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Analysis of Ipomoea (morning glory) leaf mutants

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ANALYSIS OF *IPOMOEA* (MORNING GLORY) LEAF MUTANTS

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Abstract

Ipomoea (morning glory) is the largest genus in the family Convolvulaceae. Cultivation of morning glory plants began in the late Edo period in Japan, and this horticultural success resulted in thousands of plants with varying floral displays. In addition to the different flower morphologies, leaf shape is highly variable within this family making it an ideal group in which to study leaf development. We selected four mutants that vary in lobe number and lobe depth: 1) Tokyo Standard (TKS1065; wild-type; 3 lobes), *yellow maple* (*ym1018*; 5 lobes), *delicate maple* (*dlm620*; 5 deep lobes), and *maple willow* (*m^w646*; simple leaf). These leaf mutants represent the range of leaf shapes found in this family. By using qualitative (microscopy) and quantitative (morphometric) techniques, the specific purpose of this study was to investigate leaf shape and vein homology among leaf mutants in the morning glory family. We found that veins were homologous across lobed species of *Ipomoea*.

Acknowledgments

I would like to thank the College of Humanities and Fine Arts for their support of undergraduate research at the University of Northern Iowa. I would especially like to thank them for the funding of my research through the Summer Undergraduate Research Program (SURP) and the Student Opportunities for Academic Research (SOAR) award. Thank you to Dr. Eisho Nishino at the Graduate School of Horticulture at Chiba University in Chiba, and Drs. Atsushi Hoshino and Eiji Nitasaka at the National Institute of Basic Biology (Kyushu University), Japan for their insight in morning glory research and for providing me with plant materials for this project. I also extend many thanks to Austin Jones and Bryan Hendrickson for their research, and to Dr. Jessica Moon for her counsel as head of the Honors Department. Finally, I would like to express my deepest gratitude and appreciation to Dr. Julie Kang for her unwavering guidance as my thesis mentor for the past three years. The knowledge and experience she has bestowed upon me are priceless.

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Introduction

The flowering plant family Convolvulaceae is made up of 67 genera, the largest of which is *Ipomoea* (morning glory; theplantlist.org). *Ipomoea* is a genus that includes 449 accepted species of liana, all of which grow long winding tendrils with flowers (theplantlist.org).

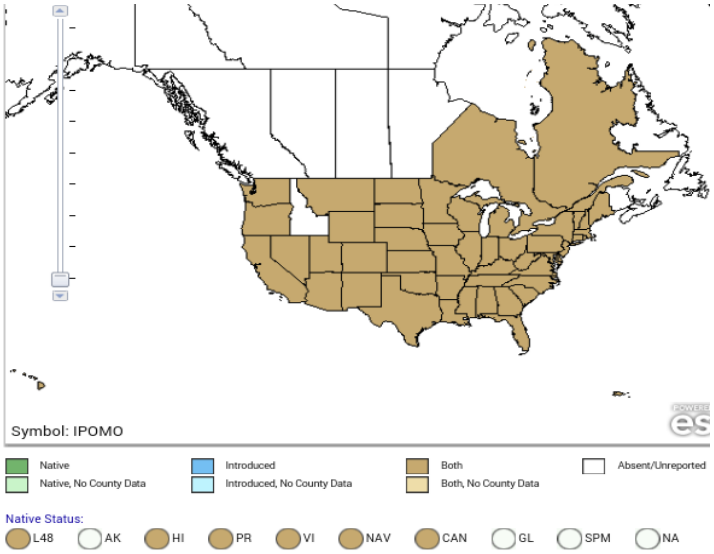

Figure 1. A map of morning glory presence across the United States.

According to the United States Department of Agriculture (USDA), *Ipomoea* is present in 49 states with the exception of Idaho (no information is available), and is considered to be both a native and introduced species (see Figure 1). In the United States, the perennial plant is designated as a noxious weed, and in some states such as Arizona, most species are prohibited (USDA.gov). *Ipomoea* is also known for its toxicity and is considered a poisonous plant. Polyhydroxylated alkaloids in the leaves, flowers, and seeds often lead to natural intoxication in livestock and domestic animals (Haraguchi, Gorniak, Ikeda, Minami, Kato, et al., 2003).

The Convolvulaceae is a food crop family that belongs in the order Solanales, an order that includes many important food crops such as sweet potato (*Ipomoea batatas*) and its relatives, the potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*). Since these crops are important to the US economy and economy world-wide, understanding the general biology of these plants will aid in understanding the overall growth habits of these plants. Since leaves are the primary structures that function in photosynthesis, the process of converting light energy to

food energy for the plant and understanding how leaves develop will allow us to better understand the development of food crops, in general.

This research was performed to investigate leaf shape (lobing) and vein homology in four leaf mutants from the *Ipomoea* genus. The four mutant species used in this study differ greatly in number and the depth of the lobes. We tested the hypothesis that, despite the differences in leaf morphology, vein patterning is homologous across the mutant species. Understanding the homologous nature of the vasculature in these mutants may contribute to a greater understanding of leaf function and efficiency. These concepts are important to comprehending plant growth in general, which is vital to our existence, but more specifically they can provide us with more leaf knowledge to apply to food crops that are important to the world food supply.

Literature Review

Ipomoea nil (morning glory) was first noted for its medicinal uses in China over 1000 years ago. From China *Ipomoea* moved to Japan where it was highly regarded as a garden-variety plant. *Ipomoea* was horticulturally important during the Edo Period, approximately 200 years ago, but genetic studies of the genus only started in the early 20th century (Kajita & Nishino, 2009). Japanese scientists began looking into morning glory genetics in 1897, prior to Mendelian genetics being generally recognized. The first morning glory publication was released in 1916; this spurred an influx of *Ipomoea* genetics research into the early 1930s from scientists in Japan, America, England, Holland, and Germany (Imai, 1930). Of note, *Ipomoea* researcher Dr. Yoshitaka Imai looked into genetic variance in *Ipomoea*. Within this study, Imai established ten groups of genes that he deemed "linkage groups." The ten linkage groups were considered independent of one another, but there were 70 genes associated with the linkage groups and 50 loci (Imai, 1930).

In 1925, Imai published his observations about the *willow* gene. Imai noted that *willow* seeds would grow into a willow shape with slender flowers. Some leaves had a slightly round shoulder while others did not. From this Imai concluded that the *willow* gene derived from the "heart factor" that gave rise to highly rounded heart-shaped leaves. If a willow plant had the heart factor the leaves would have rounded shoulders, but without the heart factor the leaves remained slender and were considered "normal willows." When these two genes were crossed Imai observed that the "normal willow" was recessive and the ratio of non-willow to willow plants was 3:1, most likely because of recessive deaths (Imai, 1925).

Imai then looked into the relationship between the *Ipomoea* willow form and the maple form. Imai asserted that the willow was often used as a maple parent, and that when these two

gene types were crossed they gave rise to narrowly lobed leaves and a 5-petal flower. This mutant was sterile. With further study, it was also noted that the willow form tried to mutate towards the maple form, bringing forth questions about a possible allelomorphic (alternative forms of the same gene occupying a given position on a chromosome) relationship between the willow gene and the maple gene (Imai, 1925).

Modern *Ipomoea* researcher, Dr. Eisho Nishino, has been studying *Ipomoea* since the early 1970s. Nishino's publications have focused on the morphology and anatomy of *Ipomoea* as compared to the heavy classical genetic research from earlier in the century. Nishino's most recent studies compare the *maple-willow* (*m^{w}Q0646*) mutant with the wild-type species (TKS1065) to genetically analyze developmental and anatomical differences in morphology between these two species. The *m^{w}646* has the strongest change in phenotype among the gene mutants, which makes it a prime mutant to study. *m^{w}646* is a recessive allele of the *MAPLE* gene that controls growth along the medial lateral axis of lateral organs. Nishino surmised that a single gene mutation in the *m^{w}646* seems to affect all lateral organs (Kajita & Nishino, 2009). This mutation decreases the width of the lamina of all lateral organs. The narrowing of leaves, sepals and petals was also determined to be a result of an inactive marginal meristem. Smaller and defective reproductive organs in the *m^{w}* mutant also arise from inadequate marginal growth. Based on histological observations, it was determined in this study that there were certain homologous elements in *m^{w}646.* Homologous parts of the mutant species include the lateral and floral organs, and overall give rise to unique characteristics that will need further study to understand developmental and gene-related expression during morphogenesis (early development; Kajita & Nishino, 2009).

Angiosperm (flowering plants) leaves differ greatly in morphology, expressing a wide range of characteristics including variations in number and/or form of marginal serrations, lobes, and leaflets. Leaves are typically flattened structures that come in a variety of shapes and sizes, which lends to their purpose of light capture and energy production (production of sugars). Without leaves and veins to transport the products of photosynthesis, plant growth would be gravely inhibited. Therefore, studying leaf shape development is at the core of understanding the overall growth habit of plants.

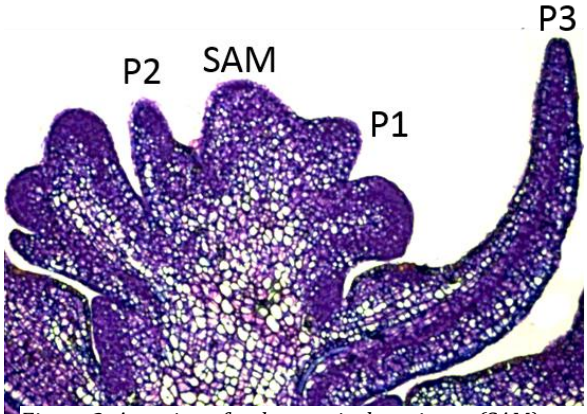

Figure 2. A section of a shoot apical meristem (SAM). Youngest primordia are closest to the SAM (P1 and P2). Older leaf primordia (P3) undergoing primary morphogenesis are developing leaf polarity.

Leaves develop from the shoot apical meristem (SAM), a dome-shaped structure that contains a population of meristematic (stem) cells located at the apex of the shoot (see Figure 2). The SAM gives rise to all the different tissues and organs (e.g. leaves, stems, flowers) of a plant. Leaf primordia initiate on the outer edge of the SAM and go through three phases of development: 1) leaf initiation, 2) primary morphogenesis, and 3) secondary morphogenesis or leaf expansion (Dengler & Tsukaya, 2001). As leaf primordia initiate from the SAM, cell identity in the primordia changes from indeterminate to determinate, establishing adaxial (the side of the leaf closest to the SAM) and abaxial leaf polarity, and differentiation of internal tissues (e.g. vascular tissue). It is during secondary morphogenesis that leaves increase in surface area. During this time, leaves may maintain or change their shape through differential patterns of expansion. The leaf margins (edges of leaves) still maintain meristematic properties that allow for the development of leaf serrations or lobes during secondary morphogenesis.

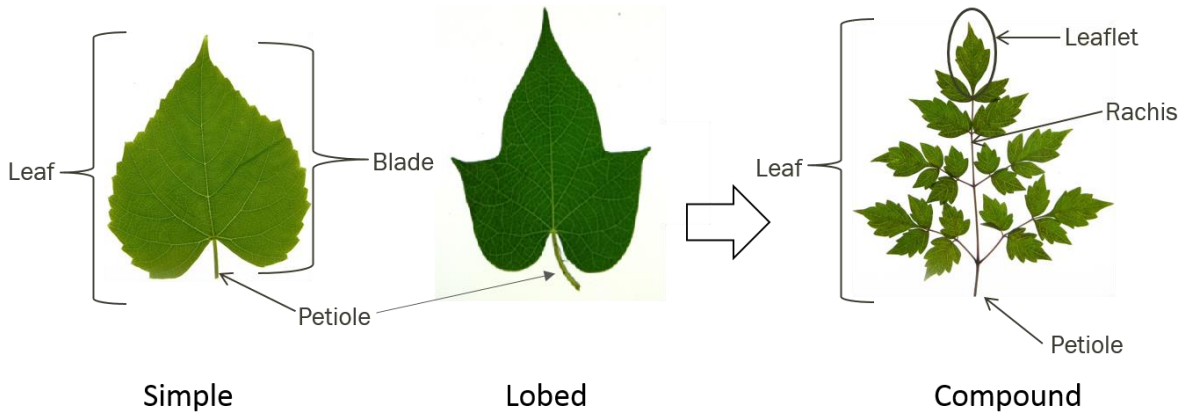

Figure 3. A diagram depicting the anatomy and diversity of a simple (left), lobed (middle), and compound leaf.

Despite of the vast array of leaf morphologies in the angiosperm family, leaves are predominately classified into two broad groups, simple and compound. Simple leaves are distinguished by having a single continuous lamina (blade), while compound leaves have multiple separate laminar units called leaflets (see Figure 3**)**. In both cases, leaf margins can be smooth, serrated, or deeply lobed. Thus simple leaves can also be lobed depending on the amount of changes that occur along the leaf margin.

Ampelography is a technique that is used to assess typical traits among groups of species to determine if heritable traits like leaf shape are correlated with other measured traits. Use of this technique was first published in 1952 by Galet (Précis d'Ampélographie Practique) and was subsequently translated in 1979 (A Practical Ampelography: Grapevine Identification; Galet, 1952; Galet,

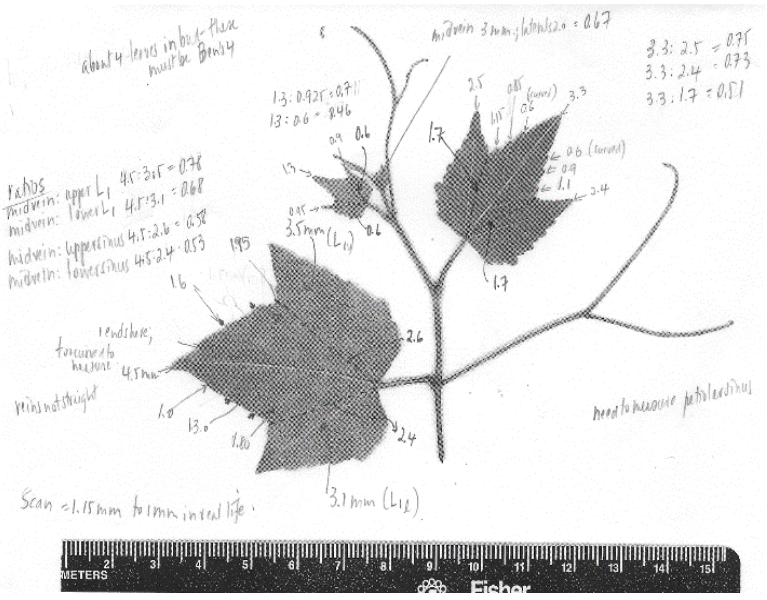

Figure 4. An example of the Ampelography technique defining leaf traits of interest in an Ampelopsis leaf.

1979; see Figure 4). Data produced from this technique can then be applied to morphometric techniques such as Principle Component Analysis (PCA; Klingenberg, Duttke, Whelan & Kim, 2012; Chitwood, Ranjan, Martinez, Headland, Thiem, et al., 2014). Currently, little is known about leaf shape development in the genus *Ipomoea.* Thus, by using an ampelographic technique, along with a developmental analysis, a comprehensive analysis of leaf shape can be conducted.

Morning glory interest and importance have spanned across centuries. While scientific research of the plant did not start until the beginning of the 20th century, it expanded full force for the first half of the century, focusing on the genes that lead to such great variance within the family. As time has gone on the research interest in genetics has shifted to an attention towards anatomy and morphology of *Ipomoea* and how these features may be affected by genetics. Moving forward, similar studies of morphology and homology of morphology will be performed. This study aims to contribute to current findings about morning glory vein homology by observing vasculature patterns across four different morning glory species. With new knowledge about vein homology in *Ipomoea* we hope to connect our findings to ideas about leaf vasculature in related agricultural species, such as sweet potato, potato, and tomato. Leaf vasculature is one of the most important components contributing to plant growth and plant efficiency. Understanding the homologous nature of *Ipomoea* mutant vasculature may lead to a more comprehensive outlook on producing the healthiest and most efficient agricultural species.

Materials and Methods

Plant Material and Growth

Tokyo Kokei Standard (TKS1065; wild-type), *yellow maple* (*ym1018*), *maple willow* (*m^w646*), and *delicate maple* (*dlm620*) seeds were obtained from Dr. Eisho Nishino at the Graduate School of Horticulture, Chiba University, Chiba, Japan and from Drs. Atsushi Hoshino and Eiji Nitasaka at the National Institute of Basic Biology (Kyushu University), Japan. All plants were cultivated at the University of Northern Iowa (UNI), Cedar Falls, Iowa, United States. Soil was obtained from the UNI Greenhouse and Botanical Center, and the plants were watered via an automated watering system.

Seed Treatment

Seeds were treated before transplanting to allow for germination. A small section of the seed coat was removed with scissors on the end of the seed opposite of the micropyle (containing female reproductive organs) on all seeds. Seed color differs amongst species, where darker seed coat color is correlated with a tougher coat. Seeds with a black seed coat, primarily TKS1065 and *ym1018*, were placed in 10N sulfuric acid for one hour following seed coat snipping to allow for further degradation. Seeds soaked in 10N sulfuric acid were rinsed with distilled water (diH_2O) five times to remove excess acid. All seeds were then transplanted directly into moist soil approximately 2 centimeters (cm) below the surface. Large flats were watered and fertilized using Miracle Grow fertilizer and covered with plastic dome (3-4 days) under growth lights. Young plants remained under growth lights until the first vines started to sprout. At this point the young plants were transplanted into quarter gallon pots and placed in the UNI Greenhouse and Botanical Center research house.

Treatment of Leaves

Immature leaves (~3-10mm) were placed in 70% ethanol (EtOH) for 24 hours. After fixation in 70% EtOH the young leaves were put directly into saturated chloral hydrate and

incubated at 60°F for approximately one week to allow for clearing of chlorophyll. Longer incubation periods (up to 3 weeks) allowed for further clearing. After clearing, the immature leaves were mounted on microscope slides using a 50-50 mixture of saturated chloral hydrate and 100% glycerol. Slides were kept at room temperature and photographed using a Zeiss microscope and camera.

Mature leaves were placed directly into 70% EtOH and photographed using a Nikon digital camera and light box. To better view vein patterns mature leaves were boiled in 70% EtOH until white and then were dyed in safranin for 3-5 hours, or until leaves were medium pink color. Leaves were stored in a 50-50 mixture of 70% EtOH and diH_2O, and then once again photographed using a Nikon digital camera and light box.

Pictures of mature leaves dyed in safranin were cropped and brightened in Adobe Photoshop and then measured in ImageJ. Measurements taken included first-secondary vein length (2°-1L) and second-secondary vein length (2°-2L), angle between each of the secondary veins and the mid-vein (2°-1A, 2°-2A), sinus length, perimeter, and area. These data were used to create box plots in Sigma Plot 12 in order to view cross species comparisons. Mid-vein length (1°L) was also measured and a total vein count was taken by counting all veins coming off of the mid-vein on one side (right or left) of the leaf. The averages of these numbers were compiled in a figure table.

Flowers

Flowers were documented with a Nikon digital camera. Pictures of the flowers were taken on the plant and also on a black or white background.

Results

Mature Phenotype

Mature plants in general all looked very similar, with only slight variations in height (around 1m). All mature plants had multiple long and winding tendrils that would often exceed a meter in length. There also appeared to be similar numbers of leaves and flowers formed on each plant (see Figure 5).

Mature leaves differed in size, number of lobes, and even color between the four species. TKS1065 leaves were broad light green leaves and averaged approximately 8.05cm in length. TKS1065 leaves had three lobes, one terminal lobe and two lateral lobes. Sinus length of TKS1065 was not severe; the average length of the sinus, measured from the petiole to the lowest point of the sinus, was approximately 3.60cm.

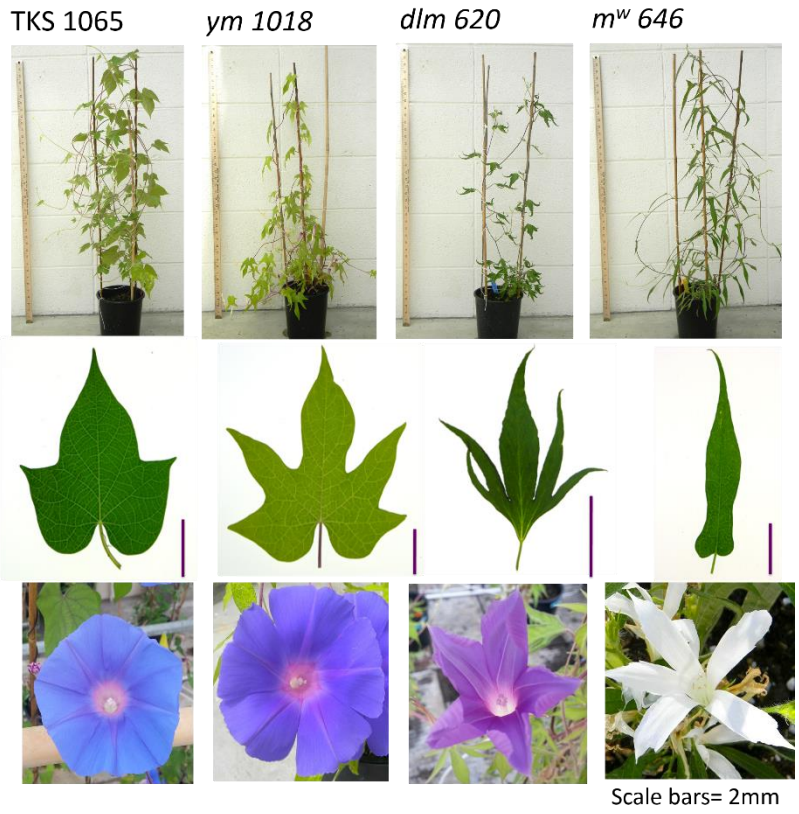

Figure 5. Mature phenotypes of Ipomoea species used in study; Mature plants, leaves, and flowers: a, e, i) TKS1065, b, f, j) ym1018, c, g, k) dlm620, d, h, l) mʷ646.

Leaves from both *ym1018* and *dlm620* had five lobes, one terminal lobe and two lateral pairs of lobes. Although the leaves of these two species have the same lobing, they appear very different. Leaves from *ym1018* are broad yellow-green leaves that averaged 7.29cm in length and have deeper sinuses than TKS1065, but not as deep as *dlm620*. Average sinus length for *ym1018* sinus one (sinus 1-L, first sinus from the mid-vein) was 2.33cm and sinus two (sinus 2-L, second sinus from the

mid-vein) was 2.51cm. In contrast, *dlm620* leaves are smaller dark-green curled leaves with very deep sinuses. *dlm620* leaves only average 5.17cm long and have a sinus 1-L of about 1.62cm and a sinus 2-L of 1.31cm. Leaves from the *m^{w}646* species had no lobes and are therefore are considered simple leaves. Due to the lack of lobes, *m^{w}646* leaves are much thinner than the other varieties, and they tend to be slightly longer, on average 9.49cm long. They are light green in color, similar to TKS1065 (see Figure 6).

It has been observed that flower morphology in *Ipomoea* mimics leaf morphology.

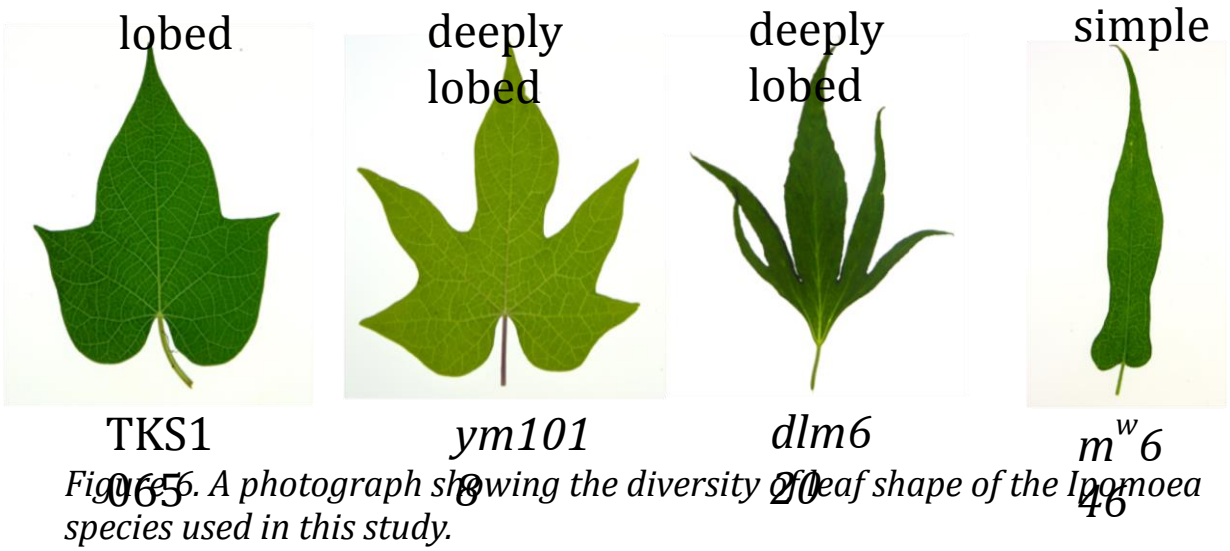

Figure 6. A photograph showing the diversity of leaf shape of the Ipomoea species used in this study.

Greater number of lobes and severity of sinus length in leaves leads to more complex flowers with highly separated petals. The flowers of the *Ipomoea* mutants vary from completely fused petals to thin and highly separated petals. Color variation remains, for the most part, in the blue, pink and purple family; although, the *m^{w}646* plant produces white flowers. TKS1065 flowers are blue and have a completely fused corolla, the trumpet bell shape often seen in garden-variety morning glories. The flowers of *ym1018* are similarly blue, with a slight purple tinge, and at first glance they also appear to have a completely fused corolla. Upon closer observation, though, it

becomes apparent that there are slight "tears" in the corolla that now give rise to slightly separated petals on an otherwise fused funnel. The difference in petal separation of the *dlm620* flower compared to that of the *ym1018* flower imitates the difference between the two species in sinus depth. The *dlm620* flower is purple and has obvious petal separation that leads to the appearance of a star. The most contrasting flowers of the four species studied were from the *m^{w}646* plant. As mentioned earlier, the *m^{w}646* plant produces a white flower compared to the three other species that have blue-purple flowers. Also different from the other flowers is the complete separation of the *m^{w}646* petals; these petals are thin and have a shaggy appearance.

Homology

Looking at our young leaves that were cleared using saturated chloral hydrate, leaf shape (including number of lobes) was completely determined by at least 3mm in length (see Figure 7). Along with leaf shape, vein patterns seemed to follow suit in being fully present by the time the leaf reached 3mm in length. All of the species studied had a mid-vein that we designated to be the primary vein (1°), but each of the four species had different secondary vein placement (2°-1, 2°-2). TKS1065 secondary veins both go into the single lateral lobe. The similarities in lobing of *ym1018* and *dlm620* determined the identical secondary vein placement in these two species. In both species there are 2°-1 and 2°-2 veins, but one secondary vein goes into each of the lobes in the lateral lobe pairs of these leaves. Once again the outlier of the four species we studied was the *m^{w}646.* As was previously mentioned *m^{w}646* does have a 1° vein, but because of the absence of lobing there is a single vein that we have designated as a secondary vein. It is a very small vein compared to other species' secondary veins and is located approximately where other secondary veins would be in the lobed species. These characteristics were also found in mature leaves, confirming that they stay the same through leaf maturity.

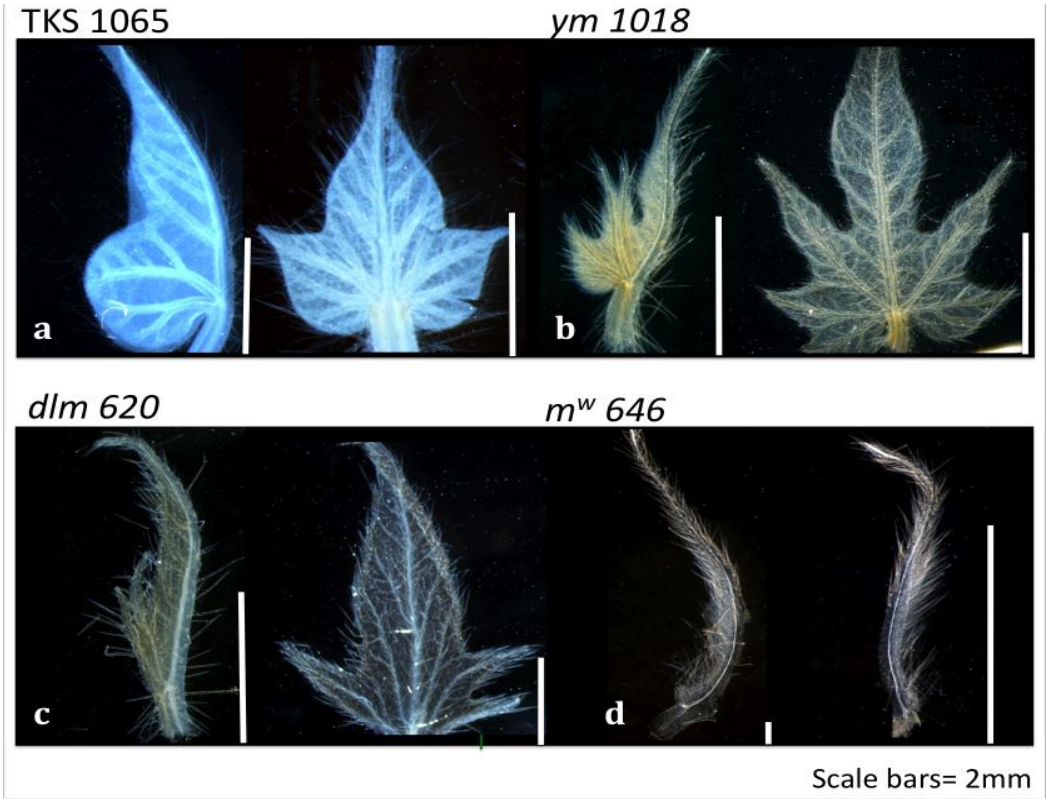

Figure 7 Vein homology in cleared young leaves. a) TKS1065, b) ym1018, c) dlm620, d) m^w646

Ampelography

Using morphometric analysis, we approached our hypothesis in a quantitative fashion to examine these traits (see Figure 8). A one-way ANOVA statistical test was run to analyze significant differences in these traits between every species; for these tests $p<0.05$. When comparing 2°-1L our statistical results show that all species comparisons had a significant difference with one exception, only TKS1065 and *ym1018* had no significant difference in 2°-1L. There was a significant difference in 2°-2L between all species except TKS1065 and *ym1018* and between *ym1018* and *dlm620*.

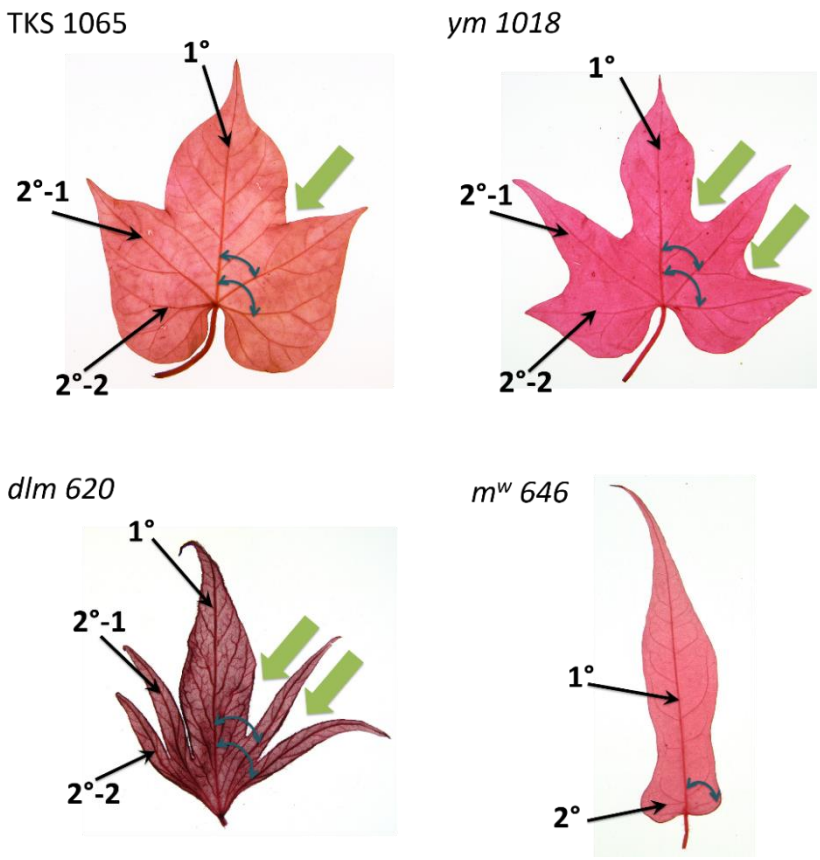

Figure 8. Features measured for morphometric analysis. Midvein length (1 ^{0}), sinus depth, first secondary vein (2 ^{0}-1L), second secondary vein length (2 ^{0}-2L), and secondary vein angles.

The 2°-1A significance comparison once again showed significant differences between all of the studied species except between TKS1065 and *m^{w}646*. A similar proportion of significance occurred when comparing 2°-2A. There were significant differences in 2°-2A for all species comparisons with the exception of *ym1018* and *dlm620*.

Sinus 1 length was significantly different in every species comparison, however this was not in true in sinus 2 length. *ym1018* and *m^{w}646*, TKS1065 and *ym1018*, *dlm620* and *m^{w}646*, and TKS1065 and *dlm620* all have significant differences in length, while *ym1018* and *dlm620*, and TKS1065 and *m^{w}646* do not have significant differences in sinus 2 length between the species.

The cross-species comparison of area once again had mixed results of significance. Neither TKS1065 and *ym1018*, nor *dlm620* and *m^{w}646* have statistically significant differences. All other cross-species comparisons did have statistically significant differences in area.

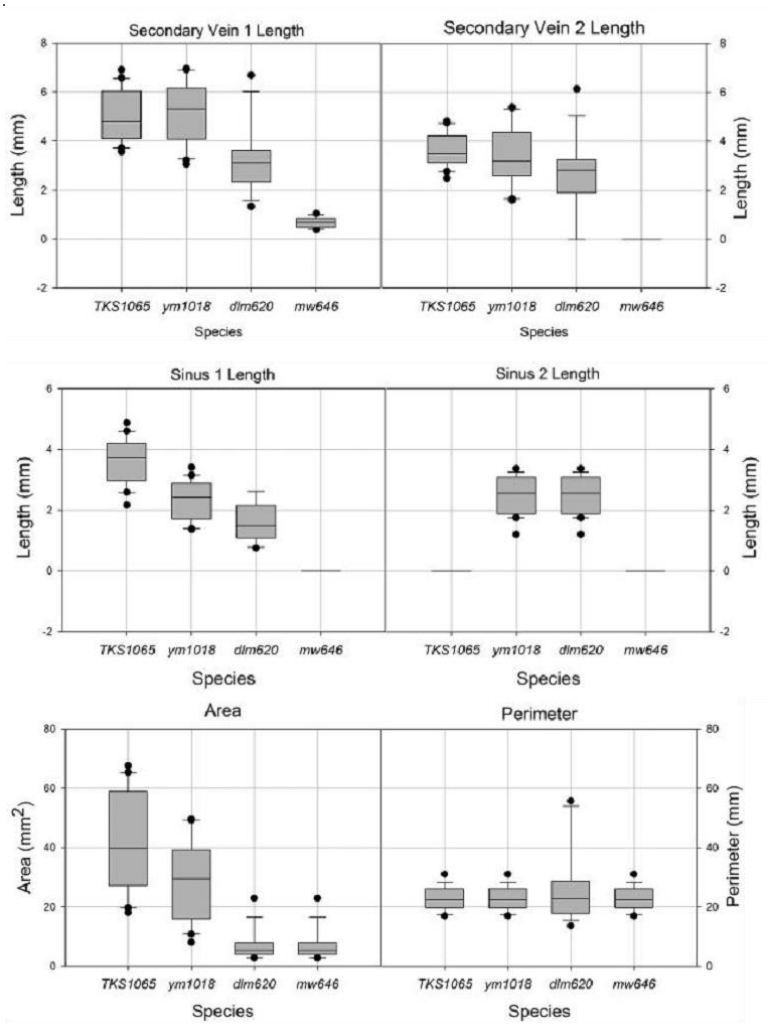

Figure 9. Box plots representing quantitative analysis of morphometric measurements. Cross species comparisons of secondary vein length, sinus length, secondary vein angles, area, and perimeter.

Finally, perimeter was examined and no statistically significant differences were observed between any of the cross-species comparisons. The statistical differences of area, despite no statistically measured differences in perimeter, will be discussed later (see Figure 9).

The average mid-vein length shows that *m^{w}646* is the longest of the species and also has the greatest number of veins coming off of the mid-vein. TKS1065 and *ym1018* are very similar in both length and vein numbers. *dlm620* has proven to be the smallest leaf overall, but has the second highest vein count (see Figure 10).

	Avg. 1° Length (cm)	**Avg. # Veins**
TKS1065	8.05	7.95
ym1018	7.29	8.20
dlm620	5.17	10.21
m^{w}646	9.49	13.53

Figure 10. Average mid-vein length and average number of veins per leaf.

Discussion

As previously described, our series of studied leaves ranged from simple to severely lobed. While it may appear that the lobed leaves are more complex relatives of the simple leaf, *m^{w}646*, we have concluded that this may be the other way around. In our series of leaves TKS1065, the wild-type, had three medium lobes. From there the leaves increased in lobe number and lobe severity. *dlm620* had the most severe lobe depth; the next plant in the series, *m^{w}646*, is a simple leaf. Our observations about plant characteristics and genetic background of these four plants point to the simplistic nature of *m^{w}646* being caused by such deep lobing that the connective tissue disappeared altogether and lobes were no longer formed. As loss of tissue and loss of surface area occured, general plant function continued. Mature plants of all species were approximately the same size with similar numbers of leaves and flowers. If the loss of leaf surface area affected leaf function or energy productivity, mature plant morphology would most likely not be so similar. For all of these traits to remain the same, it would seem that leaf vasculature must also be the same, despite these major differences.

Ampelography results brought forward interesting points that supported the idea of homologous vasculature. The first findings confirmed similarities of 2°-1L and 2°-2L. 2°-1L and 2°-2L were the same in TKS1065 and *ym1018*. These leaves were similar in size and area, which made sense. However, because TKS1065 had three lobes compared to *ym1018*, which had five lobes, it demonstrated how vein length was not affected by differing shape. 2°-2L in *dlm620* was also related to TKS1065 and *ym1018*. The size difference between *dlm620* and the others made this finding surprising, but it continued to support the idea that the veins are not affected by leaf shape.

Since dramatic variation in lobe severity is what made these specific species of *Ipomoea* useful we expected sinus length values to be very different. The first sinus length varied

dramatically between the species as expected. It was determined that the first sinus is where the greatest change occurred as species become more highly lobed. Sinus 2 was only present in *ym1018* and *dlm620*. Here we determined that more tissue was lost as lobing becomes more severe. These drastic increases in sinus depth ultimately led to complete loss of lobes. Area and perimeter analysis have shown that surface area is definitely lost.

Interestingly, in spite of a loss in surface area, perimeter was the same across the board. After careful observation of surface area, average leaf length, and average secondary vein number data we concluded that these data were in fact correlated. Not only were these data correlated, their relationship confirmed our hypothesis, that there is homology in *Ipomoea* veins. Longer leaves such as TKS1065 and *ym1018* also have much greater surface areas. These similarities resulted in very similar average leaf lengths and average minor secondary vein numbers. As the leaves became smaller, either in average length (*dlm620*) or surface area (both *dlm620* and m^w646), an extreme increase in average minor secondary vein number accompanied these changes. Greater numbers of minor secondary veins appeared to be compensation for the loss of leaf tissue in the lobed leaves, and compensation for loss of lobes and loss of a major secondary vein in m^w646. Regulation of minor secondary vein number, despite loss of leaf tissue supported vein homology in the lobed leaves. It also suggested that leaves adapted to loss of tissue or veins by increasing the number of minor secondary veins and fulfilling the same necessary vascular functions with these minor veins.

Our findings validated our claims that vein homology occurred in these morning glory mutants, at least across the lobed species. These data, especially those that demonstrated the correlation between surface area and average secondary vein number, exhibited important elements of homology. Our new understanding of the prominent role that minor secondary veins

had in maintaining vein homology and leaf function for lobed leaves can be translated to food crop species. We now also have a quantifiable method to measure influence of minor secondary veins in for all plant species.

Conclusions

Limitations

Throughout the course of this study, methods were used to observe the initiation of leaf lobing and vein patterning at very early stages of development. Scanning electron microscopy (SEM) was used to view leaf apices, the part of the plant shoot where the shoot apical meristem (SAM) is located. Once properly dissected and dried, apices are merely tissue skeletons about the size of a grain of sand.

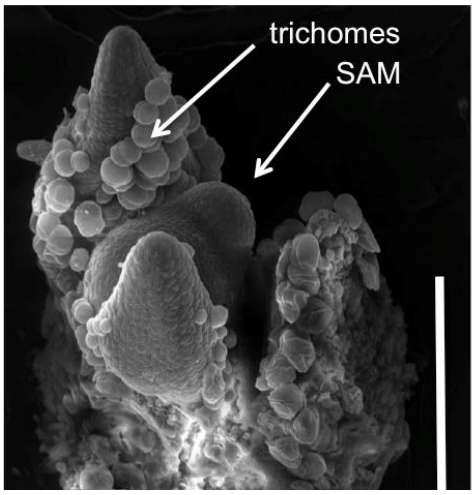

Figure 11. SEM of Ipomoea SAM with trichomes.

The fragility of apices prepared for SEM made it difficult to dissect the specimens enough to see leaf primordia initiation at the SAM. When the shoots were not properly dissected, long hair like structures called trichomes were left behind on already developing leaves (see Figure 11). These trichomes obstruct any possible visuals of leaf primordia initiation during this first stage of development. Further work will need to be done to develop the best dissection technique so trichomes no longer distort SEM images and specimens are not destroyed.

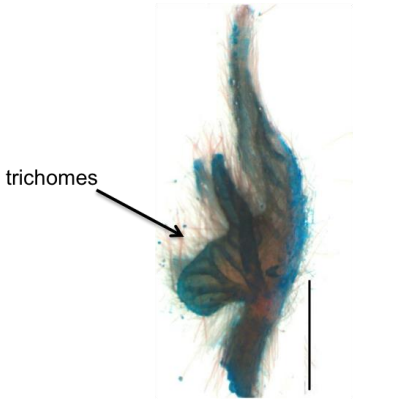

Figure 12. Young leaf stained with alcian- green. Trichomes have also stained.

For this study, clearings of young leaves were prepared following the earlier described method. Another young leaf clearing method uses a deep blue dye called alcian-green. This clearing method may be able to highlight vein patterns at an earlier stage than 3mm, compared to our current clearings. Once again, trichomes on the young leaves have proven to be challenging as they take on the color of the dye and obstruct visuals of the vein patterns (see Figure 12). In future work the concentration of alcian-green dye used will be varied to determine better coloration that doesn't also dye the

trichomes blue. A new microscope camera will also be used to elevate the quality of images captured.

Future Work

New research will be done to examine the resulting double mutant (*dlm^{w}672*) that occurs when the delicate morning glory (*dl1010*) and *m^{w}646* are crossed. Understanding the genetic background of *dlm^{w}672* may help us to understand why this extreme morphology is occurs (see Figure 13). Comparing a fifth species of *Ipomoea* also gives us more data to compare from morphometric analysis.

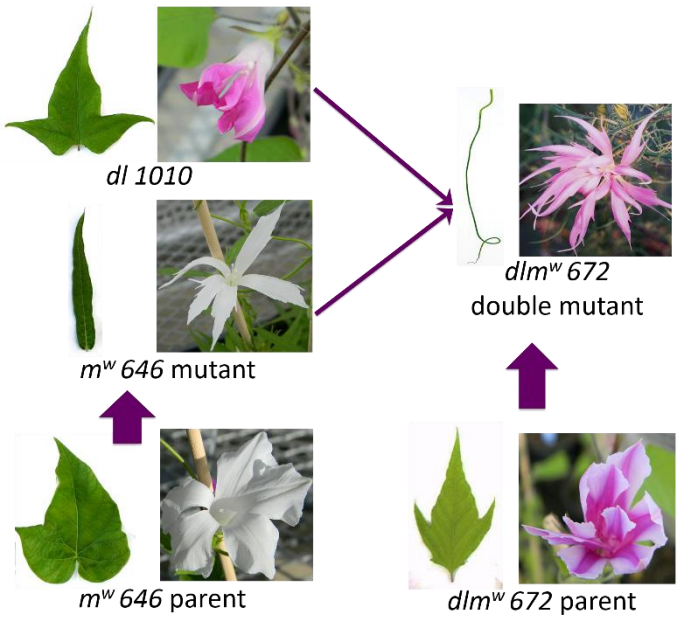

Figure 13. Genetic crosses between mw646 and dl1010 gives rise to dlmw672. Both dlmw672 and mw646 have parent plants that produce mutants. Mutants are sterile

The purpose of this study was to investigate leaf shape and vein homology in *Ipomoea* mutants to build an understanding of vasculature in morning glory plants, and to apply this knowledge to important related species (sweet potatoes, potatoes, and tomatoes). Although leaf shape differed greatly in the four species used for this study (number of lobes and depth of lobes), general function was maintained between the species. Our current observations suggest the presence of vein homology in the lobed mutant species, but leaves us with further questions about vein homology in the *m^{w}646* species. Studying leaf shape and vein homology in *Ipomoea* is vital to our understanding of plant growth in general. The widespread presence of healthy and efficient plants is hugely impactful to our existence. More specifically, though, it is important to our understanding of pertinent food crops related to *Ipomoea*. As demand for resources increases, greater competency of world food crops is necessary to develop the most economical, efficient, and productive agricultural system possible.

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