

Reproductive Biology of *Jaltomata procumbens*
(Solanaceae)

Rebecca R. Flinn

A Thesis Proposal
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Masters of Arts in Ecology and Evolutionary Biology
Department of Biology

Central Connecticut State University
New Britain

January 2016

Thesis Advisor
Dr. Thomas Mione
Biology Department

Reproductive Biology of *Jaltomata procumbens*
(Solanaceae)

Rebecca R. Flinn

An Abstract of a Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Masters of Arts in Ecology and Evolutionary Biology
Department of Biology

Central Connecticut State University
New Britain

January 2016

Thesis Advisor
Dr. Thomas Mione
Biology Department

Keywords: altitudinal trends, delayed self-pollination, floral morphology, geographic regions, *Jaltomata*, protogyny, self-compatibility

Abstract

This study investigates the reproductive biology and floral variation among 13 accessions of *Jaltomata procumbens* by noting temporal and morphological changes during anthesis. Anthesis lasts about three days; the first day of flowering the flowers are in the pistillate phase in which the anthers are not dehisced. The stigma is receptive during this period as seen with flowers that were emasculated and then hand-pollinated during the pistillate phase. The second and third day of flowering, the flowers are hermaphroditic, one or more of the anthers have dehisced. The filaments fully elongate during day two, angling outwards to varying degrees depending on the accession. Though fully dehisced, anthers do not come into contact with the stigma until the end of day two when the stamens become connivent, with the exception of accession 321 in which the stamens remain connivent during the life of the flower. The corolla position may be reflexed or partially concave while the anthers are connivent. Due to the anthers coming into contact with the stigma at the end of day two, this species can be said to show delayed self-pollination, allowing for cross-pollination prior to selfing. As seen in other members of the genus *Jaltomata*, *J. procumbens* is self-compatible. All plants were grown in a pollinator-free greenhouse ensuring no cross-pollination. Therefore, all fruit produced was from self-pollination or in the case of the emasculated flowers, hand-pollination. The variations of flower phenology among accessions were compared and noted in respect to geographic locations and altitude.

Table of Contents

Abstract.....	3
Acknowledgements.....	5
Introduction.....	6
Materials and Methods.....	15
Study Species	15
Plant Accessions.....	16
Reproductive Biology	19
Self-Compatibility.....	20
Study.....	20
Results.....	22
Discussion	42
Variation in Morphological Traits Among Accessions.....	42
Variation in Morphological and Temporal Traits Within Accessions	43
Correlation Analysis of Traits to Altitude Where Collected.....	44
Correlation Analysis of Morphological and Temporal Traits.....	45
Pollen to Ovule Ratio	45
Plant Accessions.....	46
Conclusion	51
Appendix A.....	54
Literature Cited.....	59
Biographical Statement.....	61

Acknowledgements

Thank you to Dr. Mione for initially suggesting this study, as well as his encouragement, advice, and time spent on this project. Without Dr. Mione, I would not have been able to grow plants and conduct this study during the cold months of winter.

Thank you to Dr. Donald Blume, for all of his assistance in keeping the plants alive and as pest-free as possible. This project would not have been possible without his extensive knowledge of plant care.

I am grateful for the help of Kenneth Plourd, for all of his work on pollen counts. It is a time-consuming project and could not have been completed without him.

I would also like to thank my wonderful father, Brian Flinn, whose support and help in photography provided the wonderful pictures presented here.

Thank you very much for all of my family and friends for the help, encouragement, and patience throughout this journey.

Most of all I would like to thank my mother, Deborah Flinn, who always gave me such support and encouragement to follow my heart no matter what obstacles may entail.

Introduction

A discovery in plant biology by Knight in 1799, followed by Darwin in 1868, found that cross-fertilization is advantageous. This sparked the first intensive comparison between self- and cross-fertilization. Outcrossing combines genes in novel combinations, allowing rapid evolution in populations. While this may combat events such as inbreeding depression (Vallejo-Marin et al. 2013), it does tend to cost a lot of energy in pollen production and flower morphology. In 1955, a botanist named Baker stated that successful colonization of new sites by autogamous, self-fertilizing plants would be “much more likely” than of obligately outcrossing plants. This was restated and termed Baker’s law around 1966 (Pannell and Barrett 1998). Most self-fertilizing plants have been thought to have evolved due to lack of pollinators, yet could also have developed due to lack of mating partners which can occur following long-distance dispersal (Pannell and Barrett 1998). A self-pollinating individual is able to set seed under pollen-limited conditions such as a spatially or temporally variable pollination environment. Due to this ability, it is theorized that natural selection should favor self-fertilization over cross-fertilization (Vallejo-Marin et al. 2013). The evolution of autogamous plants is due to many factors such as exiguous pollinators, low energy budget, isolation, or coevolution with nectar robbers (Kaczorowski et al. 2005).

This type of evolution can be seen in several species, including *Fragaria chiloensis* which is dioecious along the Pacific Coast of North and South America but is hermaphroditic in Hawaii (Pannell and Barrett 1998). *Mercurialis annua* is another dioecious species in which self-compatibility has evolved in regions requiring reproductive assurance (Pannell and Barrett 1998). Self-compatibility refers to the ability of the pollen to fertilize an egg within the ovary of a genetically identical plant (Kaufman et al. 1983). Baker originally thought that the evolution of self-compatibility would occur mostly in plants with long dispersal methods, yet more recent

studies have found that the distance of dispersal is unimportant (Pannell and Barrett 1998). Moreover, it is theorized that individual autogamous plants will always be more successful than obligate outcrossers in founding new colonies and therefore should be expected to be seen in species that frequently colonize new sites or go through cycles of extinction and recolonization (Pannell and Barrett 1998).

With much research and debate under way, it was acknowledged that certain plants have adapted to self-fertilization including possessing floral syndromes opposite of those identified with outcrossing. It is theorized that selection for increased self-fertilization in colonizing plants will occur more frequently in annuals as a lack of perennial life, and clonal reproduction will prevent the species from buffering any costs that can happen in a season of reproductive failure (Vallejo-Marín et al. 2013). These colonizing species also have a tendency to immigrate to ephemeral or disturbed habitats, such as *Solanum ptycanthum* Dunal which is found mostly in agricultural fields (Vallejo-Marín et al. 2013). A general statement about short-lived colonizing plants is that they are very often defined by low levels of outcrossing (Vallejo-Marín et al. 2013).

Around half a century later, the debate on self- and cross-fertilization was brought up again with the development of genetic studies. The genetic studies of the floral mating system were found to contrast greatly with the previous functional studies (Lloyd and Schoen 1992). Genetic studies bring to light the effects of inbreeding as well as the advantages of outcrossing. Autogamous species have been shown to display low levels of heterozygosity which, after generations of autogamy can fix alleles or chromosome rearrangements. Therefore, any cross-pollination that may occur would reduce the overall fitness of the seed and increase postzygotic costs (Bryant et al. 2016). The genetic studies do not look into the ecological variables presented to species.

Many functional factors lead to self-fertilization such as prepotency in which a self-compatible species has more success at setting fruit when cross-pollinated, pollen discounting, reproductive assurance (Lloyd and Schoen 1992), paucity of pollinators, energy costs, and other abiotic factors. Colonizing species in disturbed pollinator-free environments have shown pronounced shifts in floral traits and mating systems (Brys et al. 2013). There is also the aspect of differing self-fertilization mechanisms including cleistogamy, geitonogamy, facilitated self-pollination, and several modes of autonomous self-pollination (Lloyd and Schoen 1992). A species can adapt from outcrossing to self-pollination or self-pollination to outcrossing or even to mixed-mating due to a variety of ecological, morphological, or physiological factors. All of these factors affect the amount of possible self-fertilization that could take place.

Though outcrossing may seem beneficial in a genetic sense, self-pollination with its low energy costs due to low pollen production and minimal floral attractants can be advantageous (Lloyd and Schoen 1992). As seen in the study of *Centraium erythraea*, *C. littorale*, and *C. pulchellum* (Brys et al. 2016), the overall proportion of hybrid seeds was 62.5% lower in intact flowers than in emasculated flowers, suggesting a high level of competing or prior selfing (Brys et al. 2016). This adds to the evidence that selfing species are not only adapted to assuring reproduction but can hinder heterospecific pollen deposition and prevent cross-pollination (Brys et al. 2016). Even if pollinators are able to bring pollen of a selfing species, prior or competing selfing may have already clogged the stigma (Brys et al. 2016).

There are several modes of self-fertilization including cleistogamy, geitonogamy, facilitated selfing, and autonomous selfing (Lloyd and Schoen 1992). At times, cleistogamy has been found to be distinct due to pronounced morphological differences of self-fertilizing flowers from chasmogamous (potentially outcrossing) flowers on the same plant. The selfing flowers are

specialized in which the corolla is permanently closed, many species producing seeds of a different size than their chasmogamous counterparts (Schoen and Brown 1991). This enables these flowers to be easily identified (Lloyd and Schoen 1992). The advantages of cleistogamy include low energy costs for pollen and attractants, whereas chasmogamous flowers are required to entice pollinators through flower shape, color and aroma, and need to provide more pollen to ensure fertilization.

Geitonogamy, the most widespread form of self-pollination, occurs when pollen from one flower fertilizes another flower on the same genetically identical plant (Lloyd and Schoen 1992). Unlike cleistogamy, this mode usually requires the same pollination vector as cross-pollination. Due to this similarity, a small amount of geitonogamy is unavoidable in any self-compatible plant (Lloyd and Schoen 1992).

Facilitated self-pollination is analogous to geitonogamy in that it often occurs at the same time as outcrossing. As another inevitable occurrence in self-compatible species, pollinators are likely to inadvertently pollinate a stigma with pollen from the same flower's anthers unless there is a mechanism to ensure stigma receptivity strictly precedes pollen presentation such as in some protogynous species (Lloyd and Schoen 1992).

The fourth mode of self-pollination is autonomous self-pollination, which is broken up into three systems: prior selfing, competing selfing and delayed selfing (Lloyd and Schoen 1992). Prior selfing occurs when the stigma becomes receptive alongside the dehiscing of the anthers before cross-pollination can occur (Lloyd and Schoen 1992). This spatial and developmental overlap occurs either prior to anthesis, such as when opening has been postponed due to poor weather which is also known as bud pollination, or just after anthesis has begun (Brys et al. 2016). The difference between prior selfing and cleistogamy, is that in cleistogamy the flowers have

significant morphological differences than the chasmogamous flowers on the same plant and do not open. Whereas prior selfing flowers may undergo bud pollination but do open with no significant morphological differences to any other flowers on the plant. This form of selfing significantly abates the chances of cross-pollination (Brys et al. 2016). Competing selfing is similar to facilitated selfing in that both modes occur in the same period as cross-pollination due to the stigma receiving self and outcrossed pollen from a pollinator (Schoen and Brown 1991). Unlike facilitated selfing, competing selfing also occurs when the anthers are in close proximity to the stigma, also known as plesiogamy (Neson 2012), and without the aid of a pollinator (Brys et al. 2013). Competing selfing is found in species in the Brassicaceae (Lloyd and Schoen 1992) due to temporal variation in the maturity of the anthers and stigmas, known as dichogamy (Brys et al. 2013). The main component of competing selfing is that it occurs simultaneously with cross-pollination resulting in the competition between self and cross pollen (Brys et al. 2016).

The third form of autonomous self-pollination is delayed selfing, which occurs when the anthers and stigma come into contact only after the stigma has had some time to be cross-pollinated. This form of self-pollination occurs at the end of anthesis, either when the corolla is in senescence forcing the anthers to encounter the stigma (Lloyd and Schoen 1992) or on the last day of anthesis when the opportunity for cross-pollination has already passed (Brys et al. 2016).

Table 1: Modes of self-pollination (Lloyd and Schoen 1992).

Mode of self-pollination	External pollen vector required	Temporal separation of anthers and stigma required	Spatial separation of anthers and stigma required	Occurrence of bud pollination	Opportunity to outcross prior to, during, after, or none
Cleistogamy	No	No	No	Yes	None
Geitonogamy	Yes	Yes	Yes	No	During
Facilitated	Yes	Yes	Yes	No	During
Prior	No	No	No	Yes	After
Competing	No	No	No	No	During
Delayed	No	Yes	Yes	No	Prior

In 1876 Darwin demonstrated the adaptiveness of plants to become self-fertilizing when isolated. More modern experiments have yielded similar results on species with equal success in separate self- and cross-pollinations (Lloyd and Schoen 1992). This phenomenon was termed cryptic self-incompatibility, or as Darwin coined, prepotency. Prepotency is when a self-compatible species has better success at setting fruit when cross-pollinated (Lloyd and Schoen 1992). The amount of self-fertilization that occurs relies heavily on the environment and the floral morphology (Brys et al. 2016).

Whether a plant exhibits cleistogamy, geitonogamy, facilitated or autonomous self-fertilization or is not autogamous at all, flowers are morphologically adapted to attract particular pollinators or be pollinated abiotically. Floral adaptations for autonomous selfing can even inhibit heterospecific mating through mechanical protection (Brys et al. 2016). Multiple floral traits have been correlated with selfing such as minimal or no herkogamy, small floral size, and relatively low pollen production (Brys et al. 2016). The corolla can be the primary attractant to pollinators in its

size, structure, color and appearance. A reduction in the size of the corolla has been shown to reduce the number of pollinators that visit (Brys et al. 2016). Some flowers such as those of *Pedicularius denrothauma* have spots on the corolla known as petal spots, which act as an attractant and guide for pollinators to the nectar (Subodh and Kumar 2010). *Jaltomata procumbens* (Cav.) J. L. Gentry has green petal spots on its corolla, the extent of which varies among accessions from different geographic regions. For example, accession 599, collected in Morelos, Mexico, has up to 70% of its corolla covered in petal spots whereas accession 587 from Distrito Federal, Mexico, has only 11% of its corolla covered in petal spots. This could indicate the type and amount of available pollinators in each accession's region, as well as a possible shift towards higher amounts of self-pollination in the accession having fewer spots.

In addition to the availability of pollinators, species adapt to the quality of pollination they receive. As noted by Williams (1985, page 92) in his master's thesis, in Tlaxcala, Mexico honeybees have been observed visiting *Jaltomata procumbens* and potentially acting as a pollinator. Honeybees are not native to the region and therefore the quality of visitation is unknown. Muchhala et al. (2008) studied not only the types of pollinators for *Aphelandra acanthus*, but the quality of their pollination and found that what is considered a high-quality pollinator not only frequently visits and is in abundance but also provides the most conspecific pollen. *A. acanthus* has two main pollinators, bats and hummingbirds. While the bats frequented the flowers as often as the hummingbirds, the bats provided the largest abundance of heterospecific pollen, pollen from other plant species that cannot fertilize the targeted species. This heterospecific pollen is not inconsequential, as it can end up blocking or clogging the stigma or style and prevent pollination from homospecific pollen (Muchhala et al. 2008). These low-quality pollinators are termed negative pollinators as they contribute to the reduction of the plant's overall fitness

(Muchhala et al. 2008). With negative pollinators in mind, extreme specialization is not always beneficial. In theory, generalization should be favored as the abundance of pollinators can vary greatly season to season. Generalization causes a mere increase in the number of pollen vectors per unit time and allows for the flexibility to be able to withstand any spatio-temporal fluctuations in pollinator abundance (Muchhala et al. 2008).

Jaltomata procumbens exhibits self-compatibility in the pistillate (female), and hermaphroditic phases. The majority of this species has adapted to cross-pollination with an insurance of self-pollination due to delayed selfing. The stigma is receptive during day one, the pistillate phase, before the anthers dehisce. On day two of anthesis, the stamens start to dehisce, beginning the hermaphroditic phase. In most accessions, the stamens angle outwards once dehisced (Figure 8 Appendix), allowing for cross-pollination to readily occur. At the end of day two or the beginning of day three of anthesis, the stamens become connivent as the corolla starts to close for the night (Figures 9 Appendix and 10 Appendix) and begins self-fertilization. Once connivent, the anthers are in contact with the stigma, ensuring pollination and setting of fruit.

While delayed-selfing has been noted on the majority of the accessions used in this study: 318, 320, 324, 401, 506, 580, 587, 593, 599, 837, 838, and 844, one accession in particular stands out from the rest. The stamens of accession 321 dehisce while connivent and do not angle outward like the stamens of the other studied accessions, suggesting competing selfing rather than delayed selfing. Each accession has unique attributes which may be due to biotic and abiotic differences such as altitude, climate, and pollinator availability, or genetic drift. Altitude can produce significant differences within a species as found by Vitasse et al. (2009). Regardless of the proximity of the populations, the altitude affected the gene expression of phenological traits (Vitasse et al. 2009). Even traits that are under strong genetic control such as leaf morphology are

subject to environmental factors such as altitude (Hoveden and Vander Schoor 2005). High-altitude environments can infringe severe restrictions on the phenology of plants including reproduction and growth. Therefore, species can display considerable variation in morphological features along altitudinal gradients (Bresson et al. 2011, Vitasse et al. 2009).

Plants of *Jaltomata procumbens* are herbaceous with an erect or procumbent habit. There are significant variations in morphological and temporal features among accessions. *J. procumbens* displays autogamous delayed selfing. Due to this species ranging over a variety of elevations, this study tested for significant differences in traits among the accessions due to altitudinal trends. The objectives were to (1) observe developmental changes to the corolla, androecium, and gynoecium during anthesis, (2) note the specific mechanism of selfing, (3) confirm stigma receptivity and self-compatibility in both the pistillate and hermaphroditic phases and (4) test for correlation of traits with altitudinal gradients.

Materials and Methods

The genus *Jaltomata* is widely distributed from Arizona in the United States south to Bolivia, as well as in the Greater Antilles and the Galapagos Islands (Mione 1999, Mione et al. 2007). In regions of Peru, the center of diversity for *Jaltomata*, the species has been found to grow at 4,000 meters in elevation (Mione 1999, Mione et al. 2007). *Jaltomata* species have simple, alternate leaves, and the corolla has either five lobes or five lobes with five lobules (Mione, pers. com. 2016). The South American species are nearly all woody and have a full range of corolla forms including tubular, urceolate, crateriform, campanulate-rotate, and rotate (Mione et al. 2007). These species produce orange to red berries whereas, in Mexico and Central America, the plants are herbaceous with rotate corollas and berries are black or purple-black berries (Mione et al. 2007). Nectar ranges in color among the species, from transparent such as in *J. procumbens*, to blood red as in *J. aspera* (R. & P.) (Mione and Anderson 1996).

Study Species

Jaltomata procumbens is a perennial polycarpic fruiting plant belonging to the family Solanaceae. This species is widely distributed from Arizona, USA to Ecuador (Mione and Spooner 2010) and grows from 1,000 to 2,900 m in altitude (Mione and Yacher 2005). As a herbaceous plant, it can reach a height of 1.8 meters with branches ranging from green to purple in color and either five-sided or with raised longitudinal ridges. Ovate, entire to toothed leaves are arranged alternately and are either glabrous or pubescent. The inflorescence is umbellate with up to 18 flowers. A partially fused calyx is either green, or green with purple on the main veins during flowering. At fruit maturity, the calyx is green to purple and hangs in a bowl-like fashion over the fruit, partially concealing the fruit in a side view (Mione, pers. com. 2015). The corolla consists of

five lobes and at times also five lobules (Mione and Spooner 2010). The filaments elongate on the flower's second day. For *J. procumbens*, the corolla color is usually green to pale green and most collections have darker green petal spots. The stamens range from 4.5 to 7 mm long with ovate yellow anthers when undehisced, browning after dehiscence. The gynoecium is glabrous except for the stigma papillae. The nectar, unpigmented, is evident around the base of the corolla. The fruit is commonly black or a very dark purple with a few accessions producing mature green fruits (Mione, pers. com. 2015). The fruits remain attached to the parent plant and normally do not drop to the ground at maturity (Mione, pers. com. 2015). On average, it requires 39 days for a pollinated flower to become a ripe fruit (Mione, pers. com. 2015). Within the same plant, the flowers open asynchronously with anthesis of individual flowers taking place over three days. Except for accession 321, the corolla closes at night and at the end of anthesis. *J. procumbens* is self-compatible, protogynous (Mione and Yacher 2005), and displays a form of self-fertilization (Davis 1986).

Plant Accessions

Through this study, high levels of variation among accessions has been evident. This was also shown by Mione and Yacher (2005) in a plot of principal components, in which *J. procumbens* is not tightly clustered. Differences occur in growth habit, procumbent versus erect, the presence or absence of hairs on leaves, the extent of petal spots on the corolla, the maximum filament angle relative to the style, the number of flowers per inflorescence, and the petal and filament length. The majority of accessions display delayed selfing, and petal spots presumably for attracting pollinators. Accession 321, with few petal spots (Mione and Anderson 2016), presents competing selfing, suggesting a pollinator-poor or pollinator-free environment. Leaf surfaces varied by

accession and were either glabrous, nearly glabrous or pubescent; the hairs when present were never gland-tipped (though, gland-tipped hairs were seen on the hypocotyl, not on the leaves, of some accessions).

Table 2: *Jaltomata procumbens* plant accessions used in this study. Origin and altitude where collected, of accessions and whether the leaves are pubescent or glabrous. Altitudes and origins courtesy of Dr. T. Mione.

Accession Number (T. Mione)	Origin	Altitude (m)	Presence or Absence of Leaf Hairs
318	Guatemala	2,750	Pubescent
320	Guatemala	2,680	Pubescent
321	Guatemala	2,054	Pubescent
324	Guatemala	2,840	Pubescent
401	Mexico	1,650	Pubescent
506	Mexico	1,675	Nearly Glabrous
580	Honduras	1,700	Pubescent
587	Mexico	2,750	Glabrous
593	Mexico	2,600	Glabrous
599	Mexico	2,230	Glabrous except for leaf margin
837	Guatemala	no data	Nearly glabrous
838	Guatemala	2,714	Glabrous except for leaf margin
844	Guatemala	2,291	Sparsely pubescent



Figure 1: Locations where accessions were collected. Data courtesy of Dr. Thomas Mione.
(<https://www.google.com/maps/@24.1098832,-100.3957644,5z>)

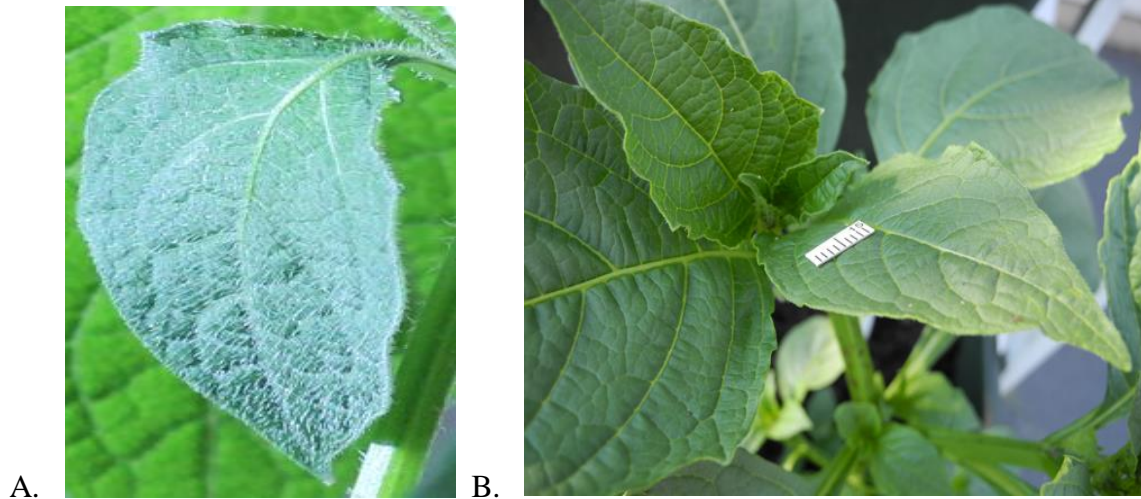


Figure 2: Variation in leaf hairs of *Jaltomata procumbens*. A. pubescent leaf, adaxial face (401), B. glabrous leaf, adaxial face (599), smallest units on segment of ruler are mm, photo by T. Mione.

Reproductive Biology

The reproductive biology of 13 accessions of *Jaltomata procumbens* was studied. Geographically widespread accessions of *J. procumbens* were grown, giving an initial total of eighty-eight plants. All the plants were grown in a pollinator-free greenhouse. To ensure there was no outside pollination, a hand lens was used to look for pollen on the stigma during the pistillate phase. Observation of flowers during anthesis took place at intervals throughout the day, using a metric ruler and hand lens. Variation occurs among accessions of *J. procumbens* in the extent of petal spots on the corolla, the maximum filament angle as well as the growth habit and vestiture. Notes were taken on all of these features for comparison. A small metric ruler was used to determine if all the stamens are the same length during and after elongation as well as whether the petals change in length from the pistillate to the hermaphroditic phase. The filaments were measured from the base of the filament to the bottom of the anther, excluding the anther. Measurements of the petals and filaments were taken on day one of flowering during the pistillate phase, and again on day two or three during the hermaphroditic phase, once all anthers had fully

dehiscence. To ensure fruit was not produced without pollination (agamospermy), randomly chosen flowers of each plant were emasculated before the anthers dehisced. By measuring the length of the filament and distance between two anthers on opposite sides of the flower with a ruler, a triangle with three known sides was created and the Law of Tangents was used to calculate the angle. The durations of the pistillate and hermaphroditic phases were recorded as before and after any of the five anthers started to dehisce.

Self-Compatibility

Jaltomata procumbens has been noted to be self-compatible and show delayed or competing selfing depending on the accession. Delayed selfing is here defined as the stamens becoming connivent for the third and final day of anthesis with the aid of the corolla, whereas competing selfing is defined as the stamens becoming connivent when anthers dehisce, leaving only one day in which the stigma was receptive and fully open to outcrossing. If prior selfing were to occur, it would have been defined as the stigma being receptive and in contact with dehisced stamens at the time anthesis begins, but this was not observed. To test for self-compatibility, one flower from each accession was emasculated during the pistillate phase and pollinated with the pollen from another flower of the same plant. Hand-pollination during the pistillate phase was also used to test if the stigma was receptive throughout the entirety of anthesis.

Study

To test for morphological and temporal differences of floral characters among accessions, a common garden study was conducted (Bresson et al. 2011, Hovenden and Vander Schoor 2005, Vitasse et al. 2009). All plants were grown from seed sown July 2015 in a pollinator-free

greenhouse. Plants were watered by drip irrigation and grown in 6” pots. Observations were taken at my home with two to ten plants at a time to observe and take measurements throughout the day. Artificial grow lights were utilized to supplement sunlight throughout the day. Osmocote slow-release fertilizer was added each time the plants were transplanted to larger pots. The study took place from July 2015 through January 2016. Both locations in which the plants were kept were pollinator-free; the only pollination that occurred was from self-pollination and hand-pollination. In order to test for self-compatibility, as well as stigma receptivity in the pistillate and hermaphroditic phases, a randomly chosen flower was emasculated and pollinated with a dehisced anther from a flower of the same plant. The techniques used for this process included a pair of tweezers and a lighted magnifying glass. A one-way ANOVA was run to test for significant differences in morphological and phenological traits among accessions. A paired T-Test was run to test for significant differences within accessions for traits such as the petal length in the pistillate and hermaphroditic phases. A Pearson correlation analysis was run in order to test for significant relationships among studied traits and the altitudes in which the accessions were collected.

Results

The majority of the studied accessions displayed delayed selfing in which the stamens angled outward away from the stigma until the end of day two (or the beginning of day three) when the corolla began to close for the night. Accession 321 was the only accession in which the stamens did not angle outwards away from the stigma, instead bent in during day two (Figure 11 Appendix), coming in contact with the stigma without aid from the corolla. Emasculated un-pollinated flowers did not produce fruits, therefore agamospermy was not demonstrated. Flowers that were emasculated and pollinated during the pistillate phase with pollen from the same plant did produce fruit, showing self-compatibility and stigma receptivity in the pistillate phase.

A Pearson correlation analysis was run to test for evidence of association between altitude and both temporal and morphological traits. As seen in Table 3, there are significant negative correlations between the altitude at which each accession was collected and the duration of the hermaphroditic phase ($P < 0.000$), the duration of anthesis ($P = 0.001$), the number of flowers per inflorescence ($P < 0.000$), the petal length during the hermaphroditic phase ($P = 0.006$), and the maximum filament length ($P < 0.000$). Trend lines show a decrease in these traits as elevation increases (Figure 17).

Table 3: Correlation analysis of floral traits and the altitude at which each accession was collected. ‘Length of filament in pistillate’ phase was omitted because there was not enough variation to run the analysis.

Floral Trait	Pearson coefficient	P-Value
Duration of pistillate phase	-0.057	0.486
Duration of hermaphroditic phase	-0.303	0.000
Duration of anthesis	-0.263	0.001
Flowers per inflorescence	-0.423	0.000
Petal length, pistillate phase	0.013	0.897
Petal length, hermaphroditic phase	-0.276	0.006
Filament length, pistillate phase	-	-
Filament length, hermaphroditic phase	-0.358	0.000
Maximum filament angle	0.070	0.556
% Petal spots	0.159	0.492

A second correlation analysis was run to look for significant associations among characters. Positive correlations were found between the duration of anthesis and the duration of the pistillate (P=0.000) and hermaphroditic (P=0.000) phases separately, yet, there was no correlation between the duration of the pistillate phase and the hermaphroditic phase (Table 4). Figure 3 shows, with trend lines, whether the correlations were negative or positive. The length of the petals in the pistillate phase was found to be correlated with the duration of the pistillate phase (P=0.000), the duration of the hermaphroditic phase (P=0.029) and the duration of anthesis (P=0.000). The number of flowers per inflorescence is correlated with the duration of the hermaphroditic phase (P=0.005), the maximum length of the petals in the hermaphroditic phase (P=0.005), as well as the

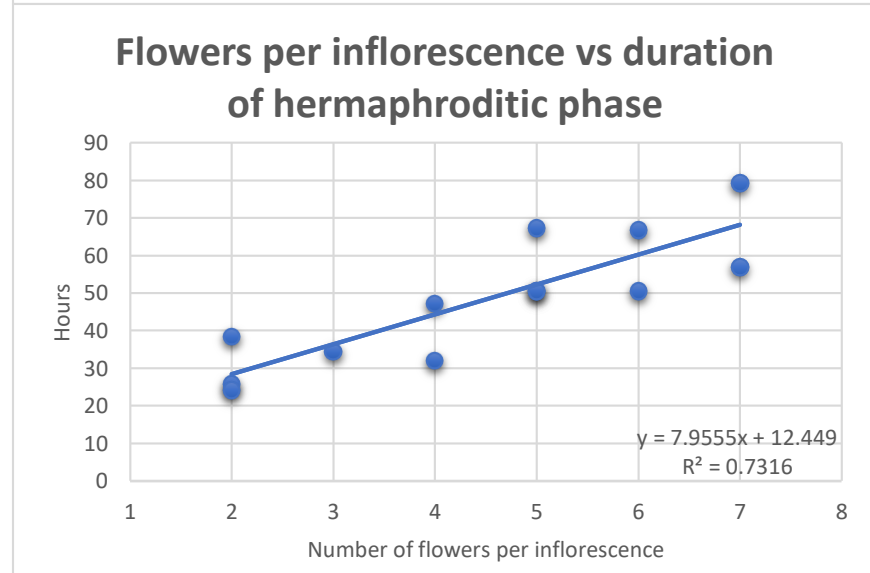
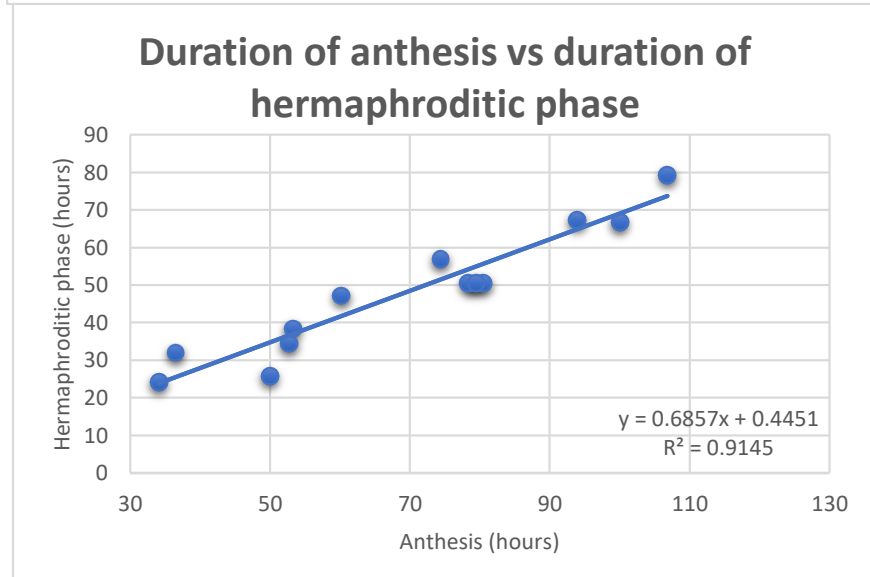
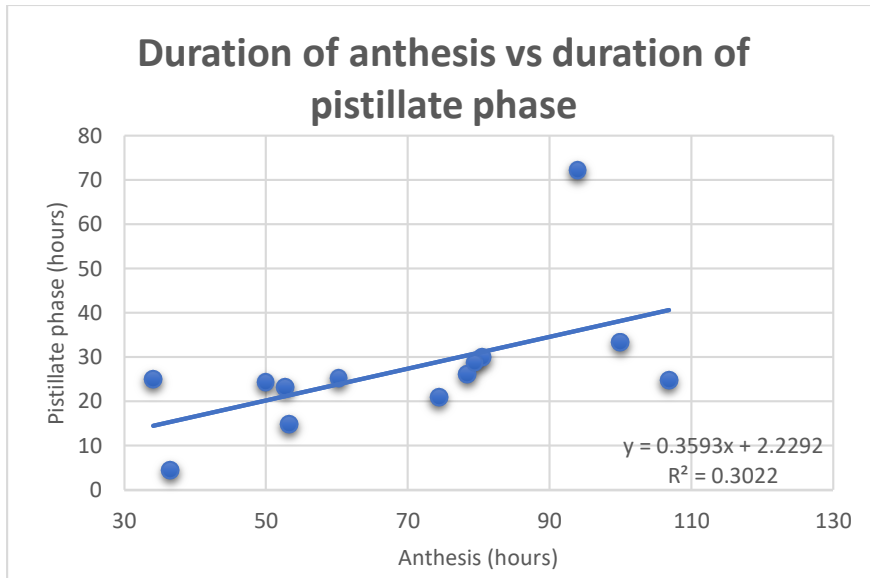
maximum length of filaments in the hermaphroditic phase ($P=0.0003$). A correlation between the petal length in the hermaphroditic phase occurred with the petal length in the pistillate phase ($P=0.000$) as well as between the maximum filament length and the angle in the hermaphroditic phase where $P=0.035$ and 0.020 , respectively. No correlations with petal spots occurred.

Table 4: Pearson correlation analysis of temporal and morphological traits^a.

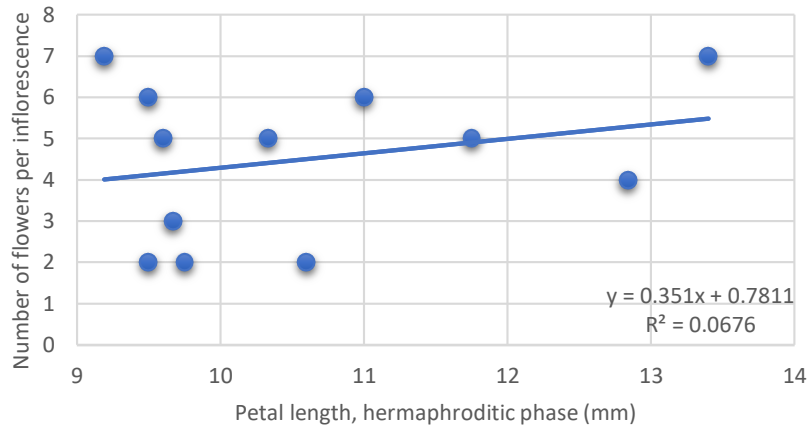
'Length of filament in pistillate' phase was omitted because there was not enough variation to run the analysis.

	<i>Duration of Pistillate Phase</i>	<i>Duration of Hermaphroditic Phase</i>	<i>Duration of Anthesis</i>	<i>Number of Flowers per Inflorescence</i>	<i>Length of Petals in Pistillate Phase</i>	<i>Length of Petals in Hermaphroditic Phase</i>	<i>Length of Filament in the Hermaphroditic Phase</i>	<i>Maximum Filament Angle</i>
<i>Duration of Hermaphroditic Phase</i>	0.053							
<i>Duration of Anthesis</i>	*** 0.643	*** 0.782						
<i>Number of Flowers per Inflorescence</i>		* 0.339	0.216					
<i>Length of Petals in Pistillate Phase</i>	*** -0.488	* -0.264	* -0.551	-0.007				
<i>Length of Petals in Hermaphroditic Phase</i>				** 0.386	*** 0.487			
<i>Length of Filament in the Hermaphroditic Phase</i>				** 0.352		* 0.256		
<i>Maximum Filament Angle in the Hermaphroditic Phase</i>						* 0.281		
<i>Extent of Petal Spots</i>				-0.027	0.218		-0.061	
	0.083	0.296	0.315	0.344	0.122	0.335	-0.008	0.018

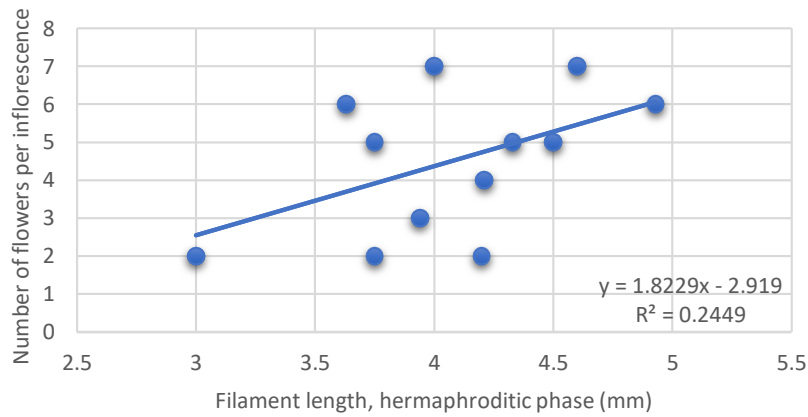
^a P values are summarized as *P= 0.01 to 0.05; **P= 0.001 to 0.01; ***P= <0.001



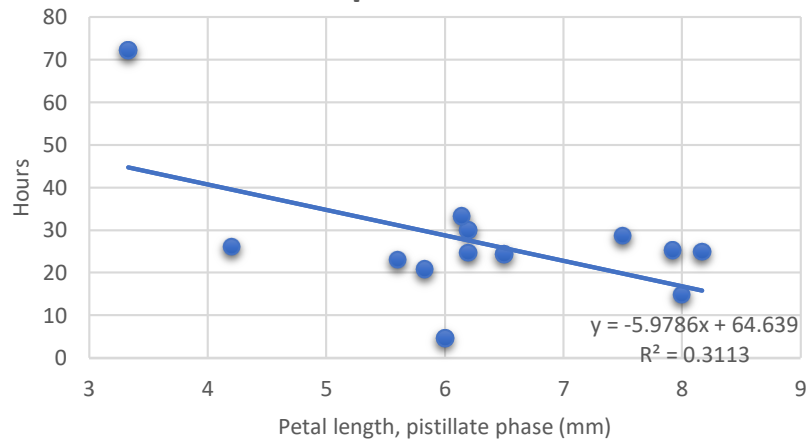
Number of flowers per inflorescence vs petal length



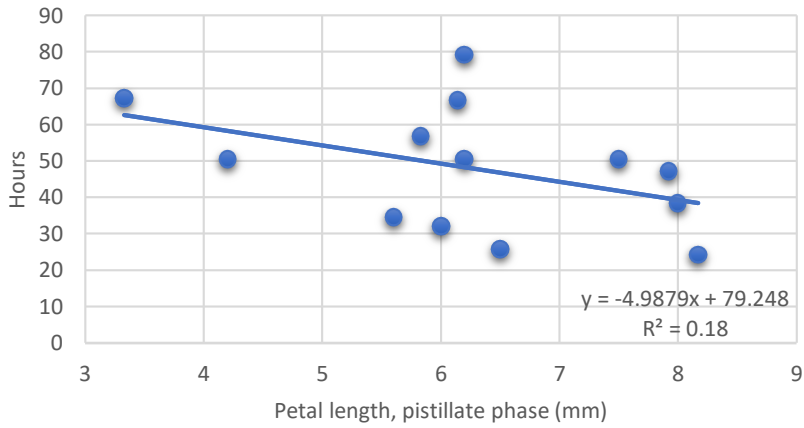
Number of flowers per inflorescence vs filament length



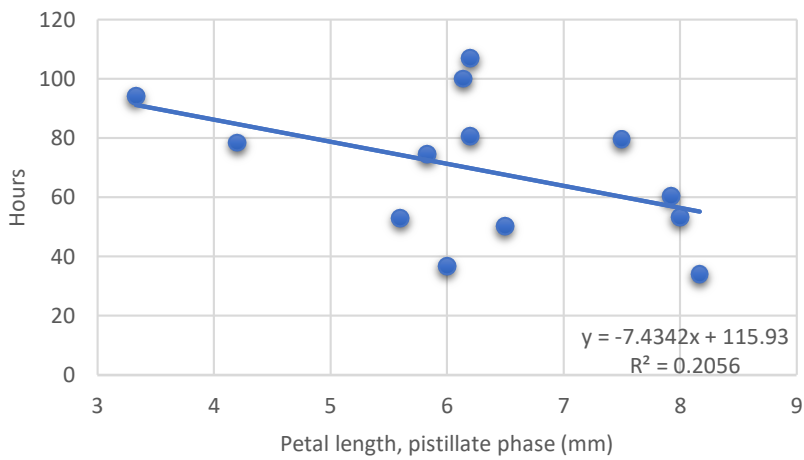
Petal length vs duration of pistillate phase



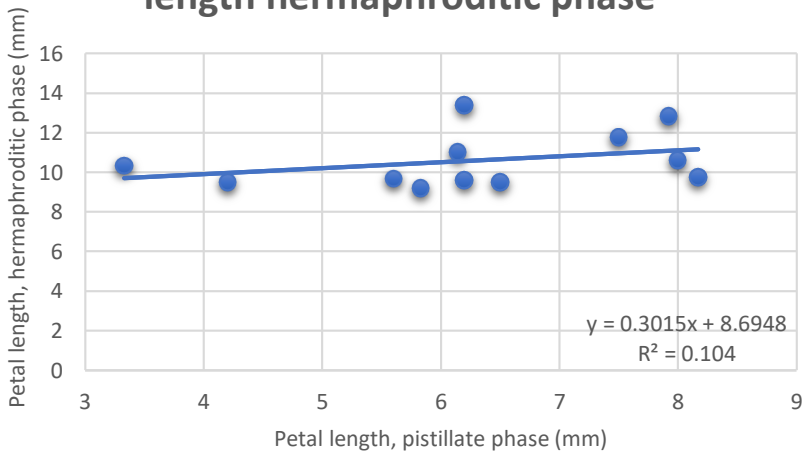
Petal length vs duration of hermaphroditic phase



Petal length vs duration of anthesis



Petal length in pistillate vs petal length hermaphroditic phase



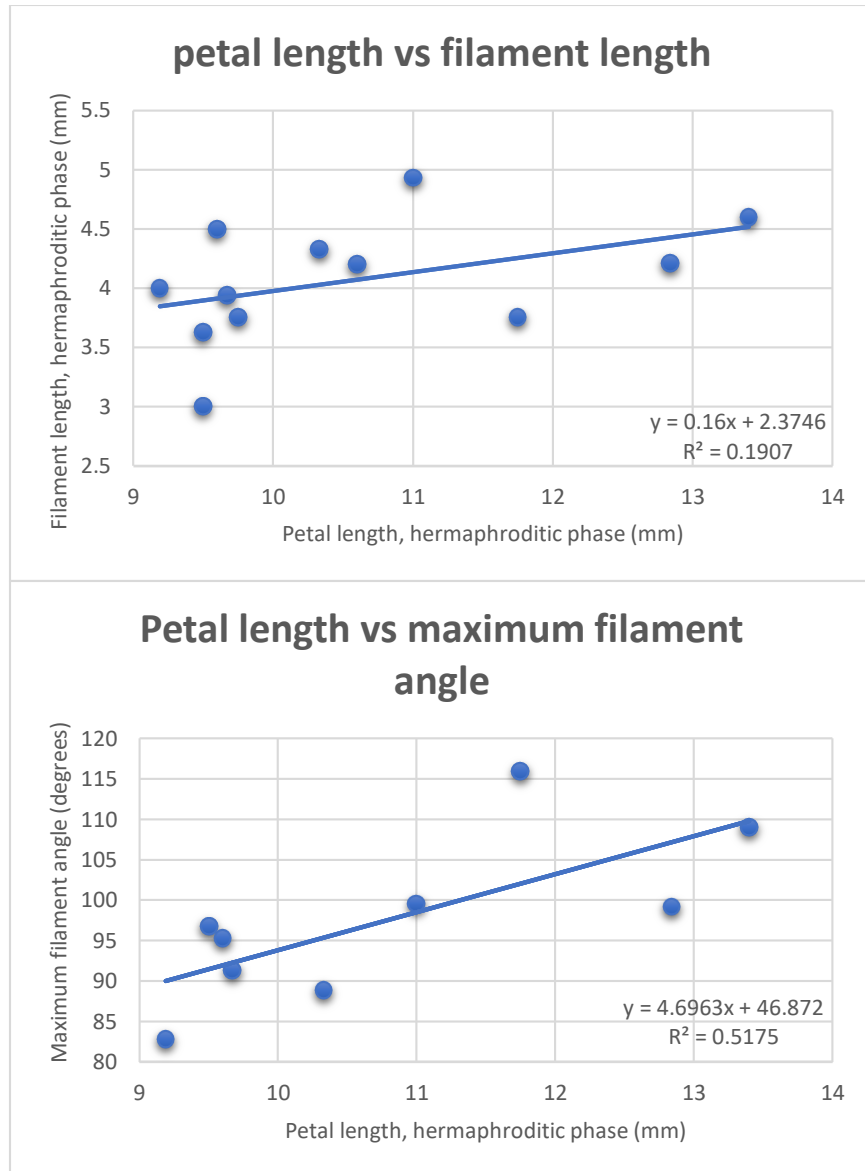


Figure 3: Scatterplots with trend lines for combinations of traits having significant Pearson correlation coefficients (correlation coefficients are shown in Table 4). Each dot represents one accession.

Paired T-Tests were done to determine if there were significant differences between the duration of the hermaphroditic and pistillate phases, as well as the length of the petals and filaments during the pistillate and hermaphroditic phases. There were significant differences (Table 5), where P was equal to or less than $P < 0.05$, for all three variables.

Table 5: Paired T-Tests for selected characters.

Paired T-Test	P-Value	T	df
Duration of pistillate phase – Duration of hermaphroditic phase	<0.0001	12.01	155
Length of petals, pistillate phase – Length of petals, hermaphroditic phase	<0.0001	21.47	101
Length of filament, pistillate phase – Length of filament, hermaphroditic Phase	<0.0001	50.66	120

A one-way ANOVA was run to test if there were significant differences among accessions for temporal and morphological traits including: duration of pistillate and hermaphroditic phases, duration of anthesis, number of flowers per inflorescence, petal length in the pistillate and hermaphroditic phase, filament length in the hermaphroditic phase, filament angle and extent of petal spots (Table 6, Figures 5-13). For filament angle there were not enough data available for accessions 506, 837, 838, and 844. For petal spots there were not enough data available for accessions 506, 593, 837, 838, or 844. Accession 506 did not produce enough data for any of the recorded morphological traits. There was a significant difference found among accessions in regards to the number of flowers per inflorescence ($P = 0.000$), length of the petals in the hermaphroditic ($P = 0.000$) and pistillate phases ($P = 0.002$), maximum filament length during the hermaphroditic phase ($P = 0.000$), maximum filament angle ($P = 0.000$), as well as the extent of petal spots ($P = 0.001$). There was a significant difference found for the duration of the hermaphroditic phase ($P = 0.000$) and duration of anthesis ($P = 0.001$), yet there was not a

significant difference among accessions for the duration of the pistillate phase ($P=0.156$). Figure 4 indicates the number of days each accession took to go through the pistillate phase, hermaphroditic phase, and the cumulative number of days to begin and complete anthesis.

Table 6: One-way ANOVA P-values for morphological and temporal traits tests for significant differences among accessions of *Jaltomata procumbens* studied.

Trait	P-Value	Significant
Number of flowers per inflorescence	0.000	Yes
Petal length in pistillate phase	0.000	Yes
Petal length in hermaphroditic phase	0.002	Yes
Filament length in hermaphroditic phase	0.000	Yes
Maximum filament angle	0.000	Yes
Extent of petal spots	0.001	Yes
Duration of pistillate phase	0.156	No
Duration of hermaphroditic phase	0.000	Yes
Duration of anthesis	0.001	Yes
Pollen Quantity	0.180	No
Ovule Quantity	0.003	Yes
Pollen to Ovule Ratio	0.104	No

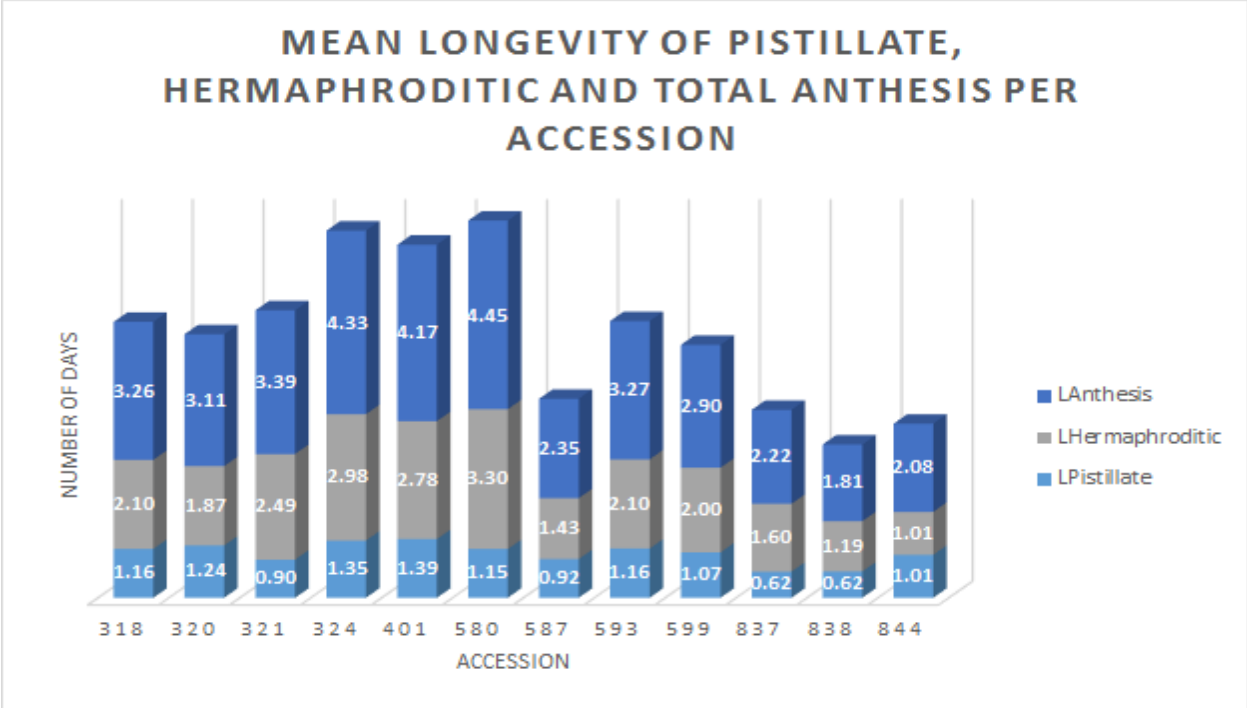


Figure 4: Average duration, in days, of each phase, pistillate and hermaphroditic, as well as the average duration of anthesis for each accession.

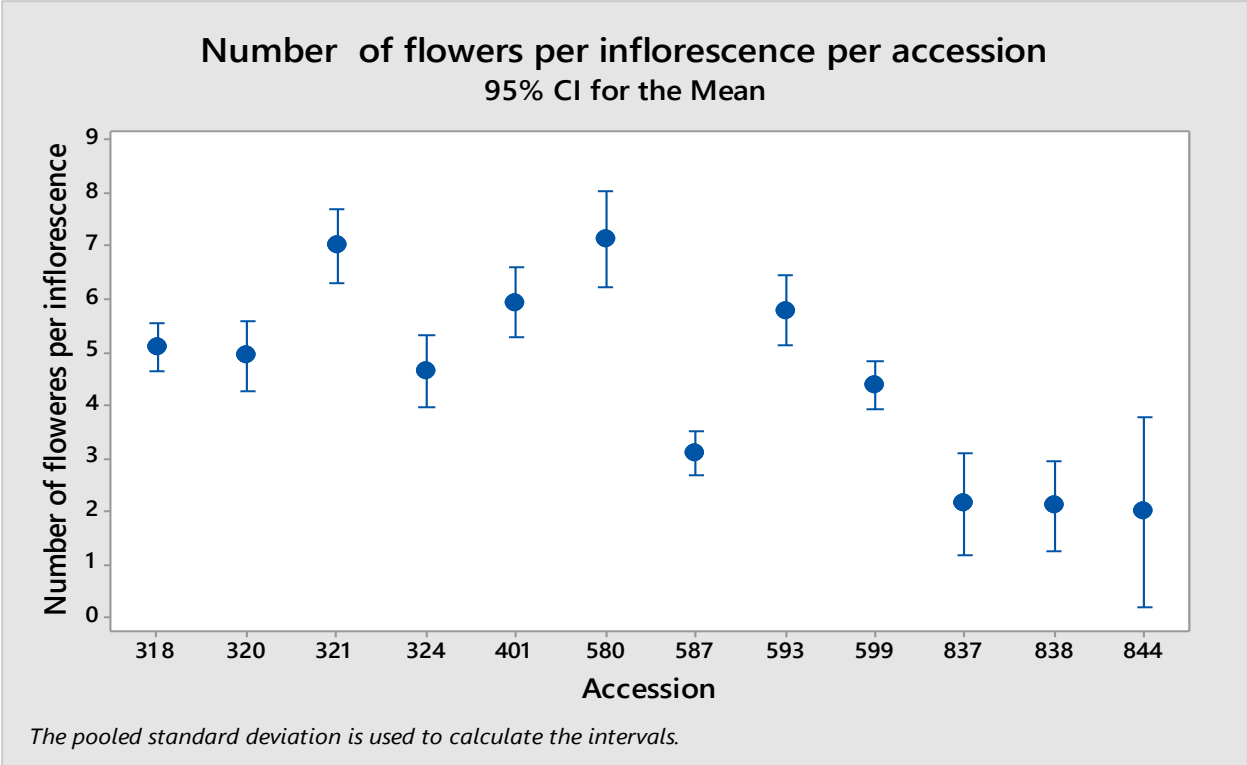


Figure 5: Number of flowers per inflorescence for each accession, with confidence intervals.

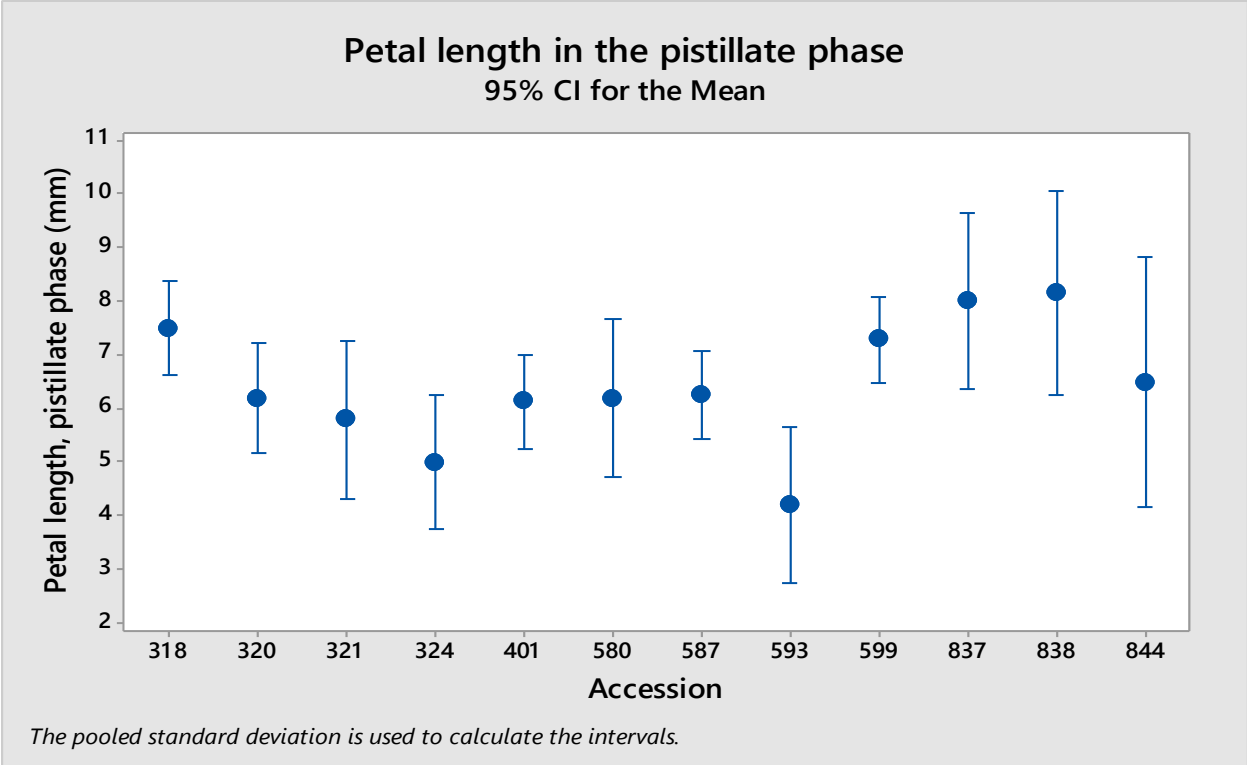


Figure 6: The petal length during the pistillate phase, of each accession, with confidence intervals.

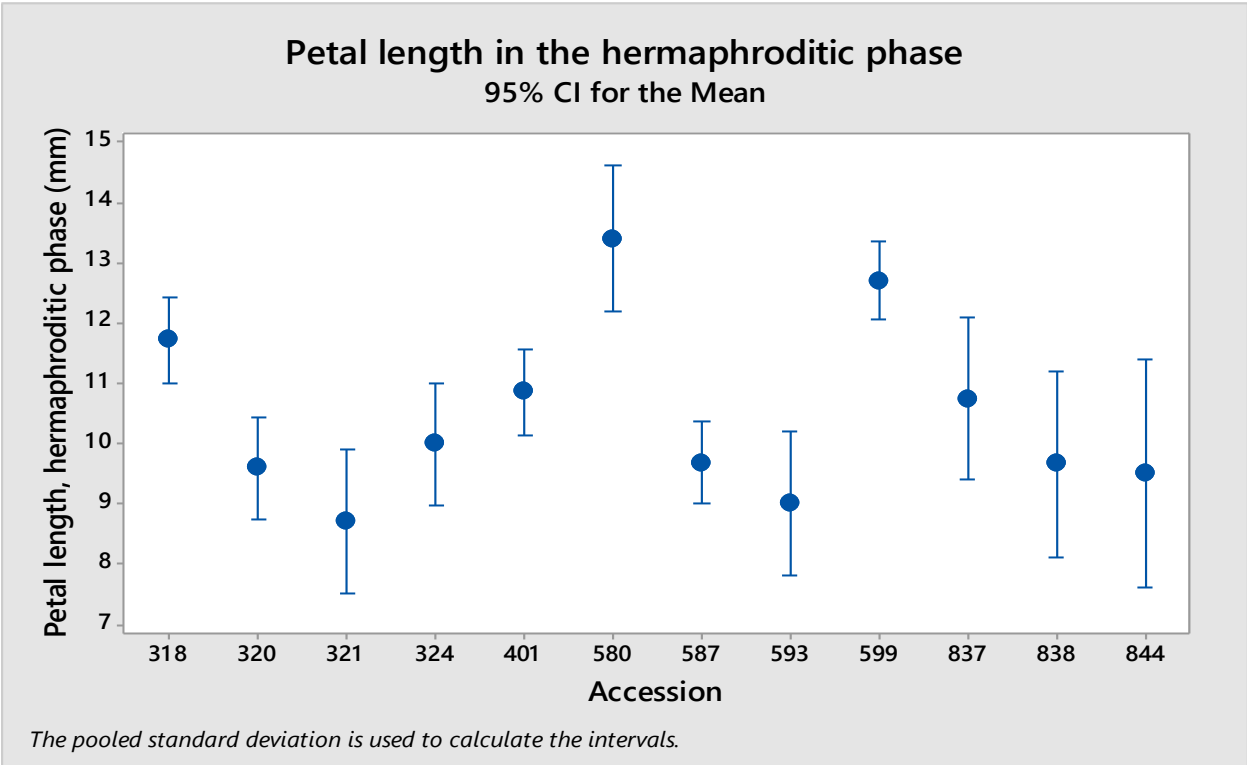


Figure 7: The maximum petal length during the hermaphroditic phase, for each accession, with confidence intervals.

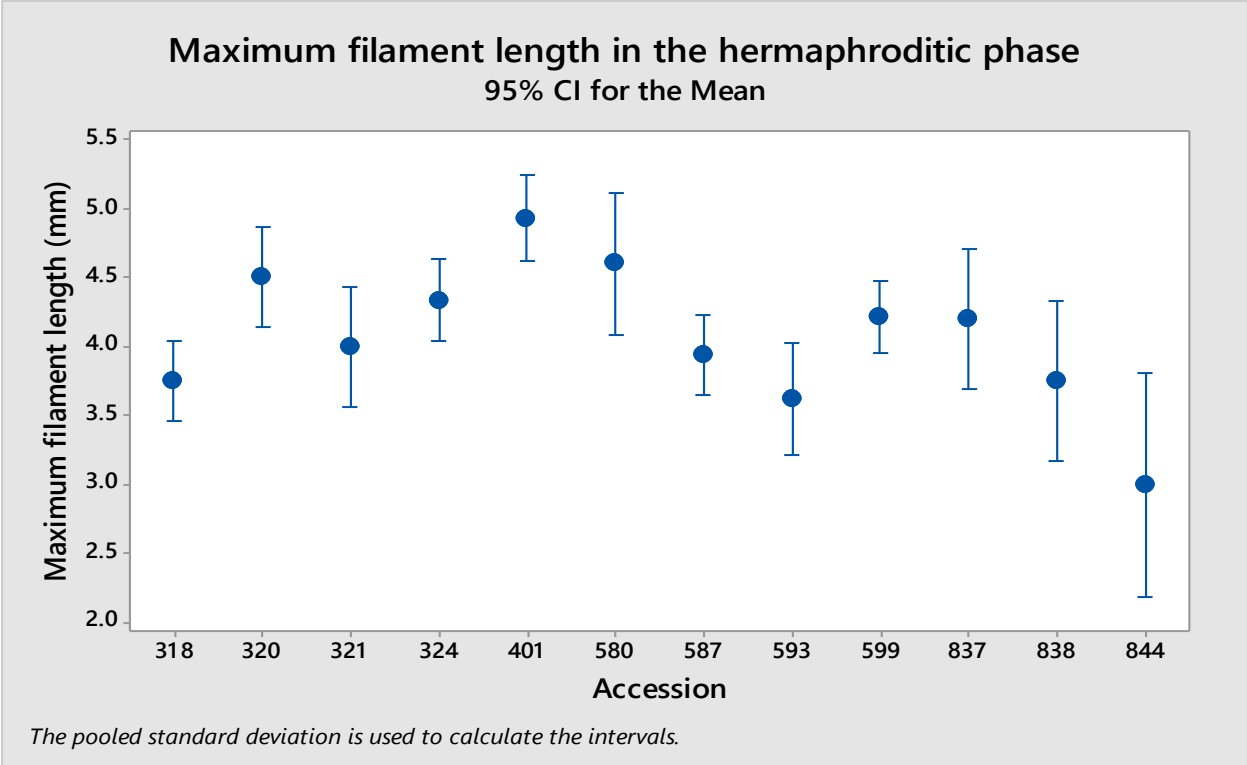


Figure 8: The maximum filament length during the hermaphroditic phase, of each accession, with confidence intervals.

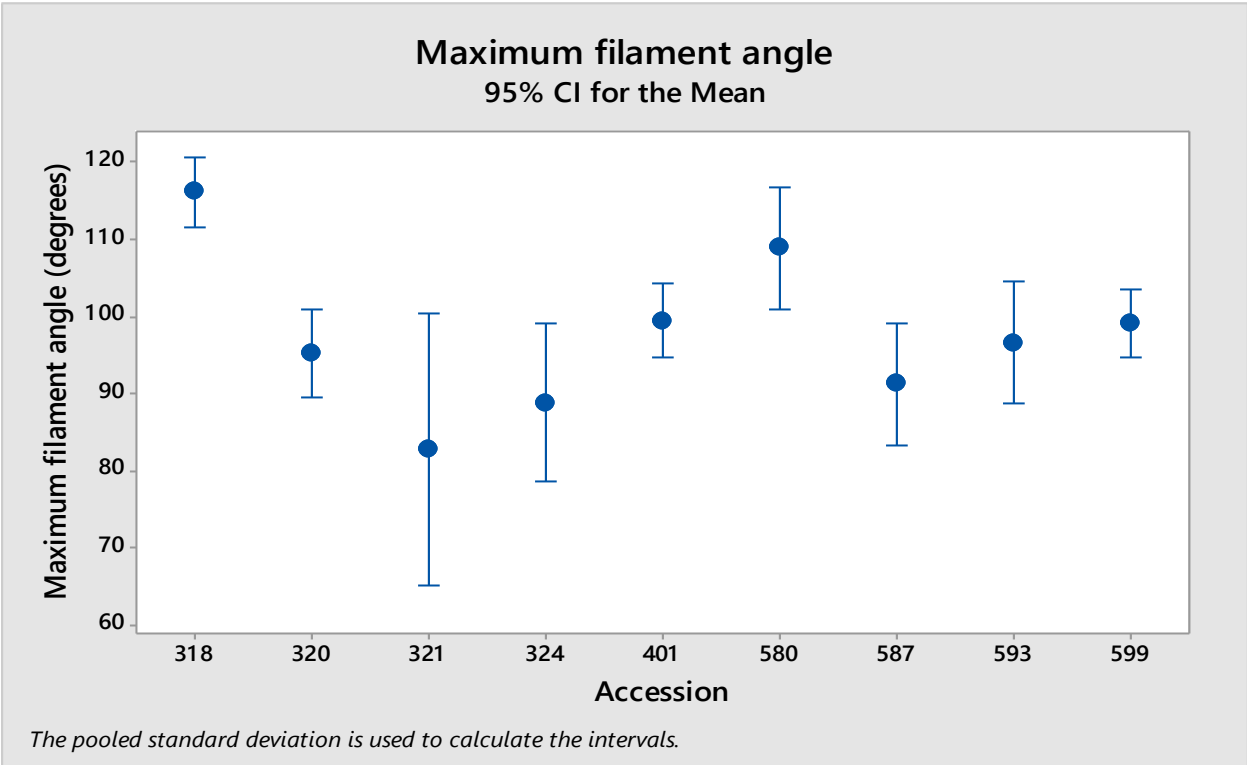


Figure 9: The maximum outward filament angle, relative to the style, during the hermaphroditic phase, for each accession, with confidence intervals.

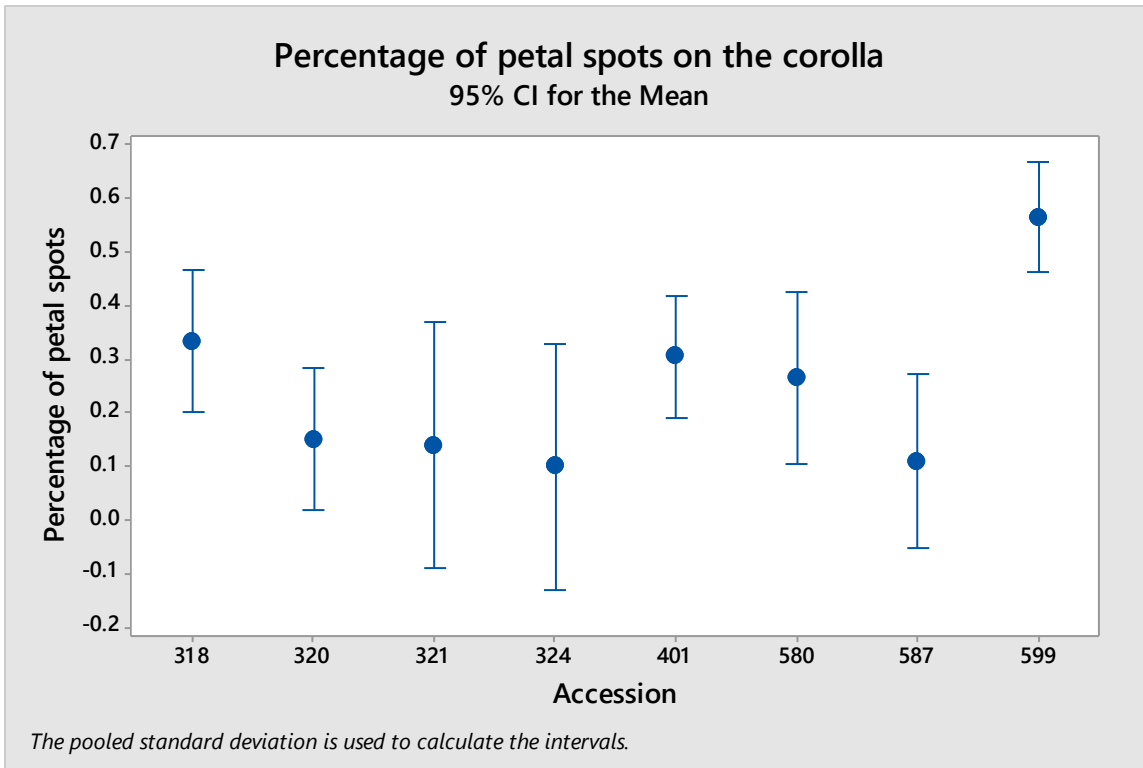


Figure 10: The extent of petal spots on the corolla, of each accession, with confidence intervals.

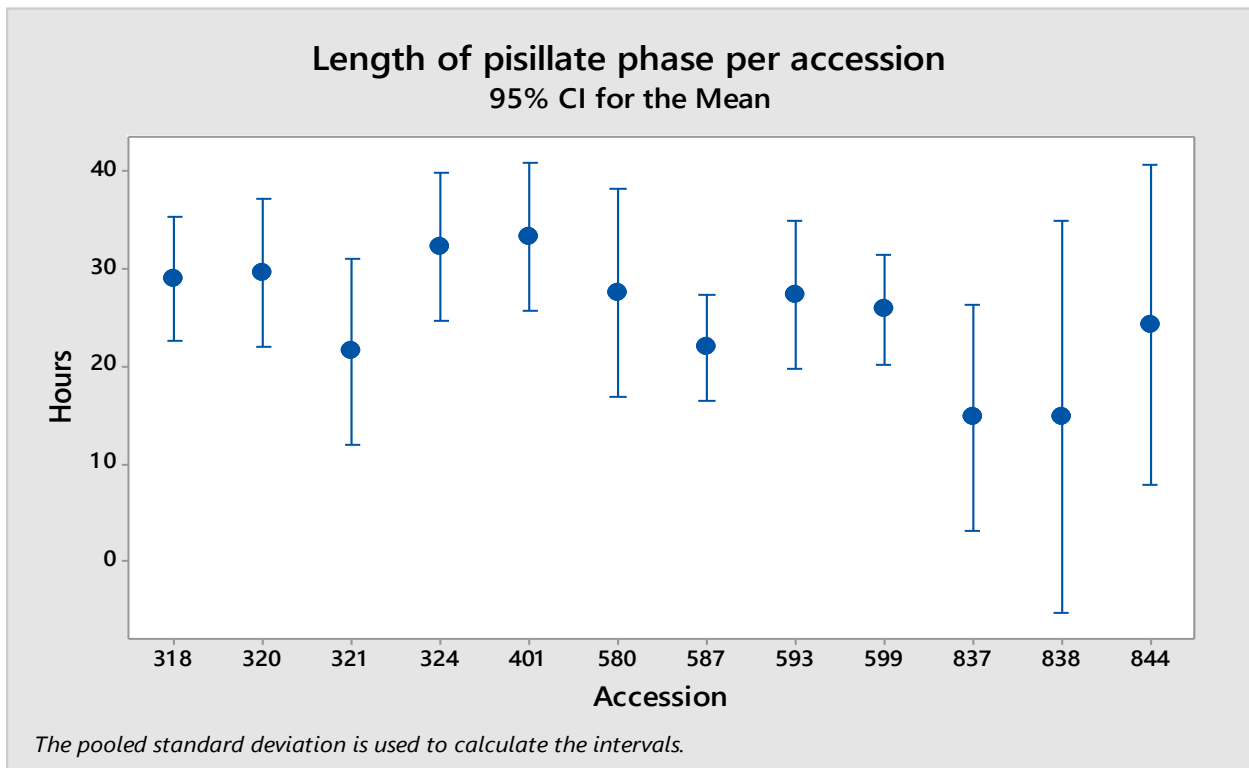


Figure 11: The duration of the pistillate phase of each accession.

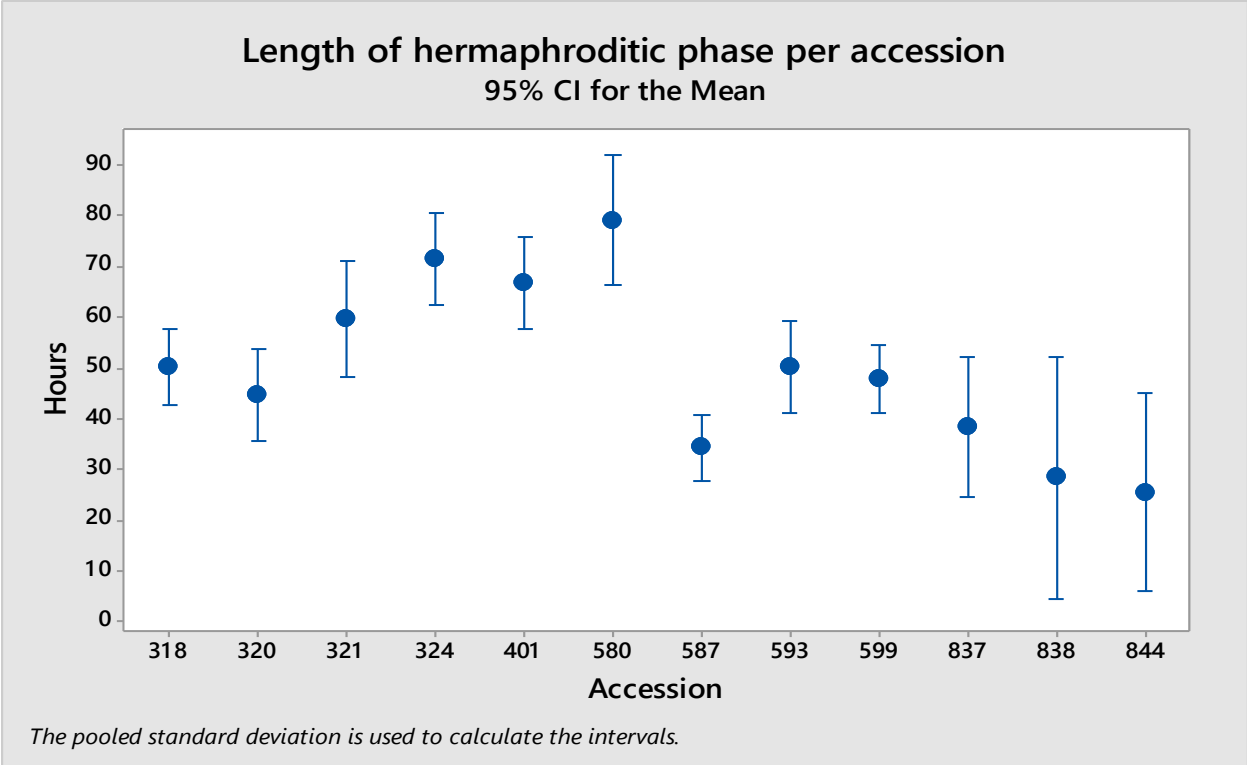


Figure 12: The duration of the hermaphroditic phase for each accession.

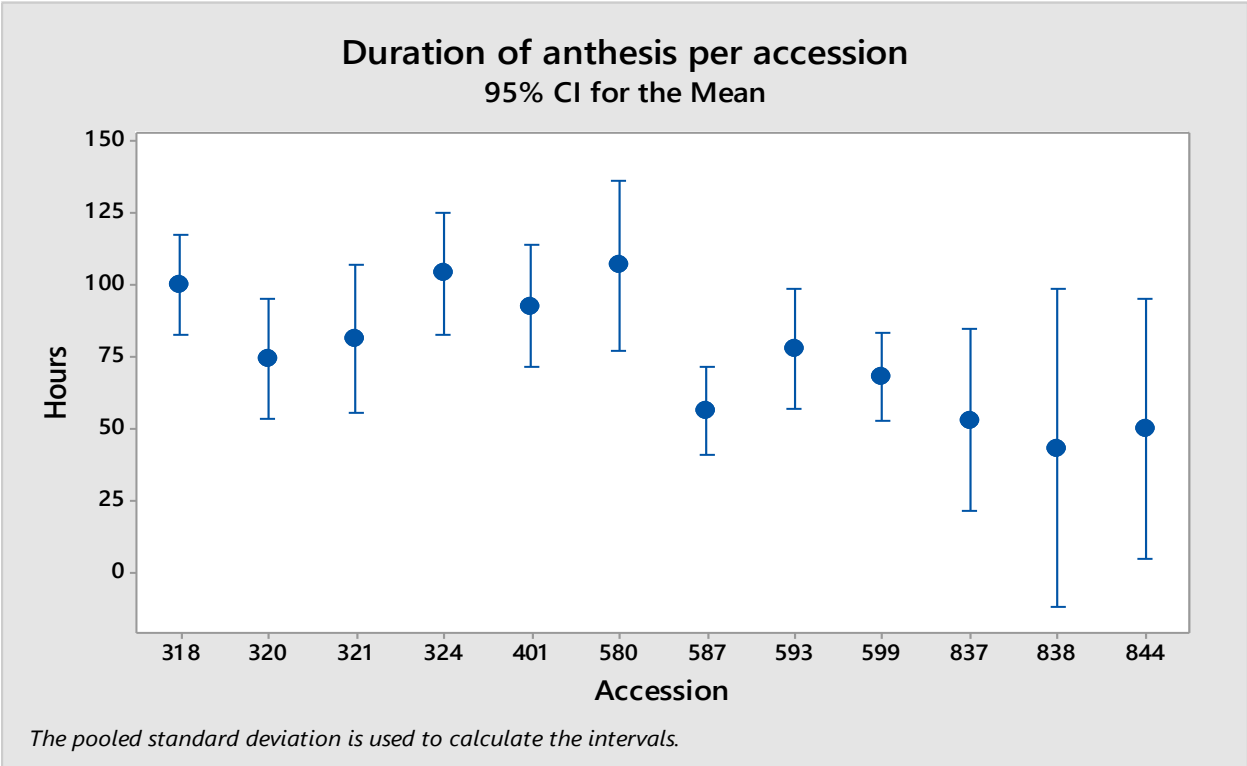


Figure 13: Duration of total anthesis for each accession.

The pollen and ovule counts (Table 7) of the studied accessions was completed by Kenneth Plourd and Dr. Thomas Mione at Central Connecticut State University. Using a one-way ANOVA, there was not a significant difference found (Figures #14-16) among accessions for pollen quantity ($p = 0.180$) and the pollen to ovule ratio ($p = 0.104$), but there was a significant difference found for ovule quantity ($p = 0.003$). Accession 401 had the largest pollen count with accession 599 having the lowest pollen count. Accession 401 also had the highest ovule count though accession 506 had the lowest ovule count.

Table 7: Pollen and ovule counts of *Jaltomata procumbens* by microscope.

Accession	Pollen Count	Ovule Count	Pollen to ovule Ratio
318	86,042	146	591
320	138,281	140	1,220
321	143,590	83	1,839
324	80,250	101	560
401	161,250	204	833
506	102,500	74	1,347
580	120,521	96	1,351
587	94,922	128	842
593	71,167	120	576
599	60,844	137	721
837	105,000	136	772
838	118,750	124	961
844	87,500	93	941

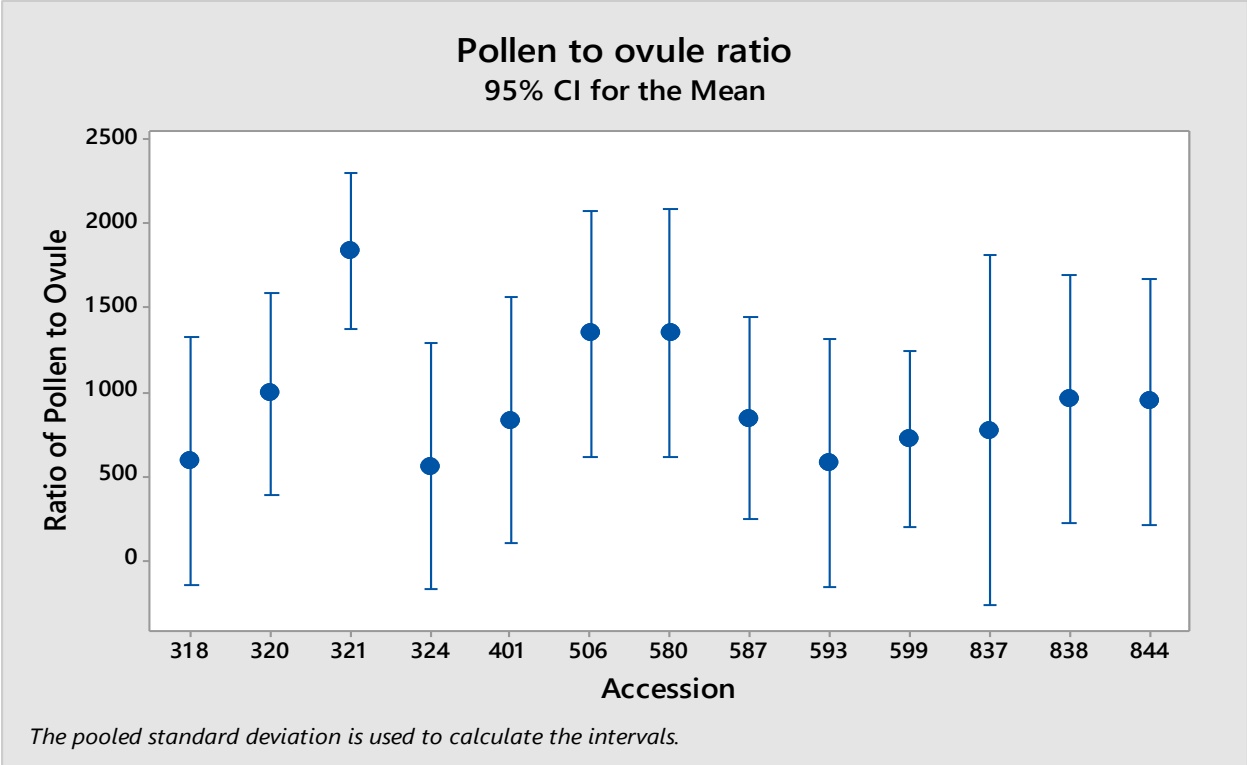


Figure 14: The pollen to ovule ratio for each accession.

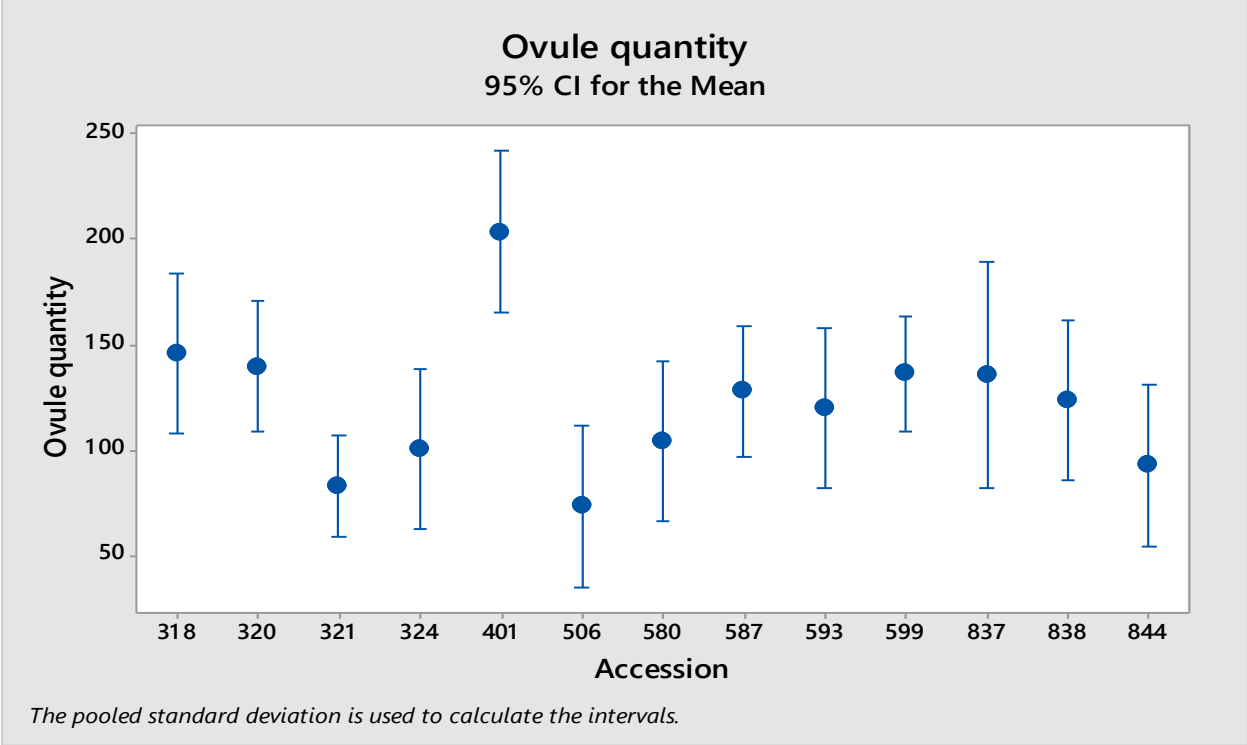


Figure 15: Ovule quantity for each accession.

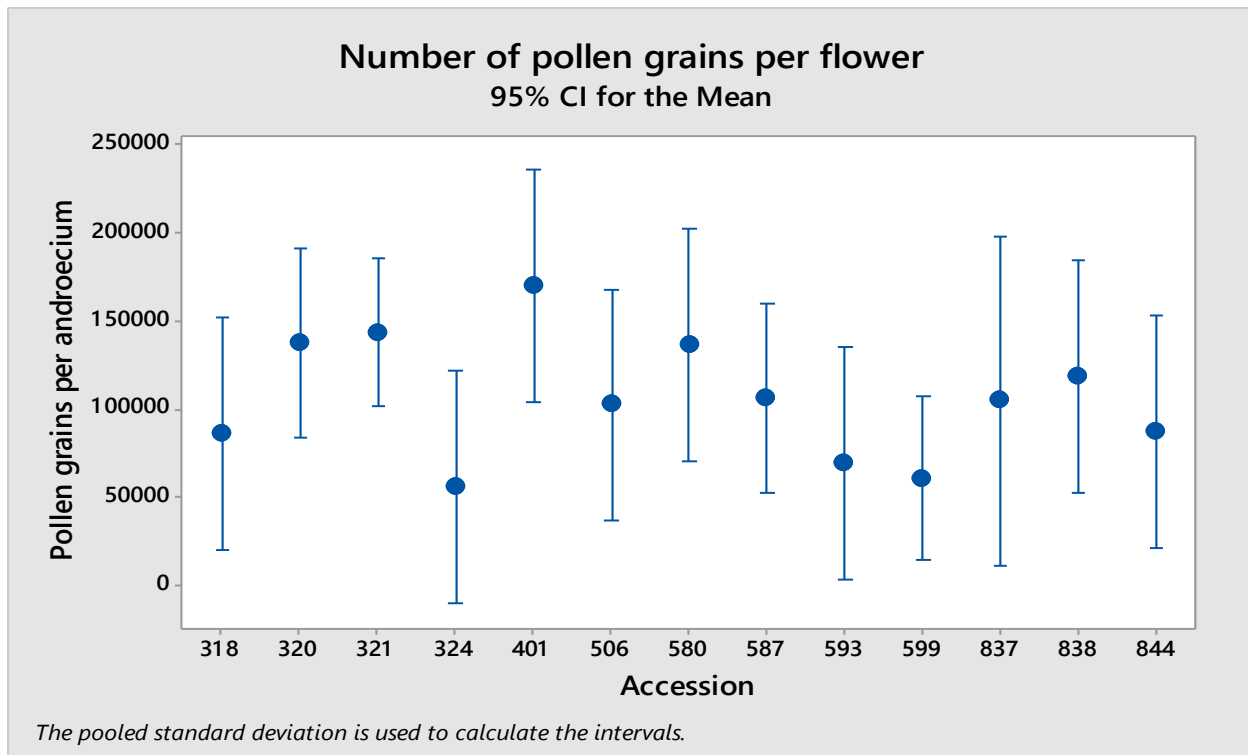
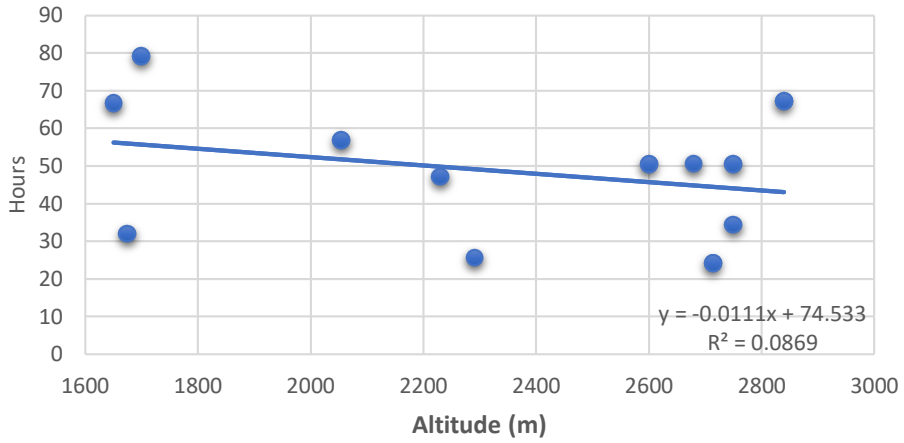


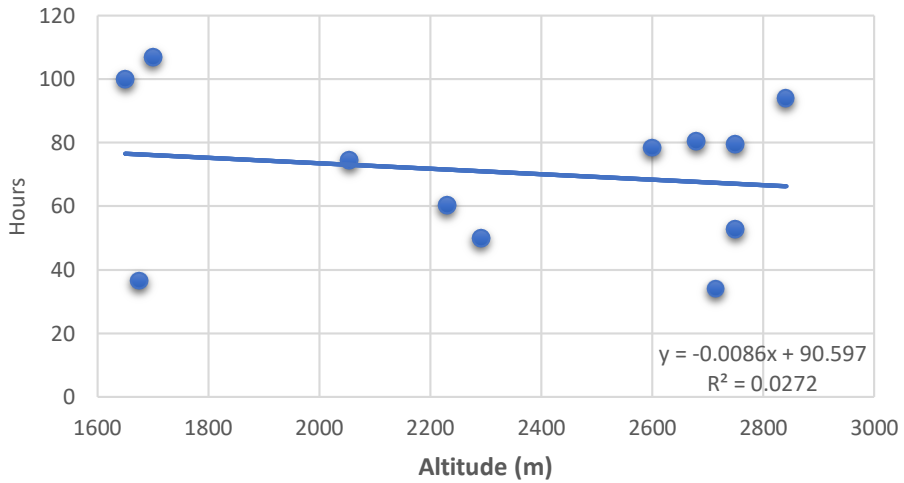
Figure 16: Pollen quantity for each accession.

A declining trend line was fit to traits found to be negatively correlated with altitude (Figure 17, Table 3). This shows that as elevation increases, the traits such as the duration of the hermaphroditic phase, the duration of anthesis, the number of flowers per inflorescence, the maximum petal and filament length during the hermaphroditic phase, all decrease. The maximum petal length and filament length are more tightly correlated with the trend line than the number of flowers per inflorescence or the duration of the hermaphroditic phase and anthesis.

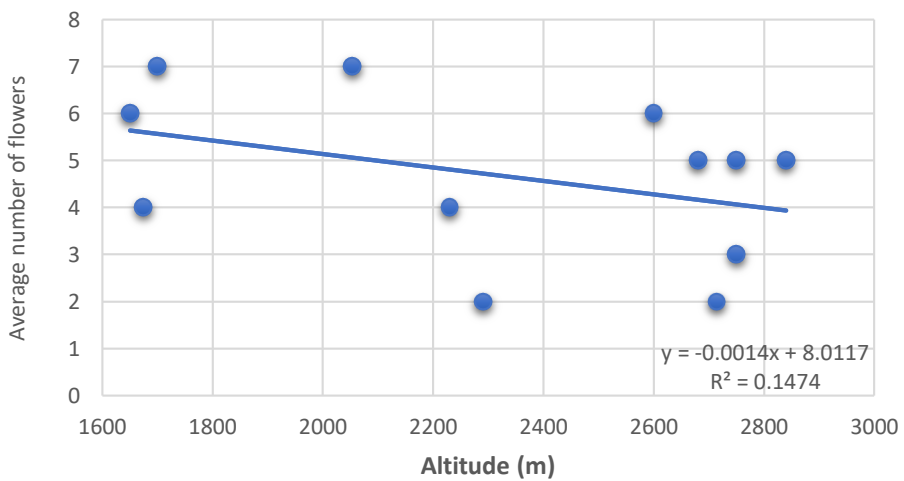
Duration of Hermaphroditic Phase



Duration of Anthesis



Number of Flowers per Inflorescence



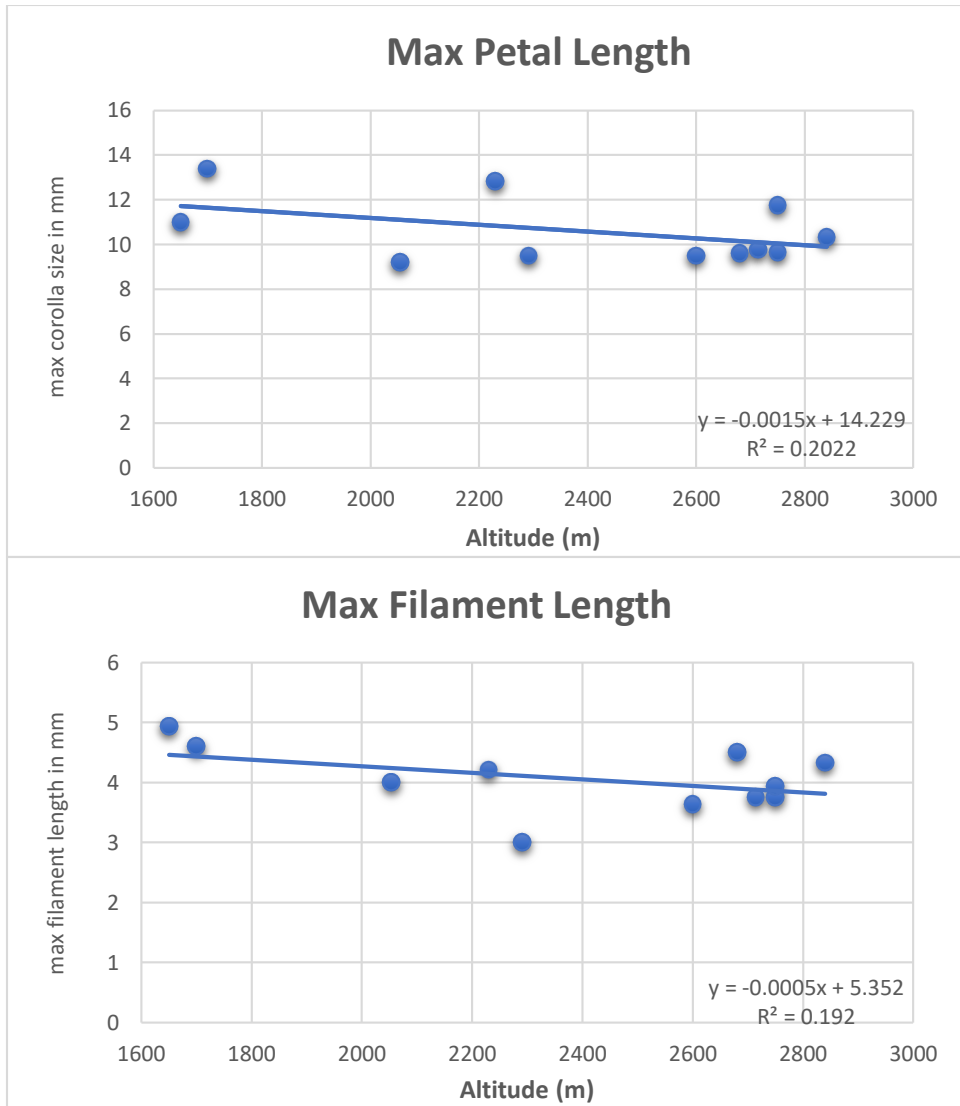


Figure 17: Scatterplots with trend lines for traits having significant negative correlations with altitude at which each accession was collected (correlation coefficients and P values are shown in Table 3).

Discussion

Variation in Morphological Traits Among Accessions

Even though *Jaltomata procumbens* is a single species, the accessions I studied vary significantly in several morphological traits including: number of flowers per inflorescence, petal length in both the pistillate and hermaphroditic phases, maximum length and maximum angle of the filaments, extent of petal spots, pollen to ovule ratio, and duration of the hermaphroditic phase and therefore the duration of anthesis. Accession 844 had the fewest flowers per inflorescence while accessions 321 and 580 had the most (Figure 5). The petal length in the hermaphroditic phase varied from 7 mm (accession 321) to 15 mm (accessions 580 and 599). The petal length in the pistillate phase reached a maximum of 10 mm (accessions 599 and 837) with an average petal length of 6.5 mm. The maximum filament length in the hermaphroditic phase varied from 3 mm to 5 mm. The maximum filament angle ranged from 70.5° (accession 587) to 128.7° (accession 318). The extent of petal spots (proximal towards distal) on the corolla varied greatly, from 10% for accession 320 to 70% on accession 599. On average, accession 580 had the longest hermaphroditic phase at 79.2 hours and anthesis at 106.9 hours whereas accession 838 had the shortest hermaphroditic phase and total anthesis at 24.9 hours and 34.1 hours, respectively. The duration of the pistillate phase did not significantly differ among accessions and averaged 26.6 hours. All these variations occurred among accessions and were tested using a One-way ANOVA.

Variation in Morphological and Temporal Traits Within Accessions

Significant differences were found in temporal attributes as well as morphological characteristics. A paired T-Test was used and significant differences were found between the length of the petals during the pistillate and hermaphroditic phases, and the length of the staminal filaments during these phases. This provides evidence for the flower enlarging as a whole throughout anthesis, allowing for a larger beacon to pollinators when fitness can be gained through both male and female functions. The average petal length went from 6.3 mm in the pistillate phase to 10.6 mm during the hermaphroditic phase, increasing 59% by the end of anthesis. On average, accession 324 (3.33 mm) had the smallest petal length during the pistillate phase with accession 838 (8.2 mm) having the largest. During the hermaphroditic phase, accession 321 (9.2 mm) had the smallest maximum petal length while accession 580 (13.4 mm) had the largest. The average filament lengthened from 1 mm to 4.07 mm, increasing approximately 4-fold. There is also a significant difference between the duration of the pistillate phase and the duration of the hermaphroditic phase. This is due to the pistillate phase lasting only one day on average (Figure 4) whereas the hermaphroditic phase lasts an average of two days.

All but one of the accessions showed delayed self-pollination. With the exception of accession 321, self-pollination without the aid of the corolla was not seen during this study. Not surprisingly, not one of the studied accessions displayed agamospermy, the production of fruits (containing asexual seeds) without pollination. When anthesis begins the stamens are very short, 1 mm in length, during the pistillate phase which usually only lasts one day. The flower is open and the stigma is receptive even though the anthers remain undehisced during the pistillate phase. On day two the stamens elongate rapidly in the morning and the anthers dehisce before the end of the day. Stamens angle outward on day two, positioning the dehisced anthers away from the stigma

(herkogamy), allowing for outcrossing though selfing by a pollinator could also take place at this time. In the early evening the corolla closes and the stamens change angle, orienting connivently. The reorientation of the stamens may be due to the corolla closing, pushing on the stamens, or the corolla and the stamens may angle towards the style/stigma together. Hence when we see complete closure of the corolla after the anthers have dehisced, pollen has come into contact with the stigma accomplishing delayed self-pollination. The third and final day of anthesis, the corolla fully opens again though the stamens remain connivent. This description of floral phenology is a generalization and summarizes the majority of observations, though it does not include accession 321.

Correlation Analysis of Traits to Altitude Where Collected

Several characteristics showed a negative correlation with the altitude of the accessions' collection sites: the duration of the hermaphroditic phase, the total duration of anthesis, the number of flowers per inflorescence, as well as the petal length and filament length during the hermaphroditic phase. As seen (Figure 17), the duration of the hermaphroditic phase and anthesis shortened as altitude increases. The same clinal trend is found in the other traits, the maximum length of the petals and filaments are shorter the higher the altitude, and the number of flowers per inflorescence decreases with an increase in elevation. Bresson et al. (2011) found similar results in which leaf size was negatively correlated with elevation. Regardless of the proximity of populations, a difference in elevation brought about genetic differences in the phenology and growth of the species, suggesting the evolution of ecotypes. Several traits including the extent of petal spots on the corolla were not correlated with altitude; this indicates that other biotic factors could be at play such as the quantity and quality of pollinators. Overall, in *Jaltomata procumbens*,

ecotypes that are adapted to higher elevations appear to invest less energy on sexual reproduction than ecotypes adapted to lower elevations.

Correlation Analysis of Morphological and Temporal Traits

The duration of the pistillate and hermaphroditic phases is positively correlated with the duration of anthesis (Figure 3). The duration of the pistillate and hermaphroditic phases as well as anthesis are negatively correlated with the petal length in the pistillate phase, yet there is no correlation to the petal length in the hermaphroditic phase. There is a positive correlation between the number of flowers per inflorescence and the duration of the hermaphroditic phase as well as the petal length and filament's length in the hermaphroditic phase. The length of the petals in the pistillate phase is positively correlated with the maximum length of the petals, which in turn is positively correlated with the maximum length and angle of the filaments.

Pollen to Ovule Ratio

Multiple studies have shown that outcrossing species produce more pollen grains than autogamous species (Mione and Anderson 1992). Autogamous species also tend to have a lower pollen to ovule ratio due to lower quantities of pollen with higher ovule production (Mione and Anderson 1992). Accession 401 had the largest pollen and ovule count whereas the lowest pollen and ovule counts were found in accessions 599 and 506 respectively. Cruden (1977) found a positive correlation in pollen to ovule ratio with the degree of outcrossing that occurred, and he maintained that the pollen to ovule ratio is a predictor of plant breeding systems. Therefore, as accession 321 had the highest pollen to ovule ratio, it can be said that outcrossing may be the main

component of its breeding. However, this does conflict with stamens dehiscing connivently into the stigma, the latter suggesting an increased penchant for selfing.

Plant Accessions

318

Native to Guatemala at an elevation of 2,750 m, this accession is procumbent in growth habit with pubescent leaves. Petal spots cover 33% of the corolla, which reaches an average maximum size of 11.75 mm. The mean number of flowers per inflorescence is five, though it can produce up to eight. The filaments elongate to 3.75 mm and angle out at 115.6 degrees. On average accession 318 has a pollen and ovule count of 86,402 and 146 respectively, with a pollen to ovule ratio of 146.

320

Originating in Guatemala at an elevation of 2,680 m, accession 320 is procumbent. The leaves are pubescent. This accession produces an average of 5 flowers per inflorescence with a maximum of eight flowers per inflorescence. Petal spots cover 15% of the 9.6 mm long petal. The filaments lengthen to 4.5 mm and angle outwards at 95.3 degrees. A pollen and ovule count showed an average of 138,281 pollen grains and 140 ovules, with a pollen to ovule ratio of 1,220.

321

Originating from Guatemala at an altitude of 2,054 m, this accession is erect in habit with pubescent leaves. An average of seven flowers per inflorescence was seen, ranging up to ten flowers. On average the petal length reaches a maximum size of 9.2 mm with petal spots covering

14% of the corolla. The filaments elongate to 4 mm and angle at 82.8 degrees. This accession has the largest pollen to ovule ratio at 1,839, with pollen and ovule counts of 143,590 and 83 respectively.

324

Accession 324 is native to Guatemala at an altitude of 2,840 m. It has a procumbent habit with pubescent leaves. An average of five flowers per inflorescence was seen, though, this accession can produce up to eighteen (Mione, pers. com. 2015). The petal length averages 10.3 mm in the hermaphroditic phase with petal spots covering 10% of the corolla. The filaments reach a length of 4.3 mm and angle outwards at a maximum of 99.6 degrees. A pollen and ovule counts showed 80,250 pollen grains and 101 ovules, with a pollen to ovule ratio of 560.

401

Native to Chiapas, Mexico at an elevation of 1,650 m, accession 401 is erect in habit with pubescent leaves. The average number of flowers per inflorescence is six, though a maximum of 13 has been seen (Mione, pers. com. 2015). The petal length in the hermaphroditic phase was found to average 11 mm with petal spots covering 31%. The filaments had a maximum length of 4.9 mm and angled outward at 99.6 degrees. This accession had the largest pollen and ovule counts at 161,250 and 204, with a pollen to ovule ratio of 833.

506

Originating from Chihuahua, Mexico, this accession was collected at an altitude of 1,675 m. It has a procumbent habit with nearly glabrous leaves. An average of four flowers per inflorescence was seen. The petal length is 6 mm during the pistillate phase. A pollen and ovule count showed 102,500 pollen grains and 74 ovules. This accession had the smallest pollen to ovule ratio at 1,347.

580

Accession 580 is native to Honduras at an elevation of 1,700 m. It is erect in habit with pubescent leaves. An average of seven flowers per inflorescence was seen with a maximum of ten. The petal length reached a maximum size of 15 mm with petal spots covering 27% on average. The filaments lengthened to 4.6 mm and angled outwards at 109.0 degrees. Pollen grains and ovules averaged at 120,521 and 96 respectively with a pollen to ovule ratio of 1,351.

587

Native to District Federal, Mexico, accession 587 was found at an elevation of 2,750 m. The leaves are glabrous, and the plant grows in a procumbent habit. It has an average of three flowers per inflorescence with a maximum of six (Mione, pers. com. 2015). It has an average maximum petal length of 9.7 mm with petal spots covering 11% of the corolla. The filaments elongate to 3.9 mm and angle outwards at 91.3 degrees. A pollen and ovule counts showed 94,922 and 128 respectively, with a pollen to ovule ratio of 842.

593

Accession 593 originates from Mexico at an elevation of 2,600 m. It has an erect growth habit and glabrous leaves. An average of six flowers per inflorescence was seen with a maximum of seven flowers. The petal length averages 9.5 mm. The filaments length to 3.6 mm and angle outwards at 96.7 degrees. A pollen and ovule counts showed 71,167 and 120 respectively, with a pollen to ovule ratio of 576.

599

Native of Morelos, Mexico, this accession has an erect growth habit and was found at an elevation of 2,230 m. The leaves are mostly glabrous with only the leaf margin being pubescent. An average of four flowers per inflorescence was seen with a maximum of nine flowers. The petal length was 12.8 mm at its maximum size with petal spots covering an average of 57% (up to 70%) of the corolla. The filaments reached a length of 4.2 mm and angled outwards at 99.2 degrees. This accession had the lowest pollen count at 60,844 with and ovule count of 137. The pollen to ovule ratio was 721.

837

Originating in Guatemala, accession 837 grows procumbent with nearly glabrous leaves. Only two flowers per inflorescence were seen on average with a maximum of three flowers. On average the petal length reached 10.6 mm in size. The filaments elongate to 4.2 mm and angle outwards. This accession has a pollen count of 105,000, an ovule count of 136 and a pollen to ovule ratio of 883.

838

Native to Guatemala at an altitude of 2,714 m, accession 838 grows in a procumbent habit. The leaves are mostly glabrous with only the leaf margin being pubescent. An average of two flowers per inflorescence was seen with a maximum of three flowers. The petal length reached 9.75 mm in size on average. The filaments lengthen to 3.75 mm and angle outwards. A pollen and ovule counts showed 118,750 and 124 respectively, with a pollen to ovule count of 961.

844

Originating from Guatemala at an altitude of 2,291 m, this accession is erect in growth habit. It has sparsely pubescent, with and an average of two flowers per inflorescence. The petals had a maximum length of 9.5 mm. The filaments reach 3 mm in length and angle outwards. Accession 844 has a pollen and ovule counts of 87,500 and 93 respectively with a pollen to ovule ratio of 941.

Conclusion

Multiple morphological and temporal traits were shown to vary significantly among and within accessions. Paired T-Tests showed significant differences for the durations of the pistillate and hermaphroditic phases, as well as the size of the filaments and corolla in the pistillate versus the hermaphroditic phases. A one-way ANOVA showed significant differences among accessions in regards to the number of flowers per inflorescence, the length of the petals in the pistillate and hermaphroditic phases, the length and angle of the filament in the hermaphroditic phase, the extent of petal spots covering the corolla, the pollen to ovule ratio, and the durations of both the hermaphroditic phase and total anthesis. Two correlations analyses were run; one revealed a negative correlation between altitude and the duration of the hermaphroditic phase, duration of anthesis, the number of flowers per inflorescence, the length of both the petals and filaments in the hermaphroditic phase. The second, found correlations among several traits indicating, for example, that petal length in the hermaphroditic phase is positively correlated with the number of flowers per inflorescence.

The data gathered shows a presence of herkogamy and dichogamy, and delayed selfing in the majority of accessions of *Jaltomata procumbens*. This was supported by the stigma being receptive prior to anther dehiscence (protogyny and dichogamy) as well as the angling out of stamens during the first day of the hermaphroditic phase (herkogamy). The first two days of anthesis allowed for outcrossing. At the end of day two or the beginning of day three, the stamens bend in towards the stigma with the aid of the corolla. The corolla does become fully open (reflexed) once more before the end of anthesis with the stamens remaining connivent (Figure 10A). The flowers that were not pollinated and emasculated did not set fruit allowing me to conclude that there was no agamospermy. The flowers that were emasculated and pollinated

during the pistillate phase by pollen from the same plant, did produce fruit. Though fruit was produced, showing self-compatibility and stigma receptivity in the pistillate phase, the fruit was unable to fully mature due to the presence of whiteflies (*Trialeurodes vaporariorum*).

Though more data needs to be gathered, one accession, in particular, 321, stands out from the rest. The most significant difference found between accession 321 and the other 12 accessions studied, was that the stamens of 321 not only dehisced asynchronously but also became connivent immediately. In all other *J. procumbens* accessions, all stamens dehisce synchronously and angle outwards, with a maximum filament angle averaging 102.2 degrees. The majority of studied accessions are thought to exhibit delayed selfing. The exception can be seen in accession 321, which displays more evidence of competing selfing due to the anthers dehiscing in a connivent manner. Along with accession 321 having the smallest maximum petal length (9.2 mm), the connivent anthers suggests that 321 may be located in a pollinator-free or pollinator-poor environment, or that a mutation leading to stamens remaining connivent throughout anthesis became fixed in a small population through genetic drift.

Though morphological features suggest the possibility of a pollinator-poor environment for accession 321, the pollen count is the second largest of the accessions studied. In competing or prior selfing, pollen counts tend to be lower due to the extra energy costs (Brys et al. 2016). Accession 321 also had the second lowest ovule count leading to the highest pollen to ovule ratio, suggesting a penchant for outcrossing. Whereas accession 599, which had the greatest extent of petal spots, had the smallest pollen count with one of the larger ovule counts. Accession 321 not only had one of the highest pollen counts but also one of the largest number of flowers per inflorescence. I speculate that this could imply a pollinator-rich environment with poor quality of pollinators. Having a large pollen count with a large number of flowers per inflorescence would

better ensure pollination in a habitat where there are nectar robbers. A further study of the native habitat of accession 321 would provide more information into pollinator quantity as 321 could have been located in a pollinator-rich environment, leading to high pollen counts. However, accession 321 may have more recently been exposed to low counts of pollinators due to environmental factors or human impact, leading to morphological changes such as a smaller corolla and connivent stamens.

There are noted points of missing data, especially for accession 506. This is due to two main factors, the first being differences in growth rates of accessions; 506 was the slowest to germinate and grow whereas accessions such as 318 and 587 were the quickest. The second and more detrimental factor was the presence of whiteflies (*Trialeurodes vaporariorum*), this greenhouse pest quickly overtook the study population and proved difficult to control with either organic (neem oil) or non-organic methods. Though the majority of accessions did still produce flowers, very few fruits fully developed. Data might vary slightly in number of flowers per inflorescence if whiteflies were not present. Another study would be necessary to conclude the analysis. Another part of a second study could be to continue investigating the differences in traits of other *Jaltomata procumbens* accessions as not all geographic areas where the species grows were represented in this study. Using the current findings as well as geostatistical analysis, predictions could be made about the remaining *J. procumbens* accessions based on origin and elevation.

Appendix A

Photographic Representatives of *Jaltomata procumbens*
All photographs by the author



Figure 1. *Jaltomata procumbens* erect growth habit (580).



Figure 2. *Jaltomata procumbens* procumbent growth habit (838)



Figure 3. *Jaltomata procumbens* flowers of an inflorescence begin anthesis asynchronously (321).



Figure 4. *Jaltomata procumbens*, flower bud not yet open (599).



Figure 5. *Jaltomata procumbens*, pistillate phase starting to transition to hermaphroditic phase (anthers starting to dehisce, not evident in photo); petal spots extend 33% of the distance from base of corolla toward lobe tips (318).



Figure 6. *Jaltomata procumbens* in the pistillate phase; petal spots extend 57% of the distance from base of corolla to corolla lobe tips (599).



Figure 7. Pubescent leaf; non-gland-tipped hairs (324).



Figure 8 and 9. The angle of the stamens changes during the life of the flower. The stigma is receptive during day one, the pistillate phase, before the anthers dehisce. On day two the stamens dehisce as seen on the left (filament angle measured as 105° ; 587), beginning the hermaphroditic phase and allowing for cross-pollination to occur. On the right (324), early day three, the stamens become connivent, with the corolla fully opening for the final day of anthesis.



Figure 10. *Jaltomata procumbens* day three of anthesis. Delayed self-pollination: partial closure of corolla near the end of anthesis reorients stamens, placing anthers into contact with stigma. This delayed-selfing is presumably an adaptation to ensure pollination occurs whether pollinators visit or not. The corolla becomes reflexed once more after the anthers are connivent (593).



Figure 11. *Jaltomata procumbens*, corolla reflexed with connivent stamens mid-day of day two of anthesis, hermaphroditic phase (321).

Literature Cited

- Bresson, C. C., Vitasse, Y., Kremer, A. and Delzon, S. 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology* 31: 1164-1174.
- Brys, R., Geens, B., Beeckman, T. and Jacquemyn, H. 2013. Differences in dichogamy and herkogamy contribute to higher selfing in contrasting environments in the annual *Blackstonia perfoliata* (Gentianaceae). *Annals of Botany* 111: 651-661.
- Brys, R., Van Cauwenberghe, J. and Jacquemyn, H. 2016. The importance of autonomous selfing in preventing hybridization in three closely related plant species. *Journal of Ecology* 104: 601-610.
- Cruden, R. W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. *Evolution* 31:32-46.
- Davis, T. IV. 1986. *Jaltomata* in the Tarahumara Indian region of Northern Mexico. In W. G. D'Arcy [ed], *Solanaceae Biology and Systematics*, 405-411. Columbia University Press, New York.
- Hovenden, M. J. and Vander Schoor, J. K. 2005. The response of leaf morphology to irradiance depends on altitude or origin in *Nothofagus cunninghamii*. *New Phytologist* 169: 291-297.
- Kaczorowski, R. L., Gardener, M. C. and Holtsford, T. P. 2005. Nectar traits in *Nicotinana* section *Alatae* (Solanaceae) in relation to floral traits, pollinators, and mating system. *American Journal of Botany* 92: 1270-1283.
- Kaufman, P. B., Mellichamp, L., Lacy-Glimn, J. and LaCroix, D. 1983. *Practical Botany*. Reston Publishing Company, Inc. Reston, Virginia, USA.
- Lloyd, D. G. and Schoen D. J. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* 153: 358-369.
- Mione, T. 1999. *Jaltomata* II: new combinations for five South American species (Solanaceae). *Brittonia* 51: 31-33.
- Mione, T. and Anderson, G. 1992. Pollen-Ovule Ratios and Breeding System Evolution in *Solanum* Section *Basarthrum* (Solanaceae). *American Journal of Botany* 79: 279-287.
- Mione, T. and Anderson, G. J. 2016 Genetics of floral traits of *Jaltomata procumbens* (Solanaceae). *Brittonia* (2016). doi:10.1007/s12228-016-9447-z.
- Mione, T., Leiva, S. G., and Yacher, L. 2007. Five new species of *Jaltomata* (Solanaceae) from Cajamarca, Peru. *Novon* 17: 49-58

Mione, T. and Spooner, D. M. 2010. *Jaltomata bohiana*: A new species and key to the *Jaltomata* (Solanaceae) of Mexico. *Novon* 20: 186-189.

Mione, T. and Yacher, L. 2005 *Jaltomata* (Solanaceae) of Costa Rica. *In*: A Festschrift for William G. D'Arcy, the Legacy of a Taxonomist, 117-130. Monographs in Systematic Botany from the Missouri Botanical Garden, St. Louis, Missouri.

Muchhala N., Caiza A., Vizuite, J. C. and Thomson, J. D. 2008. A generalized pollination system in the tropics: bats, birds and *Aphelandra acanthus*. *Annals of Botany* 103: 1481-1487.

Nelson, G. L. 2012. Plesiogamy, a term contrasting with herkogamy. *Phytoneuron* 46: 2.

Pannell, J. R. and Barrett, C. H. 1998. Barker's Law revisited: reproductive assurance in a metapopulation. *Evolution* 52: 657-668.

Schoen, D. J. and Brown, A. H. D. 1991. Whole- and part-flower self-pollination in *Glycine clandestine* and *G. argyrea* and the evolution of autogamy *Evolution* 45: 1651-1664.

Subodh, A. and Kumar, A. M. 2010. Flower phenology and pollination ecology of *Punica granatum* in Kathmandu, Nepal. *Nepal Journal of Science and Technology* 11: 115-124.

Vallejo-Marin, M., Solis-Montero, L., Souto Vilaros, D. and Lee, M. Y. Q. 2013. Mating system in Mexican populations of the annual herb *Solanum rostratum* Dunal (Solanaceae). *Plant Biology* 15: 8.

Vitasse, Y., Delzon, S., Bresson, C. C., Michalet, R. and Kremer A. 2009. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research* 39: 1259-1269.

Williams, D. E. 1985. Tres Arvenses Solanáceas Comestibles y su Proceso de Domesticación en el estado de Tlaxcala, México. Masters Thesis. Institucion de Enseñanza e Investigación en Ciencias Agrícolas, Chapingo, México.

Biographical Statement

Rebecca R. Flinn graduated in 2013 from Unity College with a Bachelor's of Arts in Wildlife and a Bachelor's of Arts in Landscape Horticulture. During her time as an undergraduate she was an animal care intern at the Greenville Zoo. Rebecca worked two summers in Oregon for Northwest Youth Corps, building and maintaining trails. During her time as a graduate student, she worked as a veterinary assistant at VCA Cromwell Animal Hospital. She currently resides in Maine and will be pursuing a career in botany upon completion of her master's degree.