.

Pollination effectiveness in fruit production and resource allocation on reproductive traits^{3/4} The number of inflorescences produced by individuals was not explained by any of the studied factors ($F = 3.69$, $p = 0.08$ type of plant; $F = 0.31$, $p = 0.80$ repeated-measure; $F = 0.42$, $p = 0.73$ interaction of type of plant $\times$ repeated-measure; Fig. 2). Nonetheless, it was observed a reduction in the number of flowers produced across time (repeated-measure; $F = 23.79$, $p < 0.0001$). That reduction in flower production was observed in the last year of survey in effectively pollinated plants (interaction between the repeated-measure $\times$ type of plant; $F = 34.11$, $p < 0.0001$; Fig. 2). Thus, effectively pollinated plants produced a lower number of flowers than ineffectively pollinated plants (means $\pm$ S.E., 13.03 ± 0.80 , $N=28$ effectively pollinated plants and 16.00 ± 0.34 ineffectively pollinated plants; $F = 27.22$, $p < 0.0001$ type of plant).

Manual pollination enhanced fruit production in effectively pollinated plants, but a reduction in fruit production was observed in the subsequent year ($F = 4.08$, $p = 0.03$ treatment nested in type of plant and $F = 2.72$, $p = 0.24$ type of plant factor). Repeated-measure analysis showed a variation in fruit production across time ($F = 15.87$, $p < 0.0001$). The fruit production variation across time was explained by the interaction of the repeated measure $\times$ treatment nested in type of plant ($F = 20.66$, $p < 0.0001$; Fig. 3). The interaction of the repeated-measure $\times$ type of plant was not significant in the model ($F = 1.01$, $p = 0.44$).

Discussion

C. integerrimum is a functionally dioecious epiphytic orchid pollinated by a single species of euglossine bee during the raining season in the study population located in Central Veracruz, Mexico.

The *C. integerrimum* floral longevity concurred with the reported floral longevity of *C. uncatum*, falling both in the category of long-lived flowers (Milet-Pinheiro et al. 2015). A commonly pattern observed in long-lived flowers is that pollination may reduce the floral lifespan (Vale et al. 2011; Huda and Wilcock 2012; Vega and Marques 2014). Floral senescence stimulated by removal and/or deposition of pollinaria have been well documented in a number of orchid species (*Chloraea alpine*, Clayton and Aizen 1996; *Acampe ochracea*, *A. papillosa*, *Eria pubescens*, *Rhyncostylis retusa*, *Dendrobium aphyllum*, *D. fimbriatum*, *D. hybrid*, *D. palpebrae*, and *Pelatantheria insectifera*, Huda and Wilcock 2012; *Epidendrum*

calanthum, *E. cochlidium*, and *E. schistochilum*; Vega and Marques 2014; *Broughtonia lindenii*, Vale et al. 2011). In *C. integerrimum*, the reduced life-time of staminate flowers after removing pollinaria were similar to the observed in *C. uncatum* (77% in *C. integerrimum* and 70% in *C. uncatum*). Nonetheless, pistillate flowers of *C. uncatum* lasted longer time without wilting than the pistillate flowers of *C. integerrimum* after pollination (68% in *C. integerrimum* and 83% in *C. uncatum*). Furthermore, the floral longevity of pistillate flowers was reduced to two days after them being visited and non-pollinated by bees.

Early flower senescence induced by pollination may be associated with the reallocation of resources to fruit production by the plant. Clayton and Aizen (1996) mentioned that reduced floral longevity in manual pollinated flowers was associated with a resource reallocation mechanism to developing fruits in *Chloraea alpina*. In *Broughtonia lindenii* was suggested that staminate flower wilting caused a reallocation of resources to fruit production, whereas pistillate flower wilting avoided re-visitation of already pollinated flowers (Vale et al. 2011). Vega and Marques (2014) recognized that floral longevity changes were determined by a trade-off between maintenance costs of floral attractiveness and allocation of resources to fruit production in several *Epidendrum* species. In *C. integerrimum*, the floral senescence may be accounted for saving-costs mechanism associated with floral maintenance rather than avoidance of re-visitation of flowers. The above because floral visitors exhibited an immediate rejection to land on a flower that was already visited, so that such flower ended to wither in the following days. In *C. uncatum* was reported the preference of pollinators for visiting non-pollinated and unvisited flowers (Milet-Pinheiro et al. 2015). Thus, the use of short-lived scent marks by bees to avoid re-visitation of flowers that were already visited by themselves and/or other bees may be possible in the genus and requires future research.

Plants' reproductive success are closely linked with the attractiveness of its floral display to attract effective pollinators. In this study, *C. integerrimum* displayed a staminate-biased ratio, attracting a single species of pollinator in the sampled population. The observed floral display size and the biased sex ratio concurred with the reported in other *Catasetum* species (*C. ochraveum*, Romero and Nelson 1986; *C. viridiflavum*, Zimmerman 1991; *C. uncatum*, Milet-Pinheiro et al. 2015; *Catasetum arietinum*, Brandt et al. 2020). Furthermore, male individuals of a euglossine bee were attracted to flowers of *C. integerrimum* as in other *Catasetum* species (Janzen 1981; Romero and Nelson 1986; Whitten et al. 1986; Milet-Pinheiro et al. 2015; Brandt et al. 2020). The above corroborates that *C. integerrimum* exhibits a specialized pollination system by bees.

In the study population, male individuals of *Eulaema polychroma* visited the pistillate and staminate flowers of the species. Nonetheless, bees visited earlier the staminate flowers but in low frequency than pistillate flowers. Del Mazo Cancino and Damon (2007) recognized that staminate flowers produced a more diverse fragrance chemistry composition than pistillate flowers (15 compounds of floral oils in pistillate flowers and 29 compounds of floral oils in staminate flowers). Thus, fragrances produced by staminate flowers may contribute to explain the early arrival of male bees to those flowers. Whilst, the forced launching of pollinaria towards bees during its floral visitation contributed to explain the early

departure and low visitation frequency of bees to staminate plants. The aversive behavior of bees caused by the forced attachment of pollinaria is coincident with the reported for several *Catasetum* species (Janzen 1981; Romero and Nelson 1986; Milet-Pinheiro et al. 2015; Nunes et al. 2017; Brandt et al. 2020).

The pollinator efficiency of *E. polychroma* in *C. integerrimum* flowers depends on matching the morphology and behavior of bees to the floral morphology and flower opening in *C. integerrimum*. In this sense, bees responded to floral morphology in the species, contacting the sexually structures of flowers. Together with floral morphology, *C. integerrimum* flowers opened in acropetalous sequence, matching with the bees' activity pattern within the inflorescence in pistillate plants (i.e., successive visits to flowers from the base to the top). In staminate plants, the aversive behavior of bees associated with the forced attachment of pollinaria avoided successive visitation of flowers, mitigating pollen mass wastage within an inflorescence. Thus, bees carrying pollinaria were potentially able to pollinated a distant plant. Overall, there is a matching between *C. integerrimum* floral traits and behavior of *E. polychroma* as pollinator. Furthermore, the observed high pollinaria deposition efficiency by euglossine bees in *C. integerrimum* concurs with the reported by Janzen (1981), who observed that the two bees bearing pollinaria were able to deposited them on the stigmas of *C. maculatum* flowers. In the same study, Janzen (1981) mentioned that only 9% of bees were returned to the same plant, suggesting that bees move over long distances. Johnson et al. (2005) observed a trend accumulation of pollinaria on a moth's proboscis by multiple visits to flowers, causing a reduction in pollinaria export, as well as obstruction of pollinaria deposition on stigmas of *Disa copperi* flowers. To my knowledge, *Catasetum* plants do no experience reproductive costs caused by an excess of pollinaria accumulation on bees during floral visitation. Thus, forced attachment of pollinaria to pollinators' body by catapult mechanism seems to play an important role in reducing reproductive costs caused by pollinators return to the same staminate plant.

Tremblay (1992) suggested that low taxonomic number of efficient pollinators per orchid species could be related to its role in diminishing the costs of failure pollen transfer. In this regard, the suitability of *E. polychroma* as pollinator of *C. integerrimum* seems to be mediated by floral trait dimorphism and dioecious nature of *C. integerrimum*. In other words, the floral trait associated with attachment of pollinaria to pollinators by catapult mechanism avoided reproductive costs associated with pollen mass wastage by interrupting stereotyped behavior of bees during its foraging within a staminate plant. In pistillate plants, bees bearing pollinaria were able to deposit them on the stigmas of flowers in every observed case.

Regardless of the efficiency in depositing pollinaria by bees, *C. integerrimum* exhibited a low fruit production by pollen limitation that was able to be released through manual pollinations. Nonetheless, the gained advantage in the fecundity by pollen limitation release seemed to lead to an over allocation of resources for fruiting that constrained the next reproductive event by reducing the production of reproductive structures. These results are consistent with the reported by several authors, who have documented that alleviating pollen limitation caused a high fruit production but it constrained future growth and/or reproduction in some tropical orchids (*Tipularia discolor*, Snow and Whigham 1989; *Epidendrum ciliare*, Ackerman and Montalvo 1990; *Dendrobium monophyllum*, Bartareau 1995).

While pollen limitation has been a topic of major research interest, little is known about ecological and evolutionary consequences of a potential maximum fruit production threshold in angiosperms. Specifically, sexual reproduction may confer fitness benefits and opportunities of adaptation, but it also may incur fitness costs for reproductive plants, as well as a low quality of produced offspring. Hence, this study highlights the importance to understand that the current pollen-limitation concept may be an oversimplification of the optimal threshold of sexual reproduction in animal-pollinated plants, so that it will require a concept re-evaluation at theoretical and procedural level.

The findings of this study showed that floral senescence and fruit production were resulted from pollen vector efficiency in picking up and depositing male gametes on conspecific stigmas, as well as in avoiding male gametes wastage mediated by floral traits in *C. integerrimum*. Although *C. integerrimum* exhibited a fruit production increase after alleviated pollen limitation, a reduction in both flower number and fruit set production were observed in the subsequent reproductive event. Together, these results were consistent with the predicted hypothesis that *C. integerrimum* would exhibit traits and cost-saving mechanism associated with its main pollinators' identity and behavior. The prevalent ecological conditions seem to favor the maintenance of low fruit production in *C. integerrimum* plants, so they may be able to adjust its fitness function across entire lifespan.

The unparallel and extraordinary floral adaptations in orchids were a source of inspiration to Charles Darwin, who devoting an entire volume to "The Various Contrivances by Which Orchids are Fertilized by Insects". Since Darwin's time to date, the pollination ecology of orchids does not stop surprising us, displaying study cases of beautiful life forms on earth.

Declarations

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Conflicts of interest

The author declares no conflict of interest.

Ethics of approval

Not applicable

Consent to participate

Not applicable

Consent for publication

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Availability of data and material

Not applicable

Code availability

Not applicable

References

1. Abacus Concepts (1996) Abacus Concepts. StatView Reference. Abacus Concepts, Berkeley, California, USA
2. Ackerman JD, Montalvo AM (1990) Short- and long-term limitations to fruit production in a tropical orchid. *Ecology* 71:262-272. <https://doi.org/10.2307/1940265>
3. Alemán M, Figueroa-Fleming T, Etcheverry A, Sühling S, Ortega-Baes P (2014) The explosive pollination mechanism in Papilionoideae (Leguminosae): an analysis with three *Desmodium* species. *Plant Syst Evol* 300:177-186. <https://doi.org/10.1007/s00606-013-0869-8>
4. Bartareau T (1995) Pollination limitation, costs of capsule production and the capsule-to-flower ratio in *Dendrobium monophyllum* F. Muell. (Orchidaceae). *Aust J Ecol* 20:257-265. <https://doi.org/10.1111/j.1442-9993.1995.tb00537.x>
5. Brandt K, Machado IC, Navarro DMAF, Dötterl S, Ayasse M, Milet-Pinheiro P (2020) Sexual dimorphism in floral scents of the neotropical orchid *Catasetum arietinum* and its possible ecological and evolutionary significance. *AoB Plants* 12(4): 1.12. <https://doi.org/10.1093/aobpla/plaa030>
6. Clayton S, Aizen MA (1996) Effects of pollinia removal and insertion on flower longevity in *Chloraea alpine* (Orchidaceae). *Evol Ecol* 10:653-660. <https://doi.org/10.1007/BF01237712>
7. Darwin C (1862) On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing. Cambridge University Press, Cambridge
8. Del Manzo Cancino A, Damon A (2007) Fragrance analysis of euglossine bee pollinated orchids from Soconusco, south-east Mexico. *Plant Species Biol* 22:129-134. <https://doi.org/10.1111/j.1442-1984.2007.00185.x>
9. Huda MK, Wilcock CC (2012) Rapid floral senescence following male function and breeding systems of some tropical orchids. *Plant Biol* 14:278-284. <https://doi.org/10.1111/j.1438-8677.2011.00507.x>

10. Janzen DH (1981) Bee arrival at two Costa Rican female *Catasetum* orchid inflorescences, and a hypothesis on euglossine population structure. *Oikos* 36:177-183. <https://doi.org/10.2307/3544443>
11. Johnson SD, Neal PR, Harder LD (2005) Pollen fates and the limits on male reproductive success in an orchid population. *Biol J Linn Soc* 86:175-190. <https://doi.org/10.1111/j.1095-8312.2005.00541.x>
12. Meléndez-Ackerman E, Ackerman JD, Rodríguez-Robles JA (2000) Reproduction in an orchid can be resource-limited over its lifetime. *Biotropica* 32:282-290. <https://doi.org/10.1111/j.1744-7429.2000.tb00471.x>
13. Milet-Pinheiro P, Navarro DMAF, Dötterl S, Carvalho AT, Pinto CE, Schlindwein C (2015) Pollination biology in the dioecious orchid *Catasetum uncatum*: How does floral scent influence the behavior of pollinators?. *Phytochemistry* 116:149-161. <https://doi.org/10.1016/j.phytochem.2015.02.027>
14. Muenchow G (1986) Ecological use of failure time analysis. *Ecology* 67: 246-250.
15. Murren CJ (2003) Spatial and demographic population genetic structure in *Catasetum viridiflavum* across a human-disturbed habitat. *J Evol Biol* 16:333-342. <https://doi.org/10.1046/j.1420-9101.2003.00517.x>
16. Nunes CEP, Gerlach G, Bandeira KDO, Gobbo-Neto L, Pansarin ER, Sazima M (2017) Two orchids, one scent? Floral volatiles of *Catasetum cernuum* and *Gongora bufonia* suggest convergent evolution to a unique pollination niche. *Flora* 232: 207-216. <https://doi.org/10.1016/j.flora.2016.11.016>
17. Raguso RA (2008) Wake up and smell the roses: the ecology and evolution of floral scent. *Annu Rev Ecol Evol Syst* 39:549-569. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095601>
18. Romero GA, Nelson CE (1986) Sexual dimorphism in *Catasetum* orchids: forcible pollen emplacement and male flower competition. *Science* 232:1538-1540. <https://doi.org/10.1126/science.232.4757.1538>
19. Snow AA, Whigham DF (1989) Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70:1268-1293. <https://doi.org/10.2307/1938188>
20. Switzer CM, Combes SA, Hopkins R (2018) Dispensing pollen via catapult: explosive pollen release in mountain laurel (*Kalmia latifolia*). *Am Nat* 191:767-776. <http://orcid.org/0000-0003-0901-169X>
21. Tremblay RL (1992) Trends in the pollination ecology of the Orchidaceae: evolution and systematics. *Can. J. Bot.* 70:642-650. <https://doi.org/10.1139/b92-083>
22. Vale A, Rojas D, Álvarez JC, Navarro L (2011) Breeding system and factors limiting fruit production in the nectarless orchid *Broughtonia lindenii*. *Plant Biol* 13:51-61. <https://doi.org/10.1111/j.1438-8677.2010.00366.x>
23. Vega Y, Marques I (2014) Both biotic and abiotic factors influence floral longevity in three species of *Epidendrum* (Orchidaceae). *Plant Species Biol* 30:184-192. <https://doi.org/10.1111/1442-1984.12046>
24. Whitten WM, Williams NH, Armbruster WS, Battiste MA, Strekowski L, Lindquist N (1986) Carvone oxide: an example of convergent evolution in euglossine pollinated plants. *Systematic Bot* 11:222-228. <https://doi.org/10.2307/2418960>

25. Zar JH (1999) Biostatistical Analysis (Fourth Edition), Prentice Hall.
26. Zimmerman JK (1991) Ecological correlates of labile sex expression in the orchid *Catasetum viridiflavum*. Ecology 72: 597–608. <https://doi.org/10.2307/2937200>

Figures

Figure 1

Comparison between staminate flowers (white circles) and pistillate flowers (black circles) of *Catasetum integerrimum* (Orchidaceae) plants in probability of being visited by male bees of *Eulaema polychroma*. The abbreviation $S(t)$ is the probability that male bees of *E. polychroma* has not yet visited a given plant. Time until male bees of *E. polychroma* arrived corresponds to the time elapsed since the start of observation period.

Figure 2

Reproductive structures produced by effectively pollinated plants and ineffectively pollinated plants across four flowering seasons (2016-2019) in *Catasetum integerrimum* (Orchidaceae). The flowers were open-intact (white bars) during the first two flowering seasons (2016-2017). The flowers were hand-manipulated (black bars) during the second two flowering seasons (2018-2019). See materials and methods for details.

Figure 3

Fruit set produced by effectively pollinated plants and ineffectively pollinated plants by treatment across four flowering seasons (2016-2019) in *Catasetum integerrimum* (Orchidaceae). The flowers were open-intact (white bars) and hand-manipulated (black bars). See materials and methods for details.