POLLINATION DECREASES LONGEVITY OF THE PROTOGYNOUS FLOWERS OF *Jaltomata sinuosa* (Solanaceae)

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AUTHORS' CONTRIBUTIONS

All authors collaborated to produce this work. Author TM designed the study and was the primary writer. Author KCP gathered data with author TM. Authors SLG and LY were partners with author TM in fieldwork in Peru. Author CTP helped to write the manuscript and provided sound advice. Author DB edited the manuscript and grew plants. All authors read and approved the final manuscript.

Short Research Article

ABSTRACT

Flowers of *Jaltomata sinuosa* are protogynous with anthers dehiscing a day after the corolla opens. Hand-pollination of flowers grown in greenhouses from field-collected (South America) seeds significantly decreases their longevity: unpollinated flowers and flowers pollinated the morning they opened lasted 4.5 and 3.6 days, respectively, when anthers were not removed, and 3.6 and 2.4 days, respectively, when all flowers were emasculated prior to anther dehiscence. Unmanipulated flowers of *J. sinuosa* set fruit abundantly in a pollinator-free greenhouse, demonstrating autogamy and self-compatibility. At the end of the corolla's life, abscission of the closed corolla (stamens are adnate to the corolla) brushes pollen onto the stigma as the corolla-androecium drops, self-pollinating flowers. Mean nectar sugar concentration was 52% with volume ranging from 0.65 to 8.5 µl (mean 2.9 µl per flower) measured once in each hermaphroditic-phase flower.

Keywords: Andes Mountains; floral attraction; floral longevity; *Jaltomata*; nectar; Peru; petal spots; sepal growth.

1. INTRODUCTION

Floral longevity can be influenced by water availability, temperature, floral maintenance costs, and pollinator density. Senescence of flowers that have been pollinated can decrease the cost (energy and water) of maintaining flowers [1]. Closure or senescence of pollinated flowers adjusts the number of flowers simultaneously open, affecting floral display size, in turn affecting the number of pollinator visits and the amount and quality of pollen received and disseminated [2]. This decreases the number of open flowers when pollinators are common consequently reducing geitonogamy [3]. Schoen and

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Ashman [4] presented floral longevity models based on tradeoffs between the cost of constructing new flowers versus maintaining existing flowers.

Pollination reduces the longevity of flowers of many species but may not generally decrease the longevity of protogynous flowers [5,6]. Our primary objective was to test this hypothesis with *Jaltomata sinuosa* (Miers) Mione, a common shrub of the Andes. Our secondary objectives were to determine if this species is self-compatible or self-incompatible, measure nectar volume and concentration in greenhouse conditions, and report post-anthesis corolla expansion and other life-history observations. The calyx of *J. sinuosa* is accrescent: after anthesis it expands and turns purplish at least in part, contrasting with the foliage. The calyx was measured regularly to document calyx expansion before and after anthesis.

2. MATERIALS AND METHODS

Jaltomata sinuosa is a common shrub growing to approximately 1 m high, in disturbed habitats of the Andes from Venezuela to Bolivia [7]. The inflorescence is umbellate and 3–5 flowered. The corolla is lavender to purple, with a purple ring surrounding five pairs of centrally located green petal spots (Fig. 1). Open flowers are oriented about 45 to 55 degrees below horizontal. Stamens are subconnivent, remaining so after filaments elongate (during day 2). There are no reports of pollinators visiting flowers in nature. However, unidentified bees were observed visiting flowers of *J. sinuosa* cultivated outdoors in Connecticut, and in Peru bees were seen by T. M. and S. L. G. visiting flowers of another Andean species having a similar floral morphology [*J. diversa* J. F. Macbr. (Mione), Department Arequipa, Peru]. *Jaltomata sinuosa* may serve as an experimental plant for a variety of studies because it

is floriferous, produces nectar, self-sets seeds that store well and readily germinate, grows well in a greenhouse, and is a shrub unlike widely utilized model plants.

Seeds were collected in the wild (Table 1) and stored at 10° C. Plants were grown in greenhouses at Central Connecticut State University where water was not limiting. Voucher specimens were deposited at CCSU. For floral longevity we tagged 28 pairs of flowers on three plants in 2015 (accession *702*). One flower of each pair was not pollinated (the control) while the other was pollinated the morning the flower opened; flowers were not emasculated. The experiment was repeated in 2017 but all flowers were emasculated prior to anther dehiscence to prevent controls from self-pollinating, and 12 pairs of flowers were used (three plants, accessions *877* and *878*). Flower longevity was recorded as the number of days a corolla was open, from first opening to final closure. For a comparison of petal length during the pistillate (anthers undehisced) and hermaphroditic (anthers dehisced) phases, paired measurements were made on different flowers on the same plant at the same time (not on the same flower at two different times). Nectar was extracted from flowers from which nectar had not been previously removed. Volume was measured with microcapillary tubes. In 2016 we used 27 flowers, approximately half in the spring and half in the fall (four plants, accession *702*), and in 2017 we used 19 flowers (accessions *877* and *878*). Nectar sugar concentration was measured with a temperature compensated digital refractometer. To 90 microliters of distilled water we added 10 microliters of nectar (1 microliter at a time), vortexed the sample and multiplied the reading by 10, on four different sampling dates (accessions *877* and *878*). Logger Pro 3, version 3.8.7 was used to model the calyx growth data.

Table 1. The geographic origins, collectors and collection numbers of the accessions of *Jaltomata sinuosa* used in this study

Collectors, collection number	Country, primary political division	Habitat	Altitude m
M. O. Dillion et al. 6193, grown as accession *534*	Peru, Department Cajamarca, province Cutervo	Garden in village	2,050
Bohs 01-58 & M. Nee 51826, grown as accession *702*	Bolivia, Department La Paz, province Larecaja	Along trail	2,475
T. Mione, S. Leiva G. & L. Yacher 869	Peru, Department Huánuco, province Huamalíes	Roadside	3,267
T. Mione, S. Leiva G. & L. Yacher 877	Peru, Department Junín, province Huancayo	Steep wall of quebrada above steam, shade	3,300
T. Mione, S. Leiva G. & L. Yacher 878	Peru, Department Junín, province Huancayo	Clearing along a trail paralleling stream, full sun	3,300

Ovules per ovary was estimated by doubling the count of one locule. Ovaries were dissected in 70% ethanol so that ovules would float away when dislodged with a needle. Pollen production was estimated using Anderson and Symon's [8] modification of Lloyd's [9] technique. To study calyx growth inflorescences were tagged and all but one flower of each was removed. Sepal length was measured with calipers every four days. We used four plants of accession *702*, and started with 12 flowers but due to attrition only 9 flowers were used during days 37 through 53, and by the last observation only four calyces were measured.

3. RESULTS AND DISCUSSION

3.1 Protogyny, Self-compatibility, Delayed Self-pollination

Flowers are autogamous (self-pollinating in the absence of pollinators) and self-compatible. Fruits containing viable seeds were abundantly produced in pollinator-free greenhouses, as reported for *Jaltomata procumbens* [10]. During the first day a flower is open its anthers remain undehisced (Fig. 1A). All (7/7) hand-pollinations prior to anther dehiscence (with pollen from hermaphroditic-phase flowers of the same plant) resulted in fruit-set, indicating self-compatibility and that the stigma is receptive prior to anther dehiscence. Thus, we describe flowers as protogynous. Flowers partially close for the night reopening the next day, with tighter closure the first night (prior to filament elongation, anther dehiscence and enlargement of the corolla) than on subsequent nights. During the second day stamens elongate markedly and anthers dehisce, transitioning the flower to the hermaphroditic phase [11, pp. 31–32]. Anthers are in close proximity to, but not in contact with, the stigma (plesiogamy [12]) (Fig. 1B). Delayed self-pollination (self-pollination after an opportunity for outcrossing [13]) without a pollinator leads to abundant fruit-set. Partial corolla closure (nightly) brings dehisced anthers close to the stigma but does not result in self-pollination. At the end of the flower's life, abscission of the closed corolla (stamens are adnate to the corolla) brushes pollen onto the stigma as the corolla-androecium drops, self-pollinating flowers. As well, wind (natural, or produced by fans in the greenhouse) may sometimes result in anthers making contact with the stigma, contributing to fruit-set in a pollinator-free environment. Development from flower to fruit takes five to five and one half weeks, likely an underestimate compared to natural conditions because many of the untagged developing fruits on the same plant were excised while tagged fruits were developing (accession *702*). From seed germination to ripe fruit took five months (accession *878*). All (11/11) flowers that were emasculated (prior to anther dehiscence) and not pollinated did not set fruit, indicating a lack of apomixis/agamospermy (accession *702*). On one 23 week old greenhouse-grown plant, 385 flower buds, flowers, developing and ripe fruits, and peduncles (from which all flowers had aborted) were counted (accession *878*).

3.2 Floral Longevity

We compared the longevity of unpollinated flowers with flowers that were manually pollinated the morning they opened. Manually pollinated flowers had significantly shorter longevity. In 2015 unmanipulated (control) flowers lasted 4.5 days, and pollinated flowers lasted 3.6 days (means), with a median difference of 1 day ($P = <.001$, two-tailed, t=4.334, df=27). In 2017 all flowers were emasculated during the pistillate phase and unpollinated flowers lasted 3.6 days while pollinated flowers lasted 2.4 days (means) ($P < .001$, two-tailed, t=7.189, df =11). In a study of spring wildflowers in North Carolina, USA, three of six protogynous species had significantly longer floral longevities when pollinators were excluded [14]. Flowers of two of the six species had longer (but not significantly longer) longevities when pollinators were excluded. And one protogynous species had the same mean longevity when insects were excluded [14]. Thus, pollination sometimes decreases the longevity of protogynous flowers, in contrast with an assertion made [5] that protogynous flowers should be insensitive to receipt of pollen because "protogyny would otherwise produce plants that are without male fitness." In contrast with our results and the results of Motten [14], the floral longevity of one protogynous species, *Dentaria laciniata* (studied in Illinois, USA) was longer if flowers were pollinated [15].

3.3 Corolla

When the sepals open, revealing the corolla for the first time, the corolla is pale-green to whitish, and then the corolla transitions to lavender a day before it opens. The adaxial face of the corolla (the face commonly encountered by pollinators) is pilosulose, the hairs 0.1–0.2 mm long, but is glabrous in the nectar zone (Fig. 1C) around the androecium. In contrast the abaxial face of the corolla is similar to the abaxial face of the calyx: both are villous with gland-tipped simple uniseriate multicellular unbranched hairs (finger hairs [16]) variable in length but up to 2 mm long. The corolla has been described as 5-lobed [17] but based on more than 10 collections in Latin America by T. M., S.L.G. and L. Y. the corolla is 10-pointed, the lobes and lobules alternating [18].

When the flower transitions from the pistillate phase to the hermaphroditic phase the corolla expands

significantly: mean petal length during the pistillate phase (day 1) was 9.7 mm, and during the hermaphroditic phase (day 2 and on) was 13.3 mm (P < .001, paired t-test and a Wilcoxon matched-pairs signed-ranks test, 15 pairs, df = 14, accession *702*). Why does the corolla expand while making the transition from the pistillate phase to the hermaphroditic phase, a day after it opens? We speculate that a pistillate-phase flower needs from one to a few visits to be pollinated and set fruit, while a flower providing pollen can contribute to a plant's fitness during multiple visits by pollinators [19]. Both phases provide nectar, but given that the pistillate phase is followed by the hermaphroditic phase, a plant can achieve greater fitness by attracting more visits to pollen-providing flowers than to pistillate-phase flowers. Having pistillate flowers the same size as hermaphroditic flowers would invite an equal number of visits per unit time. And this limited number of visits would be better spent asymmetrically by directing pollinators preferentially to flowers that provide pollen because such flowers can contribute to a plant's fitness the most by receiving multiple visits by pollinators (anther dehiscence is staggered). Given a finite number of insect visits, more than a few insect visits to a flower that is pistillate has the cost of lost visits to flowers where pollen could have been picked up and carried elsewhere resulting in the siring of seeds. This hypothesis has yet to be tested.

3.4 Androecium

Stamens elongate markedly when the flower transitions from the pistillate to the hermaphroditic phase, usually the second day the flower is open. The five stamens within a flower elongate at different rates [11, pp. 31–32]. Anther dehiscence is staggered. This was observed not only on the plants we grew for study but also on wild plants encountered in Peru. It is common to see in one flower, two (three) longer stamens having dehisced anthers and three (two) shorter stamens having non dehisced anthers (Fig. 1D). All the stamens of a flower finally become nearly the same length (Fig. 1C). In the greenhouse stamens were 9–11 mm long, including the anther, the filament and the expanded base (accessions *702* and *877*). The hairs of the staminal filament decrease in length acropetally, and are intensely purple proximally fading to unpigmented distally (Fig. 1C). Prior to dehiscence anthers are purple along their sides and bottom, are pale-yellowish otherwise, and the connective is greenish.

Anthers dehisce via longitudinal slits in the genus *Jaltomata*, unlike its sister genus *Solanum* where anthers have terminal pores [20,21]. The mean number of pollen grains per androecium was 82,058 (range 47,500 to 145,000), and ovules was 153 (range

111 to 208) per ovary (11 counts/flowers, 7 accessions). The mean pollen to ovule (P/O) ratio was 653 (the range of P/O from individual flowers was 228 to 1,306). *Jaltomata sinuosa* appears to have a mixed mating system given that it is protogynous but also self-compatible and autogamous; the P/O we found (653) falls near that of the category [22] "facultative xenogamy."

3.5 Nectar

Starting in the pistillate phase, nearly translucent nectar accumulates on the base of the corolla in shallow troughs (Fig. 1B). Dissection of flowers revealed that the ovarian disk, an expansion of approximately the bottom fourth of the ovary, is orange on open flowers (accessions *702* and *878*). However, on flowers that have not yet opened or senesced the ovarian disk is off-white. Sugar concentration ranged from 50 to 54 Brix, mean 52. From hermaphroditic phase flowers we removed 0.65 to 8.5 µl of nectar, measured once per flower in a pollinator-free greenhouse. Nectar production per flower varied among those days samples were drawn, possibly due to differences in sunshine / cloudiness and temperature (daily means were 1.18, 3.16 and 5.5 µl). Given that sample size varied among days, we present a mean (2.9 µl) of the three daily means, rather than a mean of all measurements. Given both the ubiquity of *Jaltomata sinuosa* and the hundreds of flowers a plant produces, its nectar may serve as a significant source of food and water for some insects in the Andes Mountains.

3.6 Calyx Growth

Prior to anthesis the calyx surrounds the flower bud, presumably protecting the bud, and sepals are 2.2 mm or less long. After anthesis, the calyx continues to grow (accrescence) becoming infundibular over the fruit. Sepal length grows six-fold to about 12 mm long. Sepal length is described by the expression $Ae^{-cx} + B$ fitted to the calyx growth data (A = -11.13, B = 12.14, C = 0.07, RMSE = 0.28), shown in Fig. 1E.

3.7 Leaves, Fruits and Seeds

The leaves and stems of plants (and the hypocotyl and cotyledons of seedlings) are villous with glutinous-tipped hairs; to handle *Jaltomata sinuosa* renders one's hands sticky, and we commonly saw whiteflies immobilized on leaves. We also noted that the number of whitefly eggs is markedly lower on leaves of *J. sinuosa* than on the nearly glabrous leaves of some other *Jaltomata* species grown in the same greenhouse at the same time. The fruits of *J. sinuosa* (orange at maturity, Fig. 1F) are consumed by people at least in Peru and Bolivia (herbarium specimen
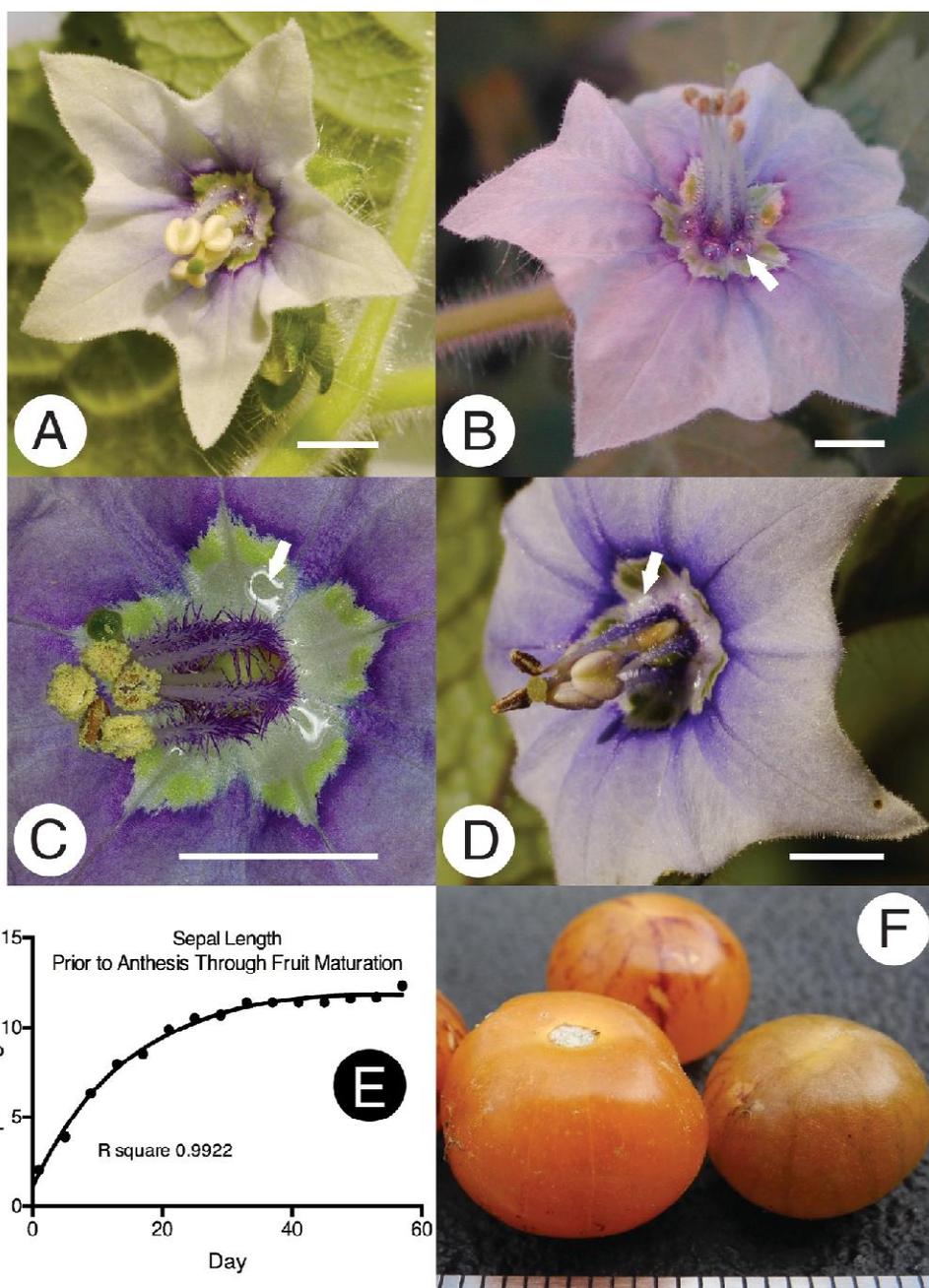
**Fig. 1. A. Flower in pistillate phase (prior to anther dehiscence); greenhouse-grown, accession *702*.
B. Flower in hermaphroditic phase. Note nectar (arrow), plesiogamy and pollen on the stigma;
greenhouse-grown, accession *534*. C. Center of a flower in hermaphroditic phase. Note nectar (arrow) on
corolla surrounding androecium. Stamens are normally subconnivent (as in B) but became connivent as
shown after removal of the flower from the plant. The stigma is dark green and usually centrally located
but in this photo it is deflected to the side of the anthers; greenhouse-grown, accession *702*. D. Staggered
anther dehiscence: The anthers of the stamens that elongate more rapidly dehisce first. Note the shallow
nectar trough (arrow) where corolla meets androecium. The stigma, green, is near the two dehisced
anthers. Photo taken in Peru, accession *878*. E. Sepal growth rate. Each plotted point is a mean sepal
length. F. Mature fruits. Photo taken in Peru, units are mm, accession *869*. All scale bars equal 0.5 cm.
Photos by T. M**

labels and fieldwork by T. M., S. L. G and L. Y.). Seeds per fruit is significantly higher in the wild than in cultivation, due either to growing conditions (e.g., greenhouse cultivation in pots clearly limits growth and may limit fecundity) and/or the absence of pollinators in the greenhouse (on wild plants there were 86–191 seeds per fruit, mean 151, n = 5 counts; on cultivated plants there were 64–114 seeds per fruit, mean 90, n = 7 counts; P = .004, two tailed, df=10, T=3.664). The first above-ground signs of seed germination were seen 9 to 25 days after seeds were sown (mean 21 days, six trials, five accessions, 1990 to 2016). It takes about 3 months from sowing seeds to flowering in greenhouses (two accessions, 1990 and 2015).

4. CONCLUSIONS

Although the longevity of protogynous flowers has been reported to be insensitive to receipt of pollen, pollinating flowers of *Jaltomata sinuosa* significantly decreased longevity. *J. sinuosa* appears to have a mixed mating system: flowers are protogynous, autogamous, and self-compatible. Flowers partially close for the night reopening the next day. Floral nectar is produced. Fruits, orange at maturity, are abundantly self-set and eaten by people.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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