FITNESS EFFECTS OF SOMATIC MUTATIONS ON MIMULUS AURANTIACUS PROGENY

by

JACK McLEAN

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Approved:_____*Matthew A. Streisfeld, Ph.D.*_____
Primary Thesis Advisor

Unlike animals, plants possess the unique ability to pass on mutations to progeny that arise both through meiotic (germline) and mitotic (somatic) division. In spite of these two sources of genetic variation, mutation rates per generation appear to be similar between plants and animals. One explanation for this discrepancy is cell lineage selection (CLS), which claims that cell lineages with deleterious somatic mutations are sieved out of the population of cells in a plant's shoot apical meristem through natural selection. On the other hand, beneficial mutations may become fixed within the population. To investigate the plausibility of CLS, I performed multiple experiments to determine the fitness effects of somatic mutations by comparing the progeny of *Mimulus aurantiacus* plants generated via self-pollination made within the same flower (autogamy) to progeny from self-pollinations made between stems on the same plant (geitonogamy). Importantly, autogamy leads to homozygosity of a proportion of somatic mutations, but progeny from geitonogamy remain heterozygous for mutations unique to each stem. Significant differences in fitness were observed among some autogamous and geitonogamous progeny. Surprisingly, autogamous progeny from several genets displayed significantly increased fitness, which challenges the assumption that differences in fitness between autogamous and geitonogamous progeny are caused solely by deleterious mutations. These results support the hypothesis that somatic mutation accumulation during vegetative growth can result in non-negligible—and possibly beneficial—changes in fitness among progeny due to CLS.

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Introduction

Mutations play a foundational role in the emergence of genetic variation within offspring. While this phenomenon is well known, the particular role of different classes of mutations remains only partially understood and appears to vary between different kingdoms of life. Among most animals, mutations that arise during meiotic division (germline) are heritable in progeny, while mutations that arise during mitotic division (somatic) are not heritable. On the other hand, plants possess the ability to pass on both germline and somatic mutations to progeny (Antolin & Strobeck. 1985). This capacity to pass on both types of mutations is possible because plants undergo indeterminate growth, where shoot and root systems continually elongate and develop throughout a significant portion of their life-cycle (D'Amato 1996).

Growth of the shoot system occurs at shoot apical meristems (SAMs). SAMs are found at the apex of elongating shoots and each possesses a population of undifferentiated, pluripotent cells. These cells differentiate into plant tissue necessary for growth and development, including the gametes involved in sexual reproduction. This reservoir of pluripotent cells is continually replenished through mitotic division, which offsets cells that have differentiated (Kwiatkowska 2008). As the shoot elongates, somatic mutations may occur due to DNA replication errors during mitotic division within this population of cells. These somatic mutations may be retained and will begin to accumulate as the stem elongates resulting in distal areas of the shoot system possessing a greater concentration of somatic mutations when compared with basal counterparts (Schultz & Scofield 2009).

The role and relevance of somatic mutations within plants remains highly contested and largely unsettled. Since plants possess the ability to pass on both germline and somatic mutations to progeny, one might expect the mutation rate per generation among plants to be noticeably higher when compared with animals. However, mutation rates per generation appear to be similar between plants and animals (Gaut et al. 2011). Multiple explanations have been offered to explain this discrepancy.

One proposed explanation for this issue is cell lineage selection (CLS), which asserts that cell lineages that possess deleterious somatic mutations are sieved out of the pluripotent cell population due to natural selection. Since the size of this localized cell population within the SAM is fixed and constantly being replenished through mitotic division, cell lineages that replicate at a slower rate due to the expression of deleterious mutations are more likely to be out competed and eventually eliminated by ancestral cell lineages, or by cell lineages with beneficial mutations that accelerate division (Pineda-Krch & Lehtila 2002). Models of stochastic growth have indicated that relatively minor differences in cell replication rates during development can result in significant differences in the proportion of mutant cells found within adults (Otto & Orive 1995; Pineda-Krch & Lehtila 2002). The findings of these models are supported by Yu et al. (2020), who performed comparisons between complements of single nucleotide polymorphisms found within the ancestral zygote and ramets (individual stems) of common eelgrass (*Zostera marina*). Furthermore, Cruzan et al. (2020) observed that seep monkeyflower (*Mimulus guttatus*) progeny exhibited extraordinary variation in fitness due to the accumulation of somatic mutations during stem growth, which in some cases led to higher fitness from potentially beneficial somatic mutations

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being inherited in progeny. These results strongly suggest that somatic mutations play a non-negligible—and possibly beneficial—role in fitness in plants, challenging earlier studies on the topic, which have asserted that beneficial mutations should be exceedingly rare (Charlesworth & Willis 2009).

In spite of these findings, CLS has been challenged by a number of studies. Some have proposed that germline segregation occurs early on in development, with future germ cells physically separated from future somatic cells within the SAM (Lanfear et al. 2018). This explanation—commonly referred to as the plant germline hypothesis—asserts that somatic mutations that arise during vegetative growth are only rarely inherited by progeny, since future germ cells would only be found in their own isolated cell lineages. These isolated populations of future germ cells could potentially have a slower rate of division than their somatic counterparts, and as a result would have a significantly lower mutation rate over time (Lanfear et al. 2018). The central zone of the angiosperm SAM has long been a candidate location for this proposed population of isolated future germ cells due to its slow cell division rate relative to the peripheral zone and rib meristem (Cutter 1965). However, further observations in angiosperm models have revealed that mitotic activity spikes both within the central zone and rib meristem during the transition from vegetative to reproductive tissue, suggesting that multiple zones contribute to the formation of the gametes (Kwiatkowska 2008). More recently, computational models based on quantitative cell lineage data from *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) were used to replicate patterns of cell division in SAMs and axillary meristems (Burian et al. 2016). These models suggested that cells were not constantly replaced within the central zone of the

SAM, and instead persisted throughout vegetative growth. Burian et al. (2016) claim that these findings demonstrate that plants possess mechanisms to prevent the fixation and eventual accumulation of deleterious genetic load. They further assert that these findings suggest that plants possess germlines analogous to those found within animals. The plant germline hypothesis has however been challenged by Cruzan et al. (2020), who demonstrate that somatic mutations can potentially have a non-negligible impact on progeny.

While the influence of somatic mutations on plant fitness remains highly contested, the relevance of somatic mutations on our daily lives remains plain to see. Gaining a more complete understanding of somatic mutations has far reaching implications on agricultural practices and conservation biology, along with numerous other fields of study. Somatic mutations have played a pivotal role in the development of many clonally-derived fruits that are grown on a commercial scale (Aradhya et al. 2003; Karamura et al. 2008). Among vulnerable and threatened species of plants with severely depleted populations, somatic mutations have been suggested as an important source of genotypic diversity that may work to counteract inbreeding depression (Gross et al. 2012).

In the hopes of further shedding light on the evolutionary consequences of somatic mutations and the plausibility of CLS, I performed multiple experiments to determine the fitness effects of somatic mutations in the progeny of the bush monkeyflower (*Mimulus aurantiacus*). *M. aurantiacus* is a woody perennial species complex that is found throughout semi-arid regions of southwestern North America. The genus *Mimulus* contains countless well established and fast emerging model

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organisms. These species complexes have long been used to investigate evolutionary processes involved in the emergence and maintenance of genomic and phenotypic diversity (Wu et al. 2008). Unlike *M. aurantiacus*, *M. guttatus* is herbaceous and is found both as an annual and a perennial based on environment (Friedman et al. 2014). By investigating the fitness effects of somatic mutations in *M. aurantiacus* progeny, I was able to compare the impact of CLS on closely related plant species that differ in important life history characteristics.

This experiment involved creating progeny through two forms of self-fertilization: self-pollinations made within the same flower (autogamy) and self-pollinations made between stems on the same plant (geitonogamy). Autogamy leads to homozygosity of somatic mutations in a proportion of progeny, while progeny created through geitonogamy remain heterozygous for mutations unique to each stem. The overall fitness of these two sets of progeny was measured using multiple direct and indirect fitness metrics, including: fruit weight, total seed weight, leaf area, and survival under terminal drought conditions. If future germ cell lineages within the SAM are not segregated from future somatic cell lineages during early development, and CLS does sieve out deleterious somatic mutations while retaining beneficial somatic mutations, one would expect autogamous progeny to display both increased variation in fitness and a significant difference in fitness when compared with geitonogamous progeny. This difference in fitness would be attributable to the accumulation of somatic mutations. These results would be consistent with those recorded by Cruzan et al. (2020) during their experiments with *M. guttatus*, and would thus reveal a prominent role for somatic mutations in plant evolution.

Methods

Crossing

Two forms of self-fertilization were employed to create progeny that could be either potentially homozygous for any somatic mutations unique to an individual stem, or never homozygous for those somatic mutations. The first method (autogamy) involved fertilizing a bisexual flower using pollen extracted from its own anthers. The second method (geitonogamy) involved fertilizing another flower on a different stem of the same genet with the same sample of pollen from the autogamous cross. Autogamous progeny are homozygous (áá) for somatic mutations (á) unique to their own stem 25% of the time, while geitonogamous progeny created on separate stems would always either be heterozygous (aá) for that same complement of somatic mutations or homozygous (aa) for the ancestral allele. The method I employed for producing autogamous and geitonogamous crosses is an improvement on the method used by Cruzan et al. (2020), where pollen from two stems was reciprocally crossed to create two autogamous and two geitonogamous crosses. I did not use that method because the fitness of the geitonogamous crosses was confounded by the combined effect of the somatic mutations found in the stem of the donor flower and by the potential for flower pairs at each node to possess different complements of somatic mutations. Instead, I used pollen from a single flower to produce one autogamous and three geitonogamous crosses, which mitigates the confounding effect of unique somatic mutations in each geitonogamous stem.
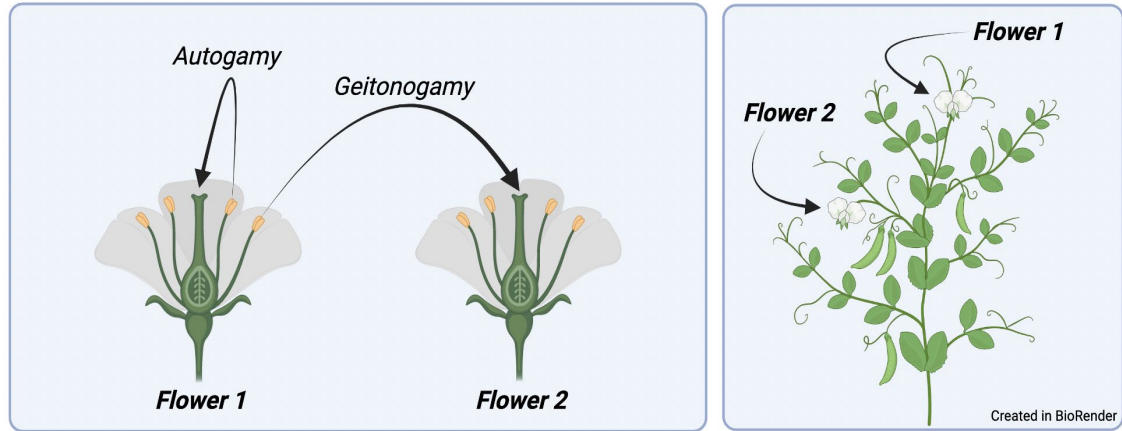
Figure 1: Diagram of Autogamous and Geitonogamous Fertilization

Autogamous cross is produced by fertilizing flower 1 using pollen from one of four anthers found on flower 1. Geitonogamous cross is produced by fertilizing flower 2 using pollen from flower 1. Both flower 1 and flower 2 are located on the same plant or genet.

Experiment Set-Up

Autogamous and geitonogamous progeny were produced from 26 *M. aurantiacus* genets located in an open plot in Eugene, Oregon. These genets are all hybrids created through the crossbreeding of red-flowered and yellow-flowered ecotypes that in the wild remain isolated primarily due to premating barriers (Sobel & Streisfeld 2015). Each cross was created by removing an anther from a flower. The pollen from the anther was then deposited onto the stigma of either that same flower (autogamy) or onto a flower from a different stem of the same genet (geitonogamy). Since each flower has four anthers, three geitonogamous crosses on three different stems were paired with each autogamous cross. Between 1 and 22 July 2021, I made 180 crosses. The flowers were then allowed to develop into fruits and dry out before

being collected in late August 2021. A total 169 crosses successfully developed into fruits and were collected in manila envelopes.

Figure 2: Development of Mimulus aurantiacus from Flower to Dried Fruit

(A) M. aurantiacus flowers in mid June 2021. Anthers are present and stigma is open. (B) Recently created autogamous cross denoted with a slip of yellow tape at the base of the flower. All four anthers have been removed and the stigma is closed. (C) Geitonogamous cross two weeks after fertilization. Visible bulge above slip of blue tape contains the growing fruit. (D) Dried fruits in late August 2021.

Fruit and Seed Weight

The dried calyx exterior was removed from each fruit before it was weighed using an analytical balance. The shell of each fruit was then carefully removed and the seeds were collected. The total weight of the seeds found within each individual fruit was then determined using an analytical balance. Previous work in the Streisfeld lab has shown that seed weight is a strong predictor of seed number (Streisfeld, unpublished).

Drought Sensitivity

Inland ecotypes of *M. aurantiacus* are drought tolerant shrubs that have adapted to endure seasonal droughts in southern California (Sobel et al. 2019). Because of these seasonal droughts, drought sensitivity likely serves as a principal agent of selection for

these ecotypes in the wild. Experimental evolution studies involving microbes have indicated that the occurrence of beneficial mutations can be accelerated in clonal populations through the usage of selective pressures on the environment (Maharjan et al. 2015). Because of its relevance and potential to drive the occurrence of beneficial mutations, a terminal drought experiment was conducted to measure the fitness of *M. aurantiacus* progeny bred in this experiment.

Six of the twenty-six genets successfully produced at least two sets of complete crosses, where three geitonogamous fruits and one autogamous fruit were generated from one pollen donor. For these 12 sets, 40 and 20 seeds were taken from each autogamous and geitonogamous fruit respectively. On 5 February 2022, seeds were sown in germination soil and placed in a grow room on a 16/8-hr light/dark cycle. After three weeks, the seedlings were transplanted into individual containers, with the location of each seedling randomly determined within racks. Additionally, the cross-type identity of each plant was kept anonymous until the experiment had ended. Optimally, 18 autogamous and 18 geitonogamous seedlings (6 from each cross) were to be used for each genet-pollen donor combination in the experiment, but due to poor seed germination the number of seedlings used for each set of crosses ranged between 5 and 18 for the autogamous crosses, and between 12 and 18 for the geitonogamous crosses. After transplantation, the seedlings were kept in the grow room for an additional week to mitigate shock. The seedlings were then moved to the University of Oregon greenhouse, where they were given water as needed until they developed additional new leaves.

At the beginning of the experiment, the soil from each plant's cone was saturated with water one last time before watering ceased. On each subsequent day, I measured each of the 343 plants for drought symptoms using a categorical score between 0 and 4, following the approach used by Sobel et al. (2019). A score of 0 indicated no sign of drought stress. A score of 1 indicated initial signs of drought stress, including the adaxial side of the leaves curling under. A score of 2 indicated the first sign of true wilting. A score of 3 indicated systemic and severe wilting. A score of 4 indicated death of the plant. The experiment for each of the six genets ended once all plants were assigned a score of 4. Plants were measured at the same time each day throughout the experiment.

Total Leaf Area

On day 0 of the drought experiment, an overhead photograph was taken of each seedling. Adobe Photoshop 2022 was then used to determine the total leaf area (cm^2) of each plant. A linear regression of drought sensitivity against leaf area for genet A seedlings revealed a strong correlation, indicating that total leaf area is a good predictor of drought sensitivity. Consistent with the findings of Sobel et al. (2019), this strong correlation suggests that drought symptoms are intensified by higher transpiration rates, which result from greater leaf area. Furthermore, biomass and growth rate are indirect metrics of fitness and strong positive correlations have been observed between these two metrics and direct metrics of fitness, including fecundity (Younginger et al. 2017).

Data Analysis

Variation in fruit and seed weight

Autogamy depression occurs when autogamous progeny demonstrate decreased viability relative to geitonogamous progeny due to the accumulation of deleterious mutations during mitotic growth (Cruzan et al. 2020). An increase in the frequency of embryo abortions among autogamous crosses is an often-observed characteristic of autogamy depression (Schultz & Scofield, 2009). Thus, autogamy depression would be indicated if autogamous crosses consistently demonstrate reduced fruit and seed weight relative to geitonogamous crosses.

The programming language R was used to determine the mean and standard deviation of fruit and seed weight among paired autogamous and geitonogamous crosses. For each paired set of crosses, the values from the geitonogamous crosses were averaged in order to mitigate any variation caused by mutations unique to each geitonogamous stem. R was then used to perform a paired t-test between cross types for both fruit and seed weight. Sets of box-plots were created for fruit and seed weight for all 26 genets, along with a boxplot with the genets combined.

Drought sensitivity and leaf area in progeny

As described above, under a model of CLS, there should be a difference in fitness between cross types, as well as increased variation in fitness among autogamous seedlings relative to geitonogamous seedlings. This difference in fitness could be interpreted as the result of the phenotypic expression of somatic mutations in some of the autogamous seedlings, since a portion should be homozygous for the unique complement of mutations from their stem of origin. Reduced drought sensitivity among autogamous progeny could be a result of the transmission of potentially beneficial somatic mutations that arose in the SAM. On the other hand, if autogamous progeny are

more negatively impacted by drought conditions, it would suggest the transmission of potentially deleterious somatic mutations.

In order to generate a metric of drought sensitivity experienced by each seedling, the slope of the linear regression between drought score and day was calculated for each plant using R. The steeper the slope, the faster a plant achieved a terminal drought score of 4, which indicates poorer drought tolerance. The y-intercept of the linear regression was forced through the origin because each plant began the experiment with a score of 0. For each genet-pollen donor combination, I performed a separate t-test to ask if drought sensitivity differed between the autogamous progeny and the mean of the three geitonogamous progeny. These t-tests were repeated for total leaf area as the response variable.

Results

Fruit and Seed Weight

There was considerable variation both among the genets and between cross types. Some genets had greater fruit and seed weight among autogamous crosses, while others had greater fruit and seed weight among geitonogamous crosses, or showed no difference between cross types (Figure 3). For all successful crosses, the mean seed weights were 0.01040 and 0.00897 (g) for autogamous and geitonogamous crosses respectively, with accompanying standard deviations of 0.00331 and 0.00292 (g). The paired t-test (df = 45) yielded a significant p-value of 0.0009496, indicating that the mean seed weight from autogamous crosses was significantly greater than from geitonogamous crosses. The mean fruit weights were 0.0486 and 0.0435 (g) for autogamous and geitonogamous crosses respectively, with accompanying standard deviations of 0.0136 and 0.0133 (g). The paired t-test (degrees of freedom 45) yielded a significant p-value of 0.001589, indicating that the mean seed weight from autogamous crosses was significantly greater than from geitonogamous crosses. Overall, while variation was high among genets and between cross types, the paired t-tests demonstrate that autogamous crosses produced significantly larger fruits with greater total seed weights when compared with their geitonogamous counterparts (Figure 4).
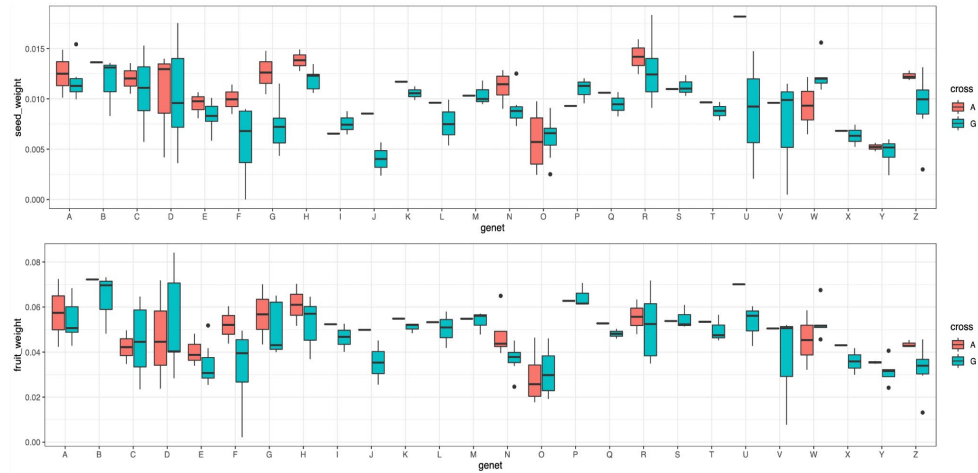
Figure 3: Box Plot of Seed and Fruit Weight Among 26 Genets

Box plot comparisons of seed (top) and fruit (bottom) weight between cross types for 26 genets. The center bar of each box represents the median value, while the upper and lower bars represent the upper and lower quartiles. The vertical lines represent upper and lower adjacent values. Dots indicate outliers. Red indicates autogamous crosses (A), while blue represents geitonogamous crosses (G).
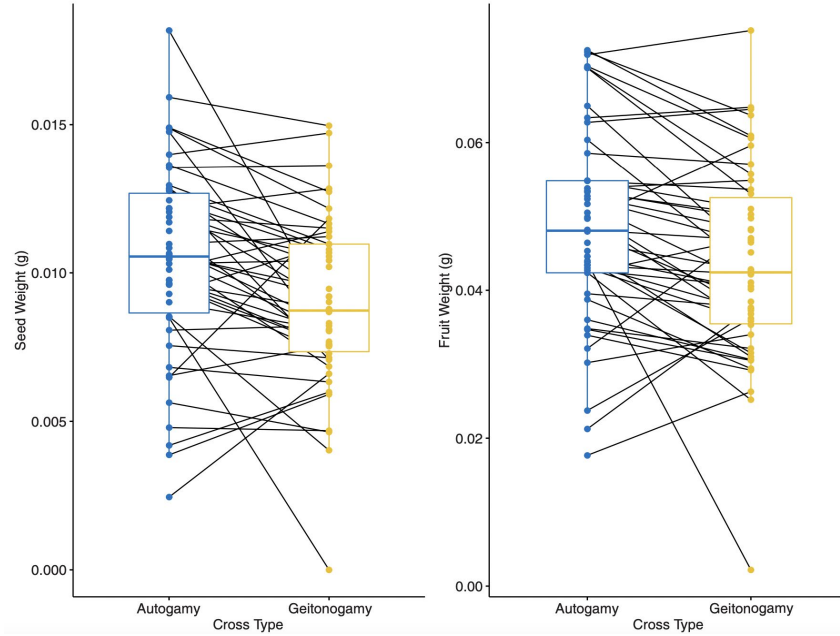
Figure 4: Box Plot of Seed and Fruit Weight Between Cross Types

Box plot comparing seed (left) and fruit (right) weight between cross types from all genets. The center bar of each box represents the median value, while the upper and lower bars represent the upper and lower quartiles. The vertical lines represent upper and lower adjacent values. Solitary dots not connected to the vertical lines indicate outliers.

Relationship Between Fruit and Seed Weight

The linear regression created between fruit and seed weight had a residual standard error of 0.002028 on 161 degrees of freedom (Figure 5). The multiple and adjusted R-squared were 0.658 and 0.6558 respectively. The model had a significant p-value of 2.2e-16, indicating a strong correlation between seed and fruit weight. While these findings reveal nothing related directly to CLS, they do provide new insight on the relationship between two indirect metrics of fitness in *M. aurantiacus*.
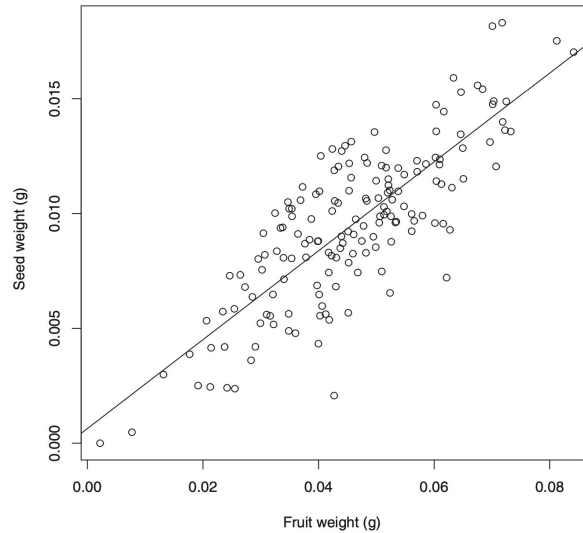
Figure 5: Scatterplot of Fruit and Seed Weight from Raw Data

Scatterplot of fruit and seed weight (g) from raw data on all successful crosses. The line of best fit was created through a linear regression model with 161 degrees of freedom.

Drought Sensitivity and Leaf Area

Each genet-pollen donor combination was treated as an independent replicate in the terminal drought experiment (Figure 6, top). Among the 12 replicates, the difference in drought sensitivity between cross types was significant ($p < 0.05$) for three sets, and near-significant ($p < 0.1$) for another two sets (Table 1). For the majority of replicates—including all with at least near-significant results—the autogamous seedlings demonstrated lower drought sensitivity. These findings indicate that autogamous progeny occasionally are more drought tolerant, potentially because of the transmission and segregation of beneficial somatic mutations.

For total leaf area, again each genet-pollen donor combination was treated as an independent replicate of the experiment (Figure 6, bottom). Among the 12 replicates,

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the difference in total leaf area between cross types was significant for three sets, and near-significant for another two sets. In general, autogamous crosses had lower total leaf area when compared with their paired geitonogamous progeny.
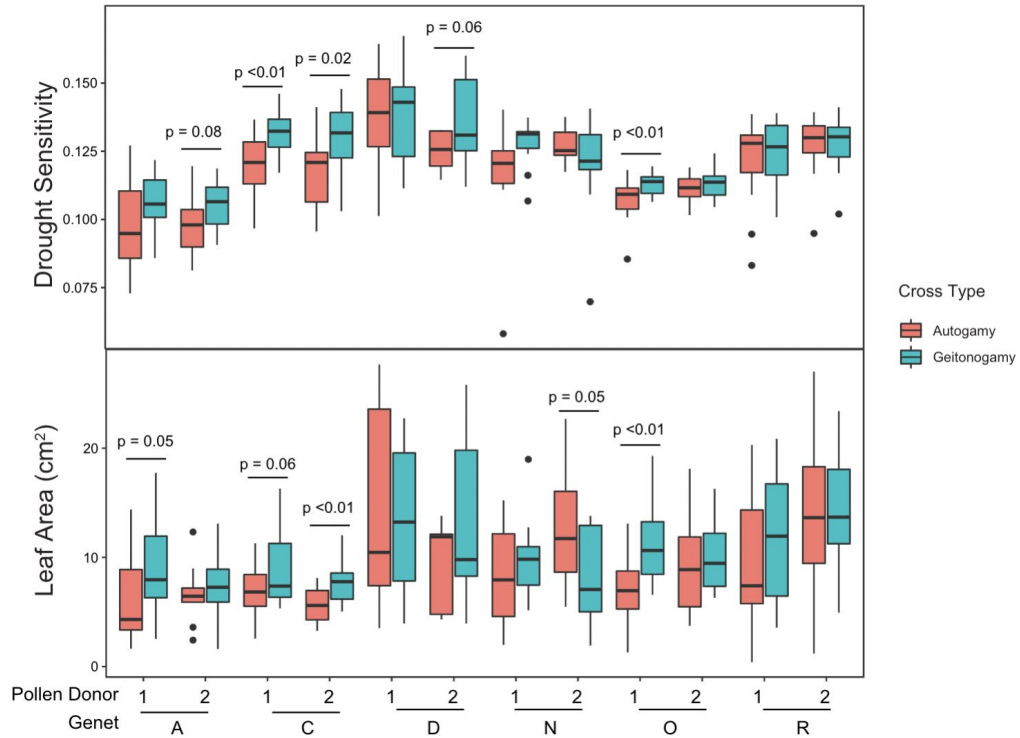
Figure 6: Box Plot of Drought Sensitivity and Leaf Area Among 12 Replicates

Box plot comparisons of drought sensitivity (top) and leaf area (bottom) between cross types for 12 pollen donors from 6 genets. Significant ($p < 0.05$) and close to significant ($p < 0.1$) p-values are denoted above respective box plots. The center bar of each box represents the median value, while the upper and lower bars represent the upper and lower quartiles. The vertical lines represent upper and lower adjacent values. Dots indicate outliers.

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Genet	Donor	Degrees of Freedom	Autogamous Mean	Geitonogamous mean	P-Value
A	1	15.569	0.0976593	0.1070763	0.1239
A	2	13.878	0.09776215	0.10584814	0.08119*
C	1	18.232	0.1196880	0.1315511	0.006955**
C	2	26.591	0.1178860	0.1294992	0.0194**
D	1	11.265	0.1378288	0.1381923	0.965
D	2	14.356	0.1250029	0.1366902	0.05786*
N	1	5.4629	0.1128102	0.1277219	0.261
N	2	17.47	0.1272692	0.1206908	0.2158
O	1	23.819	0.1071837	0.1130741	0.009625**
O	2	29.862	0.1113752	0.1131262	0.3268
R	1	31.548	0.1221248	0.1252182	0.4783
R	2	32.992	0.1276905	0.1282039	0.8798

Table 1: Statistical results for Welch's t-test of drought sensitivity between cross types.

P-values near statistical significance (*) and statistically significant (**) denoted by asterisk(s).

Genet	Donor	Degrees of Freedom	Autogamous Mean	Geitonogamous mean	P-Value
A	1	24.216	5.925002	9.046460	0.0508*
A	2	17.073	6.619369	7.396590	0.5283
C	1	28.494	6.928409	8.888985	0.06351*
C	2	27.858	5.528239	7.725620	0.003923**
D	1	9.9295	14.14232	13.39446	0.8485
D	2	12.235	9.384806	12.793121	0.2555
N	1	7.6952	8.357230	9.811865	0.5599
N	2	20.494	12.57944	8.44121	0.04698**
O	1	32.988	7.321799	10.995589	0.002159**
O	2	25.368	8.906298	10.079434	0.3699
R	1	33.975	9.251561	11.895675	0.1842
R	2	31.075	14.34704	14.12876	0.9173

Table 2: Statistical results for Welch's t-test of total leaf area (cm²) between cross types.

P-values near statistical significance (*) and statistically significant (**) denoted by asterisk(s).

Discussion

Increased Fruit and Seed Weight Among Autogamous Progeny

The data do not demonstrate evidence for autogamy depression, as autogamous crosses displayed increased fecundity relative to geitonogamous crosses. Specifically, both fruit and seed weight were significantly greater among the progeny from autogamous crosses, with p-values of 0.001589 and 0.0009496, respectively. These data are in contrast with the findings from Cruzan et al. (2020), who detected greater rates of embryo abortion among autogamous crosses compared to geitonogamous crosses but no difference in seed production between cross types. Thus, my data suggest that CLS may be altering the distribution of mutations expressed among autogamous crosses, which are occasionally homozygous for somatic mutations unique to their stem of origin. This increase in fecundity suggests that deleterious somatic mutations may be sieved out of the SAM cell population, while beneficial and neutral somatic mutations have the potential to remain, accumulate, and be inherited in the next generation.

Decreased Drought Sensitivity and Leaf Area Among Autogamous Progeny

Among most experimental replicates, autogamous progeny demonstrated reduced drought sensitivity relative to geitonogamous progeny. Autogamous progeny from three replicates displayed significantly lower drought sensitivity when compared with their geitonogamous counterparts, which indicates that they are able to better tolerate the effects of drought conditions. Another two replicates displayed lower drought sensitivity among autogamous progeny that was near-significant. There were no replicates that revealed significantly greater drought tolerance in geitonogamous

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progeny. These data are consistent with the findings of Cruzan et al. (2020), who observed that autogamous progeny can occasionally demonstrate increased fitness relative to geitonogamous progeny. These findings further support the prediction that CLS is operating and is capable of sieving out deleterious somatic mutations from the SAM cell population, while leaving beneficial somatic mutations to accumulate during vegetative growth.

Additionally, in several replicates, autogamous progeny demonstrated lower leaf area relative to geitonogamous progeny. This decrease in leaf area was significant in three replicates, and near-significant in an additional two replicates. A strong positive correlation was observed between leaf area and the severity of drought sensitivity. This finding is consistent with previous work in the red and yellow ecotypes of *M. aurantiacus* (Sobel et al. 2019), and further suggests that drought sensitivity is strongly tied to rates of transpiration from leaves.

Since the primary difference between cross types was the potential for autogamous progeny to be homozygous for mutant alleles unique to their stem of origin, the general decrease in leaf area among autogamous progeny can potentially be attributed to the expression of these somatic mutations in some portion of progeny. This decrease in leaf area would result in increased drought tolerance by reducing the amount of water lost from transpiration. Since this decrease in leaf area resulted in an increase in survival due to drought tolerance, the net effect of these somatic mutations appears to have been beneficial.

To determine whether leaf area alone explains the difference in drought sensitivity, future terminal drought experiments could pair cross types with similar leaf

areas in order to control for the variable. If the difference in drought sensitivity is no longer significant between cross types, it would strongly suggest that the transpiration rate of the plants is determined primarily by their leaf area, and that leaf area alone explains the difference in drought sensitivity observed in the original experiment. If drought sensitivity is not directly mediated via differences in leaf area, an alternative explanation would be needed for these results. For example, differences in leaf area may still be adaptive but due to another selective agent. If larger leaf area at the seedling stages results in increased fitness, perhaps due to differences in establishment or survival, then the smaller leaf areas in autogamous progeny among multiple replicates would indicate evidence for segregation of deleterious mutations in those progeny groups. Further work will be needed to identify the connection between seedling leaf area and fitness.

Limitations of the Data

Despite the findings that autogamous progeny occasionally have higher fitness than geitonogamous progeny, the sample size used in the experiment may have had an impact on these conclusions. Indeed, for two of the replicates, the difference in fitness between autogamous and geitonogamous progeny approached a p-value of 0.05, and for several others the means of autogamous progeny had lower drought sensitivity than geitonogamous progeny but were not statistically different. This could indicate limited power to detect a statistically significant effect because of a low sample size. As noted, poor germination of the seeds reduced the sample sizes in many of the replicates. Given that only a portion of progeny would be homozygous for a somatic mutation, increasing the number of seedlings used in the drought experiment would have led to greater

numbers of progeny segregating for each genotype. Edge effects also may have impacted estimates of the drought sensitivity of seedlings that lined each container compared to those seedlings that were surrounded by multiple neighboring plants. Exposure to greater airflow may have increased transpiration from those edge plants. However, the random assignment of each seedling's location in the containers likely mitigated issues caused by edge effects. Lastly, while decreased leaf area among autogamous seedlings appears to have led to an increase in fitness through decreased drought sensitivity, the pleiotropic effects of leaf area on other agents of selection remain largely unexplored in *M. aurantiacus*.

Implications and Future Directions

The significant increase in fitness observed in these experiments supports the hypothesis that CLS is capable of sieving out deleterious somatic mutations that arise during vegetative growth while retaining beneficial somatic mutations. These findings are consistent with those of several studies that have suggested somatic mutations as a source of genetic diversity among severely depleted plant populations that are vulnerable to inbreeding depression, and further provides CLS as a mechanism to explain the phenomenon (Aradhya et al. 2003; Karamura et al. 2008). Additionally, these findings further support the observations of Gross et al. (2012), who claim that somatic mutations are a potential source of unwanted variation in clonally-derived crops that are lauded for their uniform characteristics. Overall, CLS appears to be a viable explanation to address the paradox that despite a plant's ability to pass on somatic mutations to progeny, plants continue to have similar mutation rates per generation as animals.

While CLS is a plausible explanation for the findings of the experiments described in this thesis and for the findings of Cruzan et al. (2020), further CLS research is warranted. Future work should explore the influence of CLS in novel plant models, including in clades besides angiosperms, since the organization and physiology of the SAM varies drastically among clades (Lanfear 2018). Studies in microbial models also have demonstrated that it is common for multiple cell lineages to acquire multiple beneficial mutations over a short period of time (Lee & Marx 2013). These mutated cell lineages appear to compete against one another and against ancestral lineages, which generates clonal interference. Future studies in plants could investigate the competition between mutant cell lineages in SAM cell populations by using quantitative cell lineage data to construct stochastic models that account for clonal interference. These models could potentially predict the influence of CLS on division in SAM cell populations with greater accuracy than present models. Overall, while the findings of this thesis strongly support the plausibility of CLS, much remains to be uncovered on the role of CLS in plant evolution and its implications on agriculture, conservation biology, and other aspects of our daily lives.

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